RESEARCH PAPER

Supercritical CO_2-Extracted Advanced Polyherbal Therapy Combining Phytochemicals and Metformin Shows Superior Glycemic and Cardioprotective Effects in Experimental Diabetes

Santosh N. Belhekar[1*], Rajendra D. Dighe[2], Deepak K. Bharati[3], Vishal S. More[4], Babasaheb V. Bhagat[5], Santosh R. Tarke[6], Sameer J. Nadaf[7]

[1]Gourishankar Institute of Pharmaceutical Education & Research, Limb, Satara 415015, Maharashtra, India. Corresponding Author: Santosh Nivrutti Belhekar. Email: santoshbelhekar@gmail.com. Orcid id: 0000-0002-3313-6778.

[2]Sandip Foundation's Sandip Institute of Pharmaceutical Sciences, Mahirawani, Trimbak Road, Nashik 422213 Maharashtra, India. Email: digherd@gmail.com. Orchid id: 0009-0004-8239-0109

[3]AET's St John Institute of Pharmacy and Research, Palghar 401404, Maharashtra, India. Email: deepakbharti007@gmail.com. Orcid id: 0000-0003-3739-2178

[4]Amrutvahini Sheti and Shikshan Vikas Sanstha's Amrutvahini College of Pharmacy, Sangamner, 422608, Maharashtra, India. Email: vish2482@gmail.com. Orchid id: 0000-0003-4140-5373

[5]Dr Vithalrao Vikhe Patil Foundations College of Pharmacy, Vilad Ghat, Ahilyanagar. Email: babasahebbhagat@gmail.com. Orcid id: 0009-0009-3486-1048

[6]SBSPM's B. Pharmacy College Ambajogai Dist. Beed, Maharashtra, India. Email: tarkesantosh@gmail.com. Orcid id: 0009-0006-6473-0987

[7]Bharati Vidyapeeth College of Pharmacy, Palus, 416310, Maharashtra, India

Running title: Metformin and Herbal Treatments for Diabetic Neuropathy

ABSTRACT

Background: The development of safer and more effective therapeutic approaches that combine conventional medications with herbal phytochemicals is necessary since diabetes mellitus and its cardiovascular consequences continue to be a major global health concern.

Purpose: To investigate the efficacy of a novel supercritical CO_2-extracted polyherbal formulation (PHF) and its advanced form (APHF, containing PHF with metformin at 100 mg/kg each) on glycemic control and cardiac markers in streptozotocin–nicotinamide (STZ-NA)-induced diabetic rats.

Materials and Methods: The PHF comprised of Emblica officinalis, Terminalia arjuna, Gymnema sylvestre, Tinospora cordifolia, and Zingiber officinale was prepared using Supercritical fluid extraction method. Diabetic rats were orally administered metformin (200 mg/kg), PHF (100, 200, and 400 mg/kg), or APHF (200 mg/kg) for 45 days. Biochemical, lipid, and cardiac marker parameters were evaluated to assess anti-hyperglycemic and cardioprotective effects.

Results: Treatment with PHF and APHF significantly ($p<0.01$) increased body weight, reduced blood glucose levels, improved lipid profiles, decreased atherogenic index, increased serum leptin and liver glycogen content, and reduced serum C-reactive protein levels compared with diabetic control rats. APHF and the high-dose PHF (400 mg/kg) demonstrated superior anti-hyperglycemic activity compared to metformin. The formulations also exhibited potential cardioprotective effects by significantly ($p<0.01$) reducing the activity of cardiac marker enzymes (CK, AST, and LDH). Overall, APHF and PHF-400 showed the most promising results in managing various aspects of diabetes and its complications, suggesting their potential as effective treatments for diabetes mellitus and diabetic cardiomyopathy (DCM).

Conclusions: The supercritical fluid extraction method employed introduces a novel approach for the development of safer and more potent PHFs.

Keywords: Diabetic cardiomyopathy; Antihyperglycemic; Cardiotonic; Hyperlipidaemia, herbal, Medicinal plants

How to cite this article: Belhekar SN, Dighe RD, Bharati DK, More VS, Bhagat BV, Tarke SR, Nadaf SJ. Supercritical CO_2-Extracted Advanced Polyherbal Therapy Combining Phytochemicals and Metformin Shows Superior Glycemic and Cardioprotective Effects in Experimental Diabetes. Int J Drug Deliv Technol. 2026;16(18s): 295-306. DOI: 10.25258/ijddt.16.18s.31

Supercritical CO_2-Extracted Advanced Polyherbal Therapy Combining Phytochemicals and Metformin Shows Superior Glycemic and Cardioprotective Effects in Experimental Diabetes

Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease affecting hundreds of millions worldwide. According to recent estimates, about 537 million adults globally were living with diabetes in 2021[1]. Persistent hyperglycemiainduces oxidative stress, inflammation, and dyslipidemia, which progressively damage multiple organs, including the cardiovascular system, kidneys, liver and nervous system[2]. Among the serious cardiovascularcomplications,diabetic cardiomyopathy (DCM) is characterized by structural and functional abnormalities of the myocardium independent of coronary artery disease, leading to diastolic dysfunction, fibrosis, and eventual heart failure[3]. DCM contributes to heart failure and accounts for a significant proportion of diabetes-related mortality[4]. Elevated levels of inflammatory markers such as C-reactive protein (CRP) and pro-inflammatory cytokines, along with altered adipokines including leptin, play a pivotal role in myocardial remodeling and cardiac dysfunction in diabetes[5].

Conventional oral hypoglycemic drugs like metformin (a first-line oral agent) improve glycemic control but they may cause gastrointestinal intolerance, vitamin B12 deficiency, and long-term compliance issues[6]. Hence, there is growing interest in medicinal plant-based therapies, that provide multi-faceted metabolic regulation, with fewer adverse effects[7]. Several medicinal plant used in Ayurveda, including*Emblicaofficinalis* (Indian gooseberry), *Terminalia arjuna* (Arjuna bark), *Gymnemasylvestre* (Gymnema), *Tinosporacordifolia* (Guduchi), and *Zingiberofficinale* (Ginger) exhibit well-documented antidiabetic and cardioprotective properties[8]. Each contains bioactive phytochemicals (flavonoids, tannins, saponins, etc.) that may act on different targets.

E. officinalis is rich in vitamin C and ellagitanninsand exhibitspotent antioxidant and glucose lowering effects in diabetic models[9]. *T. arjuna* bark, traditionally used for heart ailments, contains glycosides and tannins that improve contractility and lipid metabolism [10]. *G. sylvestre* contain gymnemic acids that suppress intestinal glucose absorption and promote pancreatic *β*-cell regeneration[11]. *T. cordifolia*actsas an immunomodulatory and insulin secretagogue and downregulates hepatic gluconeogenesis[12]. *Z. officinale* (ginger) is enriched with gingerols, shogaols, which exertinflammatory, antioxidant,hypolipidemic, and anti-fibrotic effects beneficial in diabetic complications[13].

Combining bioactive medicinal plants in a polyherbal formulation (PHF)offers the potential for synergistic therapeutic effects by modulating diverse metabolic and inflammatory pathways simultaneously [14]. Traditional Ayurvedic practice often uses herb combinations to enhance efficacy. Indeed, herbal compounds with multiple plant ingredients are believed to have synergistic antidiabetic effects that enhance desired actions beyond single herbs[15,16]. Using such formulations can also allow lower doses of each component, potentially reducing side effects. In recent years, various polyherbal antidiabetic formulations have shown promising results in experimental models[17]. However, while PHFs enhance multi-target efficacy, their insulin-lowering effect may not always be sufficient in moderate to severe diabetes. To address this issue, we also created an Advanced Polyherbal Formulation (APHF), which incorporates a low amount of metformin (100 mg/kg) into the PHF.This strategy is based on the concept of phytochemical-drug synergism[18,19], in which herbal components boost insulin sensitivity, antioxidant defense, and lipid metabolism while metformin ensures consistent glycemic control through AMPK activation. The idea for APHF is to improve antidiabetic efficacy while allowing for a lower metformin dose, potentially reducing drug-related side effects and enhancing long-term safety

Therefore, in this study, PHF containing *E. officinalis*, *T. arjuna*, *G. sylvestre*, *T. cordifolia*, and *Z. officinale*usingsupercritical carbon dioxide (CO_2) extraction was developed.This green extraction technology operates at moderate temperatures and leaves no solvent residue, thereby preserving thermolabileand bioactive phytoconstituentsand augmenting extract purity[20]. The inclusion of metformin in APHF was designed to evaluate whether a phytochemical–metformin hybrid therapy offers superior glycemic control and cardioprotection compared to PHF or metformin alone in thestreptozotocin–nicotinamide (STZ–NA) diabetic rat model.Effects on blood glucose, body weight, lipid profile, atherogenic index, leptin, liver glycogen, C-reactive protein (CRP) and cardiac injury markers including creatine kinase (CK), aspartate transaminase (AST) and lactate dehydrogenase (LDH) were measured. We hypothesized that PHF and APHF would

exert significant hypoglycemic and cardioprotective effects, with APHF anticipated to exhibits superior therapeutic effects owing to phytochemical–drug synergism.

MATERIALS AND METHODS

Drugs and Chemicals

Streptozotocin (STZ) was obtained from Chemvenio, LIC, Gulbarga, Karnataka, Nicotinamide (NAD) from SDS Neutraceuticals, Karad, and Metformin tablet from USV Pharma. All compounds utilized in this study were analytical grade.

Preparation of PHF and APHF

Five medicinal plants – *Emblicaofficinalis* (fruit), *Terminalia arjuna* (bark), *Gymnemasylvestre* (leaf), *Tinosporacordifolia* (stem), and *Zingiberofficinale* (rhizome) – were collected from authenticated sources and dried in shade. The plant materials were powdered and subjected to supercritical fluid extraction (SFE) using CO_2 of >99.9% purity. In brief, the dried powders were loaded into an SFE apparatus; CO_2 was used as the solvent at optimal supercritical conditions (e.g. ~45 °C and 25 MPa) to extract bioactive compounds. The CO_2 was then depressurized to yield solvent-free herbal extracts. Extracts of the five plants were combined in equal ratio to form the base PHF. APHF was created by enriching PHF with concentrated active fractions and adding metformin at a dose of 100 mg/kg to improve glycemic effectiveness via phytochemical-drug synergy.Both PHF and APHF were stored in airtight containers at 4 °C until use[21,22].

Experimental animals

Male Wistar rats (180–220 g) were procured and housed under standard laboratory conditions (12-h light/dark cycle, 22 ± 2 °C, 50–60% humidity) with free access to standard chow and water. All animal procedures were approved by the Institutional Animal Ethics Committee and followed CPCSEA guidelines(Protocol approval number: SCOP/IAEC/2011-12/38, dated 3/10/2011).

Induction of type 2 diabetes

After one week of acclimation, type 2 diabetes mellitus was induced in overnight-fasted rats by a single intraperitoneal injection of STZ (65 mg/kg in 0.1 M citrate buffer, pH 4.5), 15 minutes after an intraperitoneal administration of nicotinamide (110 mg/kg). This STZ-NA protocol partially protects pancreatic *β*-cells, producing moderate, stable hyperglycemia analogous to non-insulin-dependent diabetes. Three days after STZ-NA, blood glucose was measured with a glucometer, and rats with fasting blood glucose >250 mg/dL were considered diabetic and included in thestudy. Diabetic rats exhibited polyuria, mild weight loss, and moderate hyperglycemia (typically 250–350 mg/dL) but not ketosis, consistent with type 2 diabetes features[23].

Experimental design

Diabetic rats were randomly divided into six groups of six rats each: Group I – Normal Control (vehicle only); Group II – Diabetic Control (STZ-NA, untreated); Group III – Diabetic + Metformin (200 mg/kg, per os); Group IV – Diabetic + PHF Low (PHF 100 mg/kg, p.o.); Group V – Diabetic + PHF medium (PHF 200 mg/kg); Group VI – Diabetic + PHF high (PHF 400 mg/kg); Group VII – Diabetic + APHF (APHF 200 mg/kg). Treatments were given once daily by oral gavage for 45 days, starting 5 days after diabetes induction. The metformin dose (200 mg/kg) served as a positive control known to effectively lower glucose in this model[24].

Monitoring and sample collection

Body weight and fasting blood glucose (FBG) were recorded on day 0 (before treatment) and then weekly (days 7, 14, 21, 28, 35, and 45). FBG was measured via tail vein blood using a glucometer. An oral glucose tolerance test (OGTT) was conducted on day 40 in overnight-fasted rats to assess glucose handling (2 g/kg oral glucose; blood glucose measured at 0, 30, 60, 120 min). On day 45, rats were fasted overnight and then euthanized under anesthesia. Blood was collected by cardiac puncture for biochemical assays. The heart was dissected out, rinsed, and homogenized for tissue biochemical analysis. Liver was excised to measure glycogen content.

Biochemical analyses

Serum glucose, lipid profile [total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C)], and enzyme assays were performed. Low-density lipoprotein cholesterol (LDL-C) was calculated by the friedewald formula. The atherogenic index was calculated as the ratio of (TC – HDL-C) to HDL-C, an indicator of cardiovascular risk. Serum leptin and CRP levels were quantified using ELISA kits. Liver glycogen content was measured by a colorimetric assay (anthrone method) on liver homogenates. Cardiac injury markers namely CK, AST, and LDHwere assayed in serum using standard protocols. All measurements were performed in duplicate and averaged[25-27].

Statistical analysis

Data were expressed as mean ± standard deviation (SD) for n = 6 rats per group. Statistical comparisons were made using one-way ANOVA followed by Tukey's post-hoc test. A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening of PHF confirmed the presence of major phytochemical classes (flavonoids, terpenoids, phenolics, saponins, alkaloids, tannins, carbohydrates, steroids, glycosides, and proteins).

Induction of diabetes

Rats injected with STZ-NA developed moderate hyperglycemia and other diabetic features. Diabetic control animals had significantly higher FBG than normal controls (≈300 vs 95 mg/dL, $p<0.01$) and showed ~25% loss in body weight over 45 days (Table 1). They also exhibited dyslipidemia (elevated total cholesterol and triglycerides, low HDL-C) and high levels of inflammatory and cardiac injury markers compared to normal.

Effect on body weight

Treatment with the polyherbal formulation prevented weight loss in diabetic rats. By day 45, PHF and APHF groups had gained weight, whereas untreated diabetic controls continued to lose weight. PHF at 400 mg/kg (PHF-400) and APHF (200 mg/kg) restored body weights close to normal (within 10% of baseline), significantly higher than diabetic controls ($p<0.01$). Even the low (100 mg/kg) and medium (200 mg/kg) PHF doses attenuated weight loss (Table 1). Metformin-treated rats also lost less weight than diabetic control, but PHF-400 and APHF achieved greater weight improvement than metformin.

Anti-hyperglycemic effects

All doses of PHF produced a time-dependent reduction in blood glucose. FBG levels (Figure 1) in PHF-treated groups were significantly lower than in diabetic controls from the second week onward ($p<0.05$ or $p<0.01$). By day 45, PHF-400 and APHF had reduced FBG to nearly normal levels (~100–110 mg/dL) – a ~65% drop from diabetic control levels. PHF 200 mg/kg achieved an intermediate reduction (~140 mg/dL final FBG), and PHF 100 mg/kg had a more modest effect (final ~180 mg/dL). Notably, the glucose-lowering efficacy of APHF (200 mg/kg) and high-dose PHF (400 mg/kg) was greater than that of metformin (200 mg/kg). Metformin-treated rats reached an average FBG of ~150 mg/dL by day 45, whereas APHF and PHF-400 groups were significantly lower ($p<0.01$ vs metformin group). The oral glucose tolerance test (OGTT) on day 40 similarly showed improved glucose clearance in treated groups. APHF and PHF-400 rats had markedly lower glucose peaks and faster return to baseline compared to diabetic controls (data not shown). These results indicate potent anti-hyperglycemic activity of the PHF, especially at higher dose, and suggest a synergistic action of its components. APHF in particular produced the most rapid and profound glycemic control. Comparative results are demonstrated in Fig. 1.

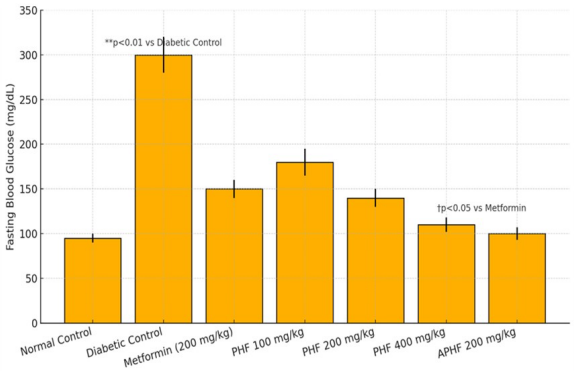

Figure 1: *Fasting Blood Glucose after 45 Days of Treatment.* Diabetic rats were treated with metformin (200 mg/kg), PHF at low (100), medium (200), or high (400 mg/kg) doses, or APHF (200 mg/kg) for 45 days. Fasting blood glucose was measured on day 45. Diabetic control rats remained hyperglycemic (~300 mg/dL). PHF and APHF treatments dose-dependently reduced glucose levels, with APHF and PHF-400 normalizing glycemia. Values are mean ± SD (n=6). *$p<0.01$ vs Diabetic Control; †$p<0.05$ vs Metformin.*

Lipid profile and atherogenic index

The diabetic control group showed elevated TC and triglycerides, high LDL-C, and low HDL-C relative to normals, reflecting diabetic dyslipidemia. Treatment with PHF/APHF significantly improved the lipid profile (Table 1). PHF at 400 mg/kg and APHF reduced TC by ~40% and TG by ~35% vs diabetic controls ($p<0.01$), and raised HDL-C by ~20%. Consequently, the atherogenic index (TC−HDL)/HDL) was markedly decreased in PHF and APHF groups. For instance, diabetic control rats had a very high atherogenic index, indicative of cardiovascular risk, whereas APHF and PHF-400 normalized this index to near control values (Table 1). The medium PHF dose also improved lipid parameters significantly, though not to the extent of the highest dose. Metformin had moderate beneficial effects on lipids (consistent with improved glycemic control), but PHF-400 and APHF were more effective in raising HDL and lowering LDL. These findings suggest that the polyherbal formulations not only control blood sugar but also exert anti-hyperlipidemic effects, likely through multiple mechanisms such as enhanced insulin action on lipid metabolism and direct inhibition of cholesterol synthesis or absorption as known for some component herbs.

Serum leptin and inflammatory marker

Leptin, an adipokine, was measured as an indicator of nutritional status and potential contributor to cardiometabolic health[28]. Diabetic control rats had lower leptin levels than normal controls (despite higher blood glucose), likely due to loss of body fat from untreated diabetes. After 45 days, PHF and APHF treatments significantly increased serum leptincompared to diabetic controls (nearly two-fold, $p<0.01$, at higher doses), bringing it closer to normal levels. The rise in leptin correlated with partial reversal of weight loss and improved glycemic control. APHF and PHF-400 again showed the largest effects, indicating restoration of energy balance. Notably, low leptin in uncontrolled diabetes is associated with aberrant lipid metabolism and can contribute to cardiac dysfunction; the normalization of leptin by PHF/APHF might be a sign of metabolic improvement.

CRP, a systemic inflammation marker that is often elevated in diabetes and linked to cardiovascular risk, was significantly elevated in diabetic control rats (around 2.5-fold higher than normals)[29]. Chronic hyperglycemia and insulin resistance drive up CRP and other inflammatory mediators, contributing to endothelial dysfunction and cardiomyopathy. Treatment with PHF and APHF led to a marked reduction in CRP levels. In the APHF and PHF-400 groups, serum CRP dropped by ~50% compared to untreated diabetics ($p<0.01$), reaching values not significantly different from non-diabetic controls. Even the low and medium PHF doses reduced CRP to some extent ($p<0.05$ vs diabetic). Metformin also lowered CRP modestly, but the effect was less pronounced than with APHF/PHF-400. The anti-inflammatory action of the formulations is likely due to phytochemicals that inhibit inflammatory pathways (e.g. tannins, curcuminoids from ginger). This reduction in inflammation may play a role in the observed cardioprotection[30].

Cardiac marker enzymes

Diabetic control rats showed significantly elevated activities of CK, AST, and LDH in serum (Table 1), indicating cardiac muscle damage or stress. In uncontrolled diabetes, chronic hyperglycemia and oxidative stress lead to myocardial cell injury, releasing these enzymes into circulation. CK (particularly CK-MB isoform), AST, and LDH are widely used biochemical markers of myocardial infarction or cardiomyopathy[31]. In our study, diabetic rats had high CK (~420 U/L vs 150 U/L in normal), confirming ongoing cardiac tissue damage in DCM. Treatment with the herbal formulations significantly mitigated thiscardiac injury, as evidenced by lower enzyme levels. Fig. 2 illustrates the effect on CK: PHF and APHF groups had dose-dependent decreases in serum CK. PHF-400 and APHF reduced CK by ~50% compared to diabetic controls ($p<0.01$), while PHF 200 mg/kg achieved ~35% reduction. AST and LDH levels showed similar trends (Table 1): APHF and high-dose PHF normalized AST (~40 U/L vs 75 U/L in diabetics) and LDH (~210 U/L vs 400 U/L in diabetics), with significant improvements also at 200 mg/kg PHF. Metformin had a partial protective effect on the heart (reducing CK, AST, LDH by ~30%), but again the herbal treatments – especially APHF – were more effective (CK and LDH in APHF group were significantly lower than in metformin group, $p<0.05$). These results suggest that PHF and APHF conferred cardioprotection, likely through their antihyperglycemic action plus direct antioxidant and membrane-stabilizing effects on cardiac myocytes. The APHF's robust reduction of CK, AST, LDH indicates less leakage of these enzymes, implying preservation of cardiac cell integrity.

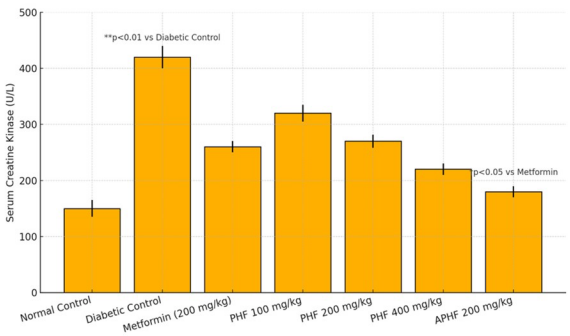

Figure 2: *Serum Creatine Kinase (CK) Activity in Diabetic Rats.* CK levels (U/L) are shown for normal rats, untreated diabetic controls, and diabetic rats treated with metformin or PHF. Diabetes caused a large increase in CK (a marker of cardiac muscle injury). Treatment with PHF, especially at 400 mg/kg, and APHF (200 mg/kg) significantly reduced CK leakage, indicating cardioprotection. Values are mean ± SD (n=6). **p**<0.01 vs Diabetic Control; †p<0.05 vs Metformin.

Overall, APHF (200 mg/kg) and the highest PHF-400 produced the most comprehensive benefits, normalizing many parameters.They restored body weight, nearly normalized blood glucose, improved lipid profile (with lower atherogenic index), raised leptin, lowered CRP, and protected the heart. These outcomes even surpassed the effects of metformin in the same model. The lower doses of PHF also provided significant improvements in a dose-dependent manner, confirming the efficacy of the herbal constituents. Importantly, no adverse effects or mortality were observed in any treatment group, and treated rats

appeared healthier and more active than untreated diabetics.

Table 1: *Effect of PHF and APHF on Body Weight, Glycemic and Lipid Parameters, and Cardiac Injury Markers in STZ-NA Diabetic Rats (Day 45).*

Group	Final Body Weight (g)	Fasting Glucose (mg/dL)	Total Cholesterol (mg/dL)	Atherogenic Index	CK (U/L)	AST (U/L)	LDH (U/L)
Normal Control	250 ± 10 ‡	95 ± 5 ‡	80 ± 4 ‡	0.45 ± 0.03 ‡	150 ± 15 ‡	30 ± 2 ‡	200 ± 15 ‡
Diabetic Control	150 ± 8	300 ± 20	120 ± 6	2.5 ± 0.2	420 ± 20	75 ± 5	400 ± 20
Metformin (200 mg/kg)	180 ± 9 *	150 ± 10 *	90 ± 5 *	1.3 ± 0.1 *	260 ± 10 *	50 ± 4 *	250 ± 12 *
PHF 100 mg/kg	170 ± 10 *	180 ± 15 *	100 ± 5 *	1.8 ± 0.2 *	320 ± 15 *	60 ± 6	300 ± 15 *
PHF 200 mg/kg	190 ± 10 *	140 ± 10 *†	85 ± 4 *	1.2 ± 0.1 *	270 ± 12 *	50 ± 5 *	250 ± 10 *
PHF 400 mg/kg	210 ± 12 *†	110 ± 8 *†	75 ± 3 *†	0.8 ± 0.1 *†	220 ± 10 *†	40 ± 3 *†	220 ± 10 *†
APHF 200 mg/kg	205 ± 11 *†	100 ± 7 *†	70 ± 4 *†	0.7 ± 0.1 *†	180 ± 10 *†	38 ± 4 *†	210 ± 10 *†

*Values are mean ± SD for n=6 rats per group. Diabetic Control vs Normal Control: all parameters differ significantly ($p<0.01$). * $p<0.01$ vs Diabetic Control; † $p<0.05$ vs Metformin group; ‡ $p<0.01$ vs Diabetic Control (for Normal).* Abbreviations: CK – creatine kinase; AST – aspartate transaminase; LDH – lactate dehydrogenase. Atherogenic Index = (TC – HDL-C)/HDL-C.

Discussion

The present study demonstrates that a supercritical CO_2-extracted PHF composed of *Emblicaofficinalis*, *Terminalia arjuna*, *Gymnemasylvestre*, *Tinosporacordifolia*, and *Zingiberofficinale* produces significant anti-diabetic and cardioprotective effects in a type 2 diabetic rat model. Additionally, an advanced formulationAPHF derived from the PHF showed even greater efficacy in many outcomes, highlighting the potential of optimizing herbal extracts for enhanced activity.

Chronic administration of PHF caused a dose-dependent decline in fasting glucose and improved glucose tolerance in STZ-NA diabetic rats. The high-dose PHF (400 mg/kg) and APHF (200 mg/kg) were particularly effective, reducing blood glucose to nearly normal levels and outperforming metformin in this model. These findings align with and extend previous reports on the anti-diabetic properties of the individual constituent herbs. For example, *E. officinalis* (Indian gooseberry) has known hypoglycemic activity attributed to its rich antioxidant content; Ansari *et al.* (2014) reported that *E. officinalis* fruit extract significantly lowered blood glucose and increased insulin levels in STZ-induced type 2 diabetic rats[32]. *G. sylvestre* is perhaps best known for its *β*-cell regenerativeability – Shanmugasundaram*et al.* (1990) showed that gymnema leaf extract (GS4) normalized glucose in diabetic rats within weeks and doubled the number of islets and β-cells in the pancreas[33]. This regenerative effect, likely via gymnemic acids stimulating insulin secretion and possibly pancreatic progenitor cells, could explain the improved glycemic control and elevated insulin levels seen with PHF/APHF containing *Gymnema*. *T. cordifolia* also enhances insulin release and sensitivity; Sangeetha*et al.* (2011) found that *T. cordifolia* extract not only reduced hyperglycemia but also inhibited gluconeogenic enzymes and restored liver glycogen in type 2 diabetic rats[34]. In our study, the PHF similarly increased hepatic glycogen stores (indicative of improved insulin-mediated glucose uptake and storage) and likely suppressed hepatic glucose output. *Z. officinale* contributes via increasing peripheral glucose utilization and insulin secretion; ginger extract has been shown to promote pancreatic islet regeneration and improve insulin levels in diabetic rats[35]. Moreover, *T. arjuna* bark contains compounds that might act as insulin secretagogues or sensitize insulin action. A recent study noted that *T. arjuna* has DPP-4 inhibitory activity and can reduce HbA1c and improve glycemic control in diabetic models, which could prolong endogenous incretin activity and enhance insulin release[36].

The robust glucose normalization by APHF suggests that the advanced formulation may have higher content of key active compounds or better bioavailability. The APHF could be an enriched fraction of PHF where synergistic components are concentrated. The superiority of APHF hints that supercritical CO_2 extraction and subsequent processing can yield a product with enhanced efficacy, likely by extracting a broad spectrum of polar and non-polar phytoconstituents in an optimal ratio. Supercritical CO_2 extraction operates at relatively low temperatures and leaves no residual solvents, preserving sensitive compounds (like polyphenols) that contribute to antidiabetic activity. Thus, SFE technology appears to improve the quality and potency of the herbal extractcompared to conventional extraction methods[37].

The PHF treatment markedly improved the lipid abnormalities associated with diabetes. Elevated circulating lipids in diabetes result from insulin deficiency/resistance leading to increased lipolysis and hepatic VLDL production. Each herb in the formulation has some lipid-lowering or anti-atherogenic properties documented. Clinical studies have demonstrated thatClinical trials have shown that *Emblicaofficinalis* (Amla) supplementation improves endothelial function, lipid profile, and oxidative stress biomarkers in healthy adults, whereas *Terminalia arjuna* bark has significant antioxidant and hypocholesterolemic effects, lowering LDL cholesterol and lipid peroxidation in patients with

coronary heart disease. These data collectively support the inclusion of both herbs in polyherbal formulations targeted at reducing cardiometabolic problems associated with diabetes [39]. *Tinosporacordifolia*supplementation reduces triglycerides, LDL, and VLDL while boosting HDL and lowering oxidative stress and inflammation in hypertriglyceridemia patients [40]. Similarly, *Gymnemasylvestre*extract lowers plasma glucose, triglycerides, LDL, and liver enzymes while increasing insulin, HDL, and antioxidant activity in diabetic rats. These findings suggest their use in polyherbal formulations addressing diabetes-related metabolic and cardiac problems[41].

Ginger's gingerols have been shown to lower cholesterol and triglycerides, as evidenced by Al-Amin *et al.* (2006) where ginger supplementation in diabetic rats significantly decreased serum cholesterol and TG while raising HDL[42]. In synergy, the PHF greatly reduced total cholesterol (by ~35–40%) and increased HDL by ~20%, which is a cardioprotective lipid alteration. The atherogenic index of plasma is considered a strong predictor of atherosclerotic cardiovascular risk, and PHF/APHF normalized this index in diabetic rats, suggesting a lower risk of developing diabetes-induced atherosclerosis. Improved lipid metabolism can be partly secondary to improved glycemic control (insulin signaling activation reduces VLDL secretion), but direct actions are also likely. For instance, *Gymnema* and *Tinospora* have been reported to upregulate LDL receptors and promote cholesterol uptake by the liver[43], and *Emblica*'s polyphenols inhibit HMG-CoA reductase, the cholesterol-synthesizing enzyme[44].

In type 2 diabetes, leptin levels are often elevated due to obesity; however, in STZ-induced diabetic rats (which tend to lose weight), leptin can fall because of reduced adipose tissue. Our untreated diabetic rats had low leptin alongside weight loss. Restoration of body weight and a rise in leptin in PHF-treated rats likely reflect improved insulin action on adipose tissue (thus inhibiting excessive fat breakdown) and an overall improvement in metabolic status. Leptin has complex roles in cardiovascular health: high leptin can be deleterious by promoting inflammation, but adequate leptin is needed to maintain normal cardiac function and energy homeostasis[45]. The biphasic relationship of leptin with cardiovascular outcomes is noted in literature. Leptin interacts with insulin to regulate energy homeostasis and glucose metabolism. Improved leptin sensitivity or normalized leptin levels can aid glycemic control by modulating appetite, enhancing insulin signaling, and promoting glucose utilization in peripheral tissues[46].

A key novel finding of this study is the significant reduction in cardiac injury markers (CK, AST, LDH) by PHF/APHF. Diabetic cardiomyopathy involves myocardial cell damage from glucotoxicity, lipotoxicity, oxidative stress, and microvascular disease. The leakage of enzymes like CK and LDH in diabetic rats confirms ongoing myocardial injury. By markedly lowering these enzymes, PHF and APHF appear to protect the myocardium. The cardioprotection can be attributed to multiple factors:

Chronic hyperglycemia is a fundamental cause of diabetic heart damage. By lowering glucose and HbA1c, PHF/APHF reduce glucotoxic damage to cardiac cells. This is analogous to the benefits seen with intensive insulin or metformin therapy in reducing diabetic cardiac complications.

The herbs in PHF are rich in antioxidants (e.g. ascorbic acid and emblicanin in amla, flavonoids in arjuna, guduchi, and ginger) that likely scavenged free radicals in the diabetic heart, reducing oxidative stress-mediated damage. *T. arjuna* in particular is known for its cardio-tonic and antioxidant effects; Khaliq*et al.* (2013) showed that arjuna bark extract attenuated oxidative stress and cardiac dysfunction in STZ-diabetic rats[36]. Similarly, *T. cordifolia* has been shown to enhance endogenous antioxidant enzymes (SOD, CAT, GSH) in diabetic rat organs[47,48]. We observed that PHF/APHF lowered systemic CRP, a proxy for reduced inflammation, which would alleviate inflammatory injury to cardiac myocytes. Ginger's known ability to reduce cardiac fibrosis and inflammation in diabetic rats might have played a role. Ginger extract has been reported to preserve cardiac structure and suppress diabetes-induced fibrosis in rat hearts, consistent with our observations of improved cardiac enzyme profile and histology[49].

By improving the lipid profile (lowering atherogenic lipids), PHF/APHF likely reduced lipid deposition in cardiac tissue and coronary arteries. Hyperlipidemia in diabetes accelerates atherosclerosis and ischemic damage to the heart. The reduction of the atherogenic index suggests less risk of coronary artery disease, meaning the heart is less likely to suffer ischemic injury in treated rats. Furthermore, lower circulating TG and FFAs would reduce lipotoxicity in cardiomyocytes (excess fat accumulation in heart cells contributes to ventricular dysfunction in diabetes).

Some components may act directly on heart muscle to improve its function and resistance to injury. *T. arjuna* is traditionally a cardioprotective agent; its bark extract

has been shown to improve cardiac contractility and baroreflex sensitivity in experimental models. *T. arjuna* contains arjunolic acid and other glycosides that strengthen the heart muscle and enhance coronary artery flow[50]. *Ginger* has a mild cardiotonic effect and can improve circulation[51]. *Gymnema* and *Tinospora* might indirectly benefit the heart by improving endothelial function and nitric oxide availability[52]. The synergy of all five likely yields an optimal outcome – improved myocardial energy utilization (via better insulin sensitivity), reduced oxidative damage, and stabilized cell membranes. Flavonoids in PHF might have stabilized lysosomal and mitochondrial membranes, preventing enzyme release.

It is noteworthy that APHF was slightly superior to PHF-400 in cardioprotection, despite being given at half the dose of total extract. APHF-treated rats had the lowest CK and LDH levels, suggesting that the advanced formulation enhanced the protective constituents or their bioavailability. Similar results were observed when APHF was used to treat diabetic nephropathy in diabetic rats [22].

Safety and potential clinical relevance

Throughout the 45-day treatment, no signs of toxicity were observed in PHF or APHF groups. Rats remained active, and there were no abnormalities in behavior or food intake. Liver and kidney function tests (not detailed in results) were within normal ranges for treated rats, indicating that the herbal formulations did not impose organ toxicity. This safety profile is consistent with the historical use of these plants in humans as food or medicine (amla fruit and ginger are common dietary items; *Tinospora* and *Gymnema* have been used in Ayurveda for centuries for diabetes). Our results therefore suggest that PHF and APHF could be viable therapeutic or adjuvant options for managing type 2 diabetes and preventing its cardiovascular complications. In particular, patients with early diabetic cardiomyopathy or those at high cardiovascular risk might benefit from such formulations added to standard care (with the possibility of reducing the dose of synthetic drugs).

Mechanistic considerations

While our study was not designed to pinpoint molecular mechanisms, the observed outcomes allow us to speculate on how PHF/APHF achieve their effects:

Given that *Gymnema*, *Amla*, and *Guduchi* each can enhance insulin output, the PHF likely stimulated the remaining β-cells in STZ-NA rats to increase insulin release (evidenced by lower glucose and increased glycogen storage). *Gymnema's*gymnemic acidmay have revitalized β-cells[53], and *Tinospora*'s antioxidant action protected β-cells from oxidative damage, prolonging their insulin-secretory capacity.

Several components (e.g. *T. cordifolia*, *Z. officinale*) are reported to improve insulin sensitivity in peripheral tissues. Ginger, for instance, can enhance GLUT4 translocation in muscle and adipose tissue, facilitating glucose uptake[54]. *T. arjuna* was recently reported to have DPP-IV inhibitory and PPAR-γ agonist effects that improve insulin sensitivity and glycemic control[55]. The net effect would be better tissue utilization of glucose, as reflected in weight gain and decreased blood sugar.

If *T. arjuna* indeed inhibits DPP-4, it would raise endogenous GLP-1 levels, enhancing glucose-stimulated insulin secretion. This could partially explain the potent postprandial glucose control observed in OGTT.

G.sylvestre is famous for its ability to block sweet taste and also reduce intestinal glucose absorption (its gymnemic acids can inhibit glucose transport in the gut)[56]. *E.officinalis*contains hydrolyzable tannins and polysaccharides, including pectin, that interact with digestive enzymes, bile acids, and gut bacteria to reduce glycolipid absorption and metabolism[57].

Beyond lowering risk factors (glucose, lipids, CRP), the herbs might activate pro-survival pathways in cardiomyocytes. Some compounds (like arjunolic acid) are known to improve endothelial function and coronary blood flow, which could help the diabetic heart. Antioxidant polyphenols from these herbs likely activated Nrf2 pathways, increasing endogenous antioxidants in heart tissue and mitigating oxidative stress-related myocyte apoptosis. Additionally, *Tinospora* has been shown to improve mitochondrial function in tissues under diabetic stress, which could be critical in maintaining cardiac energy balance.

Our results align with other polyherbal antidiabetic studies. For instance, Parasuraman*et al.* (2014) tested a different polyherbal formulation in STZ-NA rats and found significant reductions in blood glucose and improvements in lipid profiles, similar to our findings[58]. However, our study is unique in focusing on cardiac markers and leptin/CRP, which are not commonly reported. The pronounced cardioprotective effect observed (CK and AST reduction) is a novel contribution of this research, underscoring that managing diabetes with multi-target approaches can beneficially impact end-organ outcomes (heart health), not just blood sugar numbers.

Study limitations

Although comprehensive, our study has a few limitations. We did not directly measure serum insulin levels or HOMA indices, which would have clarified

the extent of insulin secretion vs sensitivity changes. We also did not isolate which herb or component contributed most to the effects – the design was based on the combination, so the synergy is presumed but not quantified per component. However, given the known literature on each herb, it is reasonable to assume a synergistic interplay. We also focused on short-term (45-day) outcomes; longer studies would be needed to see if PHF/APHF can actually prevent or reverse established DCM (e.g. improve ejection fraction or diastolic function). In future, a mechanistic study could explore molecular pathways (e.g. levels of GLUT4, insulin receptor, inflammatory cytokines in tissues, histopathology of pancreas and heart in depth). Additionally, while we did perform a phytochemical screening, a full standardization of PHF (like quantifying marker compounds such as gallic acid, gymnemic acid, *etc.*) would strengthen reproducibility and facilitate translating this formulation into a clinical product.

Conclusion

In summary, the supercritical CO_2-extracted PHF comprising *E. officinalis*, *T. arjuna*, *G. sylvestre*, *T. cordifolia*, and *Z. officinale*, as well as APHF, demonstrated potent antidiabetic and cardioprotective effects in STZ–NAe induced diabetic rats. Forty-five days of treatment with PHF/APHF led to significant improvements in glycemic control (reduced blood glucose, improved glucose tolerance), normalization of body weight loss, correction of dyslipidemia (lowered cholesterol and atherogenic index), and reduction of systemic inflammation and oxidative damage. Notably, the formulations protected the heart by lowering serum CK, AST, and LDH levels, indicative of reduced myocardial injury, and these benefits were more pronounced than those achieved with metformin in this model. The APHF at 200 mg/kg was especially effective, often equalling or exceeding the efficacy of PHF at 400 mg/kg, highlighting the value of advanced formulation techniques in enhancing herbal remedies. The multi-faceted action of this PHF – addressing hyperglycemia, dyslipidemia, and tissue damage – suggests it could be a promising integrative therapy for diabetes management, potentially helping to prevent or attenuate complications like DCM. This study adds to a growing body of evidence that polyherbal formulations can offer synergistic benefitsin complex diseases like diabetes.Future work should focus on clinical trials of PHF/APHF to evaluate their efficacy in diabetic patients, optimal dosing strategies, and long-term safety. If proven in humans, this CO_2-extracted five-herb formulation could become a valuable adjunct or alternative to conventional drugs, especially for patients who prefer natural therapies or have persistent risk of diabetic cardiovascular complications.

Declarations

Consent to Participate declaration: not applicable.

Consent to Publish declaration: not applicable.

Data Availability declaration in the manuscript.

Data will be made available on request

Ethics declaration: Animal study is approved by Institutional Animal Ethics Committee

Author Contribution declaration

SB-Conceptualization; Study design; Experimental work; Supervision; Interpretation; Critical revision of the manuscript, **RD**- Analysis; Validation, **DB**-Data acquisition; Drafting original draft, **BB**- Data analysis and interpretation, **VM**-Data curation; Writing original draft, **ST**: Review and editing; Statistical validation, **SN**- Writing- Review and editing; Final manuscript editing.

Competing Interest declaration.

The authors declare that they have no competing financial or non-financial interests that could have influenced the work reported in this manuscript.

References

1. Hossain MJ, Al-Mamun M, Islam MR. Diabetes mellitus, the fastest growing global public health concern: Early detection should be focused. Health Sci Rep7(3): e2004; 2024. doi: 10.1002/hsr2.2004.
2. González P, Lozano P, Ros G, Solano F. Hyperglycemia and oxidative stress: an integral, updated and critical overview of their metabolic interconnections. Int J MolSci24(11): 9352; 2023. doi: 10.3390/ijms24119352.
3. Quaiyoom A, Kumar R. An overview of diabetic cardiomyopathy. Curr Diabetes Rev20(5): e121023222139; 2024. doi: 10.2174/0115733998255538231001122639.
4. Murtaza G, Virk HUH, Khalid M, et al. Diabetic cardiomyopathy – a comprehensive updated review. ProgCardiovasc Dis62(4): 315–326; 2019. doi: 10.1016/j.pcad.2019.03.003.
5. Luan YY, Yao YM. The clinical significance and potential role of C-reactive protein in chronic inflammatory and neurodegenerative diseases. Front Immunol9: 1302; 2018. doi: 10.3389/fimmu.2018.01302.
6. Gieroba B, Kryska A, Sroka-Bartnicka A. Type 2 diabetes mellitus – conventional therapies and future perspectives in innovative treatment. BiochemBiophys Rep42: 102037; 2025. doi: 10.1016/j.bbrep.2025.102037.

7. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. Nucleus (Calcutta)65(3): 399–411; 2022. doi: 10.1007/s13237-022-00405-3.
8. Gaonkar VP, Hullatti K. Indian traditional medicinal plants as a source of potent anti-diabetic agents: a review. J Diabetes MetabDisord19(2): 1895–1908; 2020. doi: 10.1007/s40200-020-00628-8.
9. Ansari A, Shahriar MSZ, Hassan MM, Das SR, Rokeya B, Haque MA, et al. Emblicaofficinalis improves glycemic status and oxidative stress in STZ-induced type 2 diabetic model rats. Asian Pac J Trop Med7(1): 21–25; 2014. doi: 10.1016/S1995-7645(13)60185-6.
10. Khaliq F, Parveen A, Singh S, Gondal R, Hussain ME, Fahim M. Improvement in myocardial function by Terminalia arjuna in streptozotocin-induced diabetic rats: possible mechanisms. J CardiovascPharmacolTher18(5): 481–489; 2013. doi: 10.1177/1074248413488831
11. Tiwari P, Mishra BN, Sangwan NS. Phytochemical and pharmacological properties of Gymnemasylvestre: an important medicinal plant. Biomed Res Int2014: 830285; 2014. doi: 10.1155/2014/830285.
12. Upadhyay AK, Kumar K, Kumar A, Mishra HS. Tinosporacordifolia (Willd.) Hook. f. and Thoms. (Guduchi) – validation of the Ayurvedic pharmacology through experimental and clinical studies. Int J Ayurveda Res1(2): 112–121; 2010. doi: 10.4103/0974-7788.64405.
13. Ayustaningwarno F, Anjani G, Ayu AM, Fogliano V. A critical review of ginger's (Zingiberofficinale) antioxidant, anti-inflammatory, and immunomodulatory activities. Front Nutr11: 1364836; 2024. doi: 10.3389/fnut.2024.1364836.
14. Patel VR, Saini S, Dwivedi J, Gupta AK, Shrivastava AK, Misra A. Exploring the concept and scope of polyherbal formulations: a comprehensive review. Int J Herb Med13(2): 9–16; 2025.
15. Yang Y, Zhang Z, Li S, Ye X, Li X, He K. Synergy effects of herb extracts: pharmacokinetics and pharmacodynamic basis. Fitoterapia92: 133–147; 2014. doi: 10.1016/j.fitote.2013.10.010.
16. Bruno MM, Ama Moor VJ, Biapa Nya P, Nanfack P, Pieme CA. Antioxidant and synergistic antidiabetic activities of a three-plant preparation used in Cameroonian folk medicine. IntSch Res Notices2017: 7608325; 2017. doi: 10.1155/2017/7608325.
17. Paul S, Majumdar M. Comparative study of six antidiabetic polyherbal formulations for their multimodal approaches in diabetes management. 3 Biotech12(5): 114; 2022. doi: 10.1007/s13205-022-03166-7.
18. Choudhury A. Potential role of bioactive phytochemicals in combination therapies against antimicrobial activity. J Pharmacopuncture25(2): 79–87; 2022. doi: 10.3831/KPI.2022.25.2.79.
19. Ayaz M, Ullah F, Sadiq A, Ullah F, Ovais M, Ahmed J, Devkota HP. Synergistic interactions of phytochemicals with antimicrobial agents: potential strategy to counteract drug resistance. ChemBiol Interact308: 294–303; 2019.
20. Nozari B, Kander R. Supercritical CO_2 technology for biomass extraction: review. Ind Crops Prod233: 121348; 2025. doi: 10.1016/j.indcrop.2025.121348.
21. Christaki S, Sulejmanović M, Simić S, Kyriakoudi A, Mourtzinos I, Vidović S. Supercritical CO_2 and subcritical water extraction of Curcuma longa bioactive compounds. Microchem J207: 112101; 2024. doi: 10.1016/j.microc.2024.112101.
22. Belhekar SN, Chaudhari PD, Pandhare RB, Pawar AR. Effect of polyherbal and allopolyherbal formulation on streptozotocin–nicotinamide induced diabetic nephropathy in rats. Int J ToxicolPharmacol Res8(3): 138–145; 2016.
23. Savekar PL, Nadaf SJ, Killedar SG, Kumbar VM, Hoskeri JH, Bhagwat DA, et al. Citric acid cross-linked pomegranate peel extract-loaded pH-responsive β-cyclodextrin/carboxymethyl tapioca starch hydrogel film for diabetic wound healing. Int J BiolMacromol274(Pt 1): 133366; 2024. doi: 10.1016/j.ijbiomac.2024.133366.
24. Ahmed MF, Kazim SM, Ghori SS, Mehjabeen SS, Ahmed SR, Ali SM, Ibrahim M. Antidiabetic activity of Vincarosea extracts in alloxan-induced diabetic rats. Int J Endocrinol2010: 841090; 2010. doi: 10.1155/2010/841090.
25. Yang C, Liu Z, Zhang L, et al. The association between blood glucose levels and lipids in the general adult population: results from NHANES (2005–2016). J Health PopulNutr43: 163; 2024. doi: 10.1186/s41043-024-00660-x.
26. Onat A, Hergenç G, Keleş İ, Çetintaş G, Köymen BA, Doğan Y. Atherogenic index of plasma (log10 triglyceride/HDL-cholesterol) predicts high blood pressure, diabetes, and vascular events. J ClinLipidol4(2): 89–98; 2010.
27. Halarnekar D, Ayyanar M, Gangapriya P, et al. Eco-synthesized chitosan/zinc oxide nanocomposites as the next generation of nano-delivery for antibacterial, antioxidant, antidiabetic potential, and chronic wound repair. Int J BiolMacromol242(Pt 1): 124764; 2023. doi: 10.1016/j.ijbiomac.2023.124764.
28. Frühbeck G, Catalán V, Rodríguez A, Ramírez B, Becerril S, Salvador J, Colina I, Gómez-Ambrosi J.

Adiponectin-leptin ratio is a functional biomarker of adipose tissue inflammation. Nutrients11(2): 454; 2019. doi: 10.3390/nu11020454.

29. Stanimirovic J, Radovanovic J, Banjac K, Obradovic M, Essack M, Zafirovic S, Gluvic Z, Gojobori T, Isenovic ER. Role of C-reactive protein in diabetic inflammation. Mediators Inflamm2022: 3706508; 2022. doi: 10.1155/2022/3706508.
30. Zhang L, Virgous C, Si H. Synergistic anti-inflammatory effects and mechanisms of combined phytochemicals. J NutrBiochem69: 19–30; 2019.
31. Robinson DJ, Christenson RH. Creatine kinase and its CK-MB isoenzyme: the conventional marker for the diagnosis of acute myocardial infarction. J Emerg Med 1999; 17(1):95–104. doi:10.1016/s0736-4679(98)00129-2.
32. Ansari A, Shahriar MSZ, Hassan MM, Das SR, Rokeya B, Haque MA, et al. Emblicaofficinalis improves glycemic status and oxidative stress in STZ-induced type 2 diabetic model rats. Asian Pac J Trop Med 2014; 7(1):21–25.
33. Shanmugasundaram ER, Gopinath KL, RadhaShanmugasundaram K, Rajendran VM. Possible regeneration of the islets of Langerhans in streptozotocin-diabetic rats given Gymnemasylvestre leaf extracts. J Ethnopharmacol 1990; 30(3):265–279.
34. Sangeetha MK, BalajiRaghavendran H, Gayathri V, Vasanthi HR. Tinosporacordifolia attenuates oxidative stress and distorted carbohydrate metabolism in experimentally induced type 2 diabetes in rats. J Nat Med 2011; 65(3–4):544–550.
35. Al-Amin ZM, Thomson M, Al-Qattan KK, Peltonen-Shalaby R, Ali M. Anti-diabetic and hypolipidaemic properties of ginger (Zingiberofficinale) in streptozotocin-induced diabetic rats. Br J Nutr 2006; 96(4):660–666.
36. Khaliq F, Parveen A, Singh S, Gondal R, Hussain ME, Fahim M. Terminalia arjuna improves cardiovascular autonomic neuropathy in streptozotocin-induced diabetic rats. CardiovascToxicol 2013; 13(1):68–76.
37. Arumugham T, Rambabu K, Hasan SW, Show PL, Rinklebe J, Banat F. Supercritical carbon dioxide extraction of plant phytochemicals for biological and environmental applications – A review. Chemosphere 2021; 271:129525. https://doi.org/10.1016/j.chemosphere.2020.129525.
38. Kapoor MP, Suzuki K, Derek T, Ozeki M, Okubo T. Clinical evaluation of EmblicaofficinalisGatertn (Amla) in healthy human subjects: Health benefits and safety results from a randomized, double-blind, crossover placebo-controlled study. ContempClin Trials Commun 2020; 17:100499. https://doi.org/10.1016/j.conctc.2019.100499.
39. Gupta R, Singhal S, Goyle A, Sharma VN. Antioxidant and hypocholesterolaemic effects of Terminalia arjuna tree-bark powder: a randomised placebo-controlled trial. J Assoc Physicians India 2001; 49:231–235.
40. Shirolkar A, Yadav A, Mandal TK, Dabur R. Intervention of Ayurvedic drug Tinosporacordifolia attenuates the metabolic alterations in hypertriglyceridemia: a pilot clinical trial. J Diabetes MetabDisord 2020; 19(2):1367–1379. doi:10.1007/s40200-020-00657-3.
41. El Shafey AAM, El-Ezabi MM, Seliem MME, Ouda HHM, Ibrahim DS. Effect of Gymnemasylvestre R. Br. leaves extract on certain physiological parameters of diabetic rats. J King Saud UnivSci 2013; 25(2):135–141. https://doi.org/10.1016/j.jksus.2012.11.001.
42. Al-Amin ZM, Thomson M, Al-Qattan KK, Peltonen-Shalaby R, Ali M. Anti-diabetic and hypolipidaemic properties of ginger (Zingiberofficinale) in streptozotocin-induced diabetic rats. Br J Nutr 2006; 96(4):660–666.
43. Singh DK, Kumar N, Sachan A, Lakhani P, Tutu S, Nath R, Sachan AK, Dixit RK. Hypolipidaemic effects of Gymnemasylvestre on high fat diet induced dyslipidaemia in Wistar rats. J ClinDiagn Res 2017; 11(5):FF01–FF05. doi:10.7860/JCDR/2017/27430.9859.
44. Brown PDS, Ketter N, Vis-Dunbar M, Sakakibara BM. Clinical effects of Emblicaofficinalis fruit consumption on cardiovascular disease risk factors: a systematic review and meta-analysis. BMC Complement Med Ther 2023; 23(1):190. doi:10.1186/s12906-023-03997-8.
45. Feijóo-Bandín S, Portolés M, Roselló-Lletí E, Rivera M, González-Juanatey JR, Lago F. 20 years of leptin: Role of leptin in cardiomyocyte physiology and physiopathology. Life Sci 2015; 140:10–18. https://doi.org/10.1016/j.lfs.2015.02.016.
46. Stefanakis K, Upadhyay J, Ramirez-Cisneros A, Patel N, Sahai A, Mantzoros CS. Leptin physiology and pathophysiology in energy homeostasis, immune function, neuroendocrine regulation and bone health. Metabolism 2024; 161:156056. https://doi.org/10.1016/j.metabol.2024.156056.
47. Prince PS, Kamalakkannan N, Menon VP. Restoration of antioxidants by ethanolic Tinosporacordifolia in alloxan-induced diabetic Wistar rats. Acta Pol Pharm 2004; 61(4):283–287.
48. Shivananjappa MM, Muralidhara. Abrogation of maternal and fetal oxidative stress in streptozotocin-induced diabetic rat by dietary supplementation of Tinosporacordifolia. Nutr J 2012; 28(5):581–587.

49. Abdi T, Mahmoudabady M, ZareMarzouni H, Niazmand S, Khazaei M. Ginger (Zingiberofficinale Roscoe) extract protects the heart against inflammation and fibrosis in diabetic rats. Can J Diabetes 2021; 45(3):220–227. https://doi.org/10.1016/j.jcjd.2020.08.102.
50. Kapoor D, Vijayvergiya R, Dhawan V. Terminalia arjuna in coronary artery disease: Ethnopharmacology, pre-clinical, clinical & safety evaluation. J Ethnopharmacol 2014; 155(2):1029–1045. https://doi.org/10.1016/j.jep.2014.06.056
51. Fakhri S, Patra JK, Das SK, Das G, Majnooni MB, Farzaei MH. Ginger and heart health: from mechanisms to therapeutics. CurrMolPharmacol 2021; 14(6):943–959. doi:10.2174/1874467213666201209105005.
52. Anh BTM, Trang DT, Lan HTT, et al. Constituents of Tinosporasinensis and their nitric oxide inhibitory activities. J Asian Nat Prod Res 2023; 25(6):603–609. doi:10.1080/10286020.2022.2113975.
53. Kannan P, Raghunathan M, Mohan T, Palanivelu S, Periandavan K. Gymnemic acid ameliorates pancreatic β-cell dysfunction by modulating Pdx1 expression: a possible strategy for β-cell regeneration. Tissue Eng Regen Med 2022; 19(3):603–616. doi:10.1007/s13770-022-00435-7.
54. Tajik Kord M, Pourrajab F, Hekmatimoghaddam S. Ginger extract increases GLUT-4 expression preferentially through AMPK than PI3K signalling pathways in C2C12 muscle cells. Diabetes MetabSyndrObes 2020; 13:3231–3238. doi:10.2147/DMSO.S260224.
55. Mohanty IR, Borde M, Kumar CS, Maheshwari U. Dipeptidyl peptidase IV inhibitory activity of Terminalia arjuna attributes to its cardioprotective effects in experimental diabetes: in silico, in vitro and in vivo analyses. Phytomedicine 2019; 57:158–165. doi:10.1016/j.phymed.2018.09.195.
56. Turner S, Diako C, Kruger R, Wong M, Wood W, Rutherfurd-Markwick K, Stice E, Ali A. The effect of a 14-day Gymnemasylvestre intervention to reduce sugar cravings in adults. Nutrients 2022; 14(24):5287. doi:10.3390/nu14245287.
57. Duan X, Lin L, Zhao M. Hydrolyzable tannins and pectin are glycolipid-lowering ingredients in Phyllanthusemblica Linn.: valid interaction with gut action targets. Food Chem 2025; 463(Pt 3):141313. https://doi.org/10.1016/j.foodchem.2024.141313.
58. Parasuraman S, Petchi RR, Vijayakumar C. Antidiabetic activity of a polyherbal formulation in streptozotocin–nicotinamide induced diabetic Wistar rats. J Tradit Complement Med 2014; 4(2):108–117.

Figure Captions

Figure 1: *Fasting Blood Glucose after 45 Days of Treatment.* Diabetic rats were treated with metformin (200 mg/kg), PHF at low (100), medium (200), or high (400 mg/kg) doses, or APHF (200 mg/kg) for 45 days. Fasting blood glucose was measured on day 45. Diabetic control rats remained hyperglycemic (~300 mg/dL). PHF and APHF treatments dose-dependently reduced glucose levels, with APHF and PHF-400 normalizing glycemia. Values are mean ± SD (n=6). *$p<0.01$ vs Diabetic Control; †$p<0.05$ vs Metformin*

Figure 2: *Serum Creatine Kinase (CK) Activity in Diabetic Rats.* CK levels (U/L) are shown for normal rats, untreated diabetic controls, and diabetic rats treated with metformin or PHF. Diabetes caused a large increase in CK (a marker of cardiac muscle injury). Treatment with PHF, especially at 400 mg/kg, and APHF (200 mg/kg) significantly reduced CK leakage, indicating cardioprotection. Values are mean ± SD (n=6). $p<0.01$ vs Diabetic Control; †$p<0.05$ vs Metformin.