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Aqueous extract of wild ash gourd (*Benincasa hispida*) ameliorates diabetes and restores pancreatic, hepatic, and renal function in STZ-induced rats: Integrating ethnomedicine with modern pharmacology

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Abstract
Wild ash gourd (*Benincasa hispida*), a traditional vegetable from Kerala, India, was evaluated for its antidiabetic and organ-protective efficacy in a streptozotocin-induced diabetic rat model. The aqueous fruit extract was found to be rich in polyphenols (47.3 mg GAE/g) and flavonoids (15.0 mg QE/g). *In vitro* studies showed that the extract reduced glucose diffusion and inhibited α-amylase activity in a dose-dependent manner, achieving 74.4% inhibition, similar to acarbose. It also demonstrated antioxidant activity, neutralizing 67.7% of free radicals in the DPPH assay. In streptozotocin-induced diabetic rats, oral administration of 600 mg/kg extract for a period of 35 days lowered fasting blood glucose by 51% and HbA1c by 49%, compared to diabetic controls. Improvements were observed in lipid profiles, hepatic enzymes, and renal markers. Histological analysis indicated regeneration of pancreatic β-cells and preservation of liver and kidney tissue structures. No adverse effects were observed in acute toxicity tests up to 2000 mg/kg. These findings suggest that wild ash gourd may offer antidiabetic benefits through multiple mechanisms, supporting its potential use in managing diabetes and associated organ dysfunction.

Keywords: Wild ash gourd, *Benincasa hispida*, Type 2 diabetes, antidiabetic, antioxidant, pancreatic regeneration, ethnomedicine

1. Introduction
Type 2 diabetes mellitus (T2DM) has emerged as a critical global health issue characterized by persistent hyperglycaemia and metabolic disturbances that lead to multi-organ complications. The growing prevalence of T2DM, particularly in low- and middle-income regions, poses considerable healthcare challenges [1]. Recent estimates by the International Diabetes Federation indicate that the global diabetic population surpassed 537 million in 2021, with numbers projected to exceed 783 million by 2045 [2]. India remains among the nations with the greatest prevalence, highlighting an acute need for readily available and effective therapeutic approaches [3]. Contemporary T2DM management is anchored in dietary and lifestyle interventions, complemented by pharmacotherapy including metformin, sodium-glucose co-transporter-2 (SGLT-2) inhibitors, and glucagon-like peptide-1(GLP-1) receptor agonists which have proven benefits in glycaemic regulation and complication prevention [4,5]. Nonetheless, these therapeutic modalities are often hampered by adverse effects, limited long-term effectiveness, and economic barriers, especially in resource-limited settings [6,7]. Consequently, there is increased global interest in traditional medicinal plants that are accessible, culturally embraced, and capable of multi-targeted pharmacological actions [8,9]. Nevertheless, these medications are frequently associated with side effects, diminish in efficacy over prolonged use, and may pose significant financial burdens, particularly in economically challenged settings.

Given these challenges, there has been renewed investigation into medicinal plants that are not only accessible and widely accepted within traditional healthcare systems but also exhibit diverse mechanisms of action. Notably, wild ash gourd (*Benincasa hispida* (Thunb.) Cogn.), referred to as Ney Kumbalanga in Kerala and Budagumbala in other regions, is well-known in Ayurveda in preparations such as Kooshmanda Rasayana for metabolic health. Unfortunately, wild ash gourd is now less frequently cultivated, largely due to its slow growth and specific soil requirements. Mature fruits typically weighing about 500 grams [10].

Phytochemical studies on *Benincasa hispida* have identified bioactive compounds, such as flavonoids, phenolics, polysaccharides, and triterpenoids, with notable antioxidant and hypoglycemic activities [11,12]. However, these studies primarily focus on the cultivated variety, and similar research on the wild ash gourd remains unexplored, leaving a gap in its potential bioactivity. Despite centuries of use in traditional practice, detailed experimental evidence supporting the antihyperglycemic effects of the wild form remains sparse and is only beginning to be systematically explored.

In light of the World Health Organization's emphasis on scientifically verifying indigenous botanicals with antidiabetic potential, this study aims to rigorously evaluate the aqueous fruit extract of wild ash gourd using an established streptozotocin (STZ)-induced diabetic rat model. The work investigates key parameters including fasting blood glucose, HbA1c, oral glucose tolerance, lipid and liver function profiles, renal markers, and histological changes in target organs, thereby bridging ethnobotanical knowledge and modern pharmacology.

2. Methodology

2.1 Plant Material Collection and Preparation

Fresh fruits of wild ash gourd (*Benincasa hispida*), identified and authenticated, were harvested from Idukki district, Kerala, India. After thorough washing, the fruits were peeled, chopped, and oven-dried at 40°C for 48 h to preserve active constituents. The dried matter was ground into coarse powder using a grinder and stored in a desiccated environment at room temperature.

2.2 Extraction Procedures

The aqueous extract (WAGE) was prepared by homogenizing fresh fruit pulp, followed by filtration through muslin cloth and centrifugation at 3000 rpm for 10 minutes. Separately, powdered fruit samples were subjected to Soxhlet extraction using petroleum ether and ethyl acetate, with 10–12 complete extraction cycles per solvent to maximize recovery of respective phytochemicals. The resulting extracts were concentrated under vacuum and subsequently reconstituted with 5% dimethyl sulfoxide (DMSO) to a final assay concentration of 100 mg/mL.

2.3 Phytochemical Screening and Quantification

Qualitative analysis was conducted to detect the presence of key phytochemicals, including carbohydrates, amino acids, saponins, flavonoids, alkaloids, phenols, glycosides, steroids, and triterpenes, following standard analytical protocols [13]. For quantitative analysis, total phenolic content was measured via the Folin-Ciocalteu assay, utilizing gallic acid to generate the standard calibration curve, with results expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). The abundance of flavonoids was established through an aluminum chloride colorimetric assay, referencing quercetin as the standard and reporting concentrations in mg quercetin equivalents per gram (mg QE/g). Protein levels were determined by the Lowry method, employing bovine serum albumin as the reference standard for calibration.

2.4 *In vitro* Functional Assays

The *in vitro* functional activities of WAGE were assessed using standardized protocols for glucose diffusion inhibition, α-amylase inhibition [14], and DPPH radical scavenging. For all assays, WAGE was evaluated at concentrations of 50. 100, 150, 200, 250 and 300 µg/mL (DPPH: 200, 500, and 1000 µg/mL); each experiment included appropriate negative (vehicle-only) and positive controls (acarbose 100 µg/mL for the α-amylase assay, ascorbic acid for DPPH).

For the glucose diffusion inhibition assay, a solution of 22 mM glucose and 0.15 M NaCl with or without WAGE was placed in dialysis tubing and incubated at room temperature. Aliquots of the external medium were collected at 30, 60, 90, 120, 150, and 180 minutes, and glucose concentration was measured spectrophotometrically using the o-toluidine method.

The α-amylase inhibition assay was performed in 96-well plates: porcine pancreatic α-amylase and 1% starch (w/v) substrate were incubated with WAGE or controls, followed by color development with DNSA. Absorbance was read at 540 nm, and percent inhibition was calculated relative to the vehicle control.

DPPH radical scavenging activity was tested by mixing DPPH solution with WAGE or ascorbic acid at varying concentrations, incubating in the dark for 30 minutes at room temperature, and measuring absorbance at 517 nm. All assays were conducted in triplicate, and results were expressed as mean ± SEM.

2.5 Animals and Experimental Design

Adult male Wistar albino rats (age 10-12 weeks, weight 150-200 g) were procured from a certified animal facility in Karnataka, India. Animals were housed in polypropylene cages under controlled environmental conditions (temperature 22-25°C, humidity 55-60%, 12-hour light/dark cycle), with ad libitum access to standard pellet diet and water. Prior to experimental procedures, rats were acclimatized for 7 days under these standardized conditions.

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of St. Aloysius College, Mangalore (Approval No.: SAC/IAEC/04/2018), and experiments were performed in accordance with the CPCSEA guidelines and OECD test guideline 420 for acute toxicity.

2.6 Acute Toxicity Testing

Acute oral toxicity of WAGE was evaluated following OECD guideline 420. Twenty-four healthy rats were randomly assigned to four groups (n=6 per group) and administered single oral doses of 800, 1000, 1500, and 2000 mg/kg body weight of WAGE, respectively. A control group received physiological saline. Animals were fasted for 3-4 hours with water access before dosing, and observations for mortality, behaviour, and physical changes were made for 14 days post-treatment [15].

2.7 Diabetes Induction and Grouping

Type 2 diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) at 35 mg/kg body weight in citrate buffer (pH 4.5). Seventy-two hours post-injection, fasting blood glucose (FBG) was measured via tail vein sampling using a OneTouch® glucometer (Johnson & Johnson, India). Rats with FBG > 240 mg/dL were classified as diabetic and included in the study.

Animals were randomly allocated into six groups (n = 6 per group) as follows: Group I – Normal control (vehicle only); Group II – Diabetic control (vehicle); Groups III to V – Diabetic rats treated with WAGE at 200, 400, and 600 mg/kg body weight orally; Group VI – Diabetic rats treated with glibenclamide (2.5 mg/kg body weight orally) as positive

control. Treatments were administered once daily via oral gavage for 30 days. Body weights and fasting blood glucose were monitored weekly during the treatment period.

2.8 Oral Glucose Tolerance Test (OGTT)

All experimental rats were fasted overnight (18 hours) with free access to water. Thirty minutes after administering the test extract or standard drug, each animal was given an oral glucose load of 3 g/kg body weight. Blood glucose concentrations were measured from tail vein samples using a OneTouch® glucometer (Johnson & Johnson, India) at 30 minutes before administration, at baseline (0 hours), immediately prior to extract/drug administration, and at 30, 60, 90, 120, 150, and 180 minutes after the glucose challenge [16].

2.9 Biochemical and Histopathological Evaluation

At the end of the treatment period, animals were euthanized under mild anesthesia. Whole blood was collected, and serum was separated by centrifugation for subsequent biochemical analyses. Serum levels of liver enzymes (SGOT, SGPT), lipid profile, and renal markers (urea, creatinine, uric acid, BUN) were determined using commercially available kits.

Vital organs, including the liver, pancreas, and kidneys, were excised, washed with ice-cold saline, and fixed in 10% neutral buffered formalin for histopathological examination. Paraffin-embedded sections (5 µm) were stained with haematoxylin and eosin (H&E) and examined under light microscope (Olympus Japan) and photography using CCD camera (Jenoptic Germany) for morphological assessment.

2.10 Statistical Analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test. A p value < 0.05 was considered statistically significant.

3. Results

Phytochemical Profiling and Antioxidant Activity

Phytochemical analysis confirmed solvent-dependent extraction efficiency, with the aqueous extract (WAGE) exhibiting the broadest spectrum of bioactive constituents including carbohydrates, amino acids, glycosides, flavonoids, steroids, and phenolics (Table 1). Both petroleum ether and ethyl acetate extracts showed comparatively limited profiles. Quantitative assays demonstrated high levels of total phenolics (47.3 ± 0.02 mg GAE/g) and flavonoids (15.0 ± 0.03 mg QE/g) in WAGE, which are key antioxidants implicated in the modulation of diabetic pathophysiology. Additionally, the WAGE contained 0.071 mg/ml protein, with an ash content of 2.17% and potassium concentration of 70 ppm, suggesting potential benefits in electrolyte balance relevant to diabetic complications. The potent antioxidant capacity of WAGE was further evidenced by its concentration-dependent radical scavenging activity in the DPPH assay, achieving 67.66% inhibition at 1000 µg/mL, albeit lower than the reference standard, ascorbic acid, which exhibited 85.74% inhibition.

In vitro Antidiabetic Functional Assays

WAGE significantly inhibited glucose diffusion across dialysis membranes in a time dependent manner relative to control, supporting a hypothesized retardation of intestinal glucose absorption (Fig. 1. a). The WAGE treated samples exhibited a markedly lower glucose release compared to the control at all measured time points beyond 30 minutes. At 60 minutes, the mean glucose concentration in the WAGE group was 19.0 ± 1.2 mg/dL versus 23.5 ± 1.5 mg/dL in the control ($p \leq 0.0154$). By 180 minutes, the mean glucose diffusion reached 57.0 ± 3.5 mg/dL in the control compared to 38.5 ± 2.5 mg/dL in the WAGE group.

In the α-amylase inhibition assay, WAGE demonstrated dose-dependent suppression of enzyme activity with maximum inhibition of 74.4% at 300 µg/mL, closely paralleling the standard drug acarbose (79.2%) (Fig. 1.b). These findings indicate multi-faceted potential to reduce postprandial glycemic excursions by limiting carbohydrate digestion and absorption.

Acute Toxicity Assessment

No mortality or behavioural abnormalities were observed across the high-dose (up to 2000 mg/kg) WAGE treatment over a 14-day observation period, indicating a favourable safety profile compatible with chronic administration.

Oral Glucose Tolerance Test (OGTT)

The OGTT demonstrated a significant, dose-dependent improvement in glucose tolerance in streptozotocin-induced diabetic rats treated with WAGE (Fig. 2). Administration of WAGE at 200, 400, and 600 mg/kg markedly lowered plasma glucose levels at all measured time points (30, 60, 90, 120, and 180 minutes) following oral glucose challenge compared to diabetic controls ($p<0.01$). Notably, the 600 mg/kg dose elicited the most pronounced antihyperglycemic effect, closely approximating the efficacy of the reference drug, glibenclamide. At 180 minutes post-glucose load, glucose levels in the 600 mg/kg WAGE group were statistically indistinguishable from those in the glibenclamide-treated group ($p > 0.05$), underscoring the potent glucose-lowering capacity of the extract. These results suggest that WAGE significantly enhances glucose clearance and may improve insulin sensitivity and/or residual β-cell function in diabetic rats.

Long-Term Glycaemic Control

Chronic administration of WAGE for 35 days produced a significant, dose-dependent reduction in fasting blood glucose levels in streptozotocin-induced diabetic male Wistar rats. (Fig. 3). Notably, the highest dose, 600 mg/kg, achieved a pronounced 51% decrease, lowering glucose from 300 ± 22.41 mg/dL to 147 ± 16.75 mg/dL, which was markedly superior to the reductions seen with 200 mg/kg (12.9%) and 400 mg/kg (45.4%) doses. The glucose-lowering effect of 600 mg/kg WAGE was comparable to that of the standard drug glibenclamide, which achieved a 44.98% reduction, with no statistically significant difference observed between these two treatments ($p > 0.05$). Statistical analysis through one-way ANOVA followed by Tukey's post-hoc test confirmed that all treatment groups-WAGE at 200, 400, and 600 mg/kg as well as glibenclamide-exhibited statistically significant decreases in fasting blood glucose compared to the diabetic control group ($p<0.01$).

Serum Lipid and Renal Biomarkers

The evaluation of liver function markers revealed significant hepatoprotective effects of WAGE treatment (Figure 5). STZ-induced diabetic control rats showed markedly elevated SGOT (315.4 ± 1.66 U/L) and SGPT (195.8 ± 1.43 U/L) levels compared to normal controls ($p<0.001$). The WAGE

treatment significantly normalized dyslipidemia induced by diabetes: it decreased total cholesterol by 38.7%, LDL by 54.1%, triglycerides by 37.1% while elevating HDL by 36.9% (Table 2). These corrections are critically associated with reduced cardiovascular risk in diabetes.

Evaluation of renal functional parameters demonstrated a clear dose-dependent nephroprotective action of WAGE in STZ-induced diabetic rats (Table 3). Among the tested doses, 600 mg/kg exerted the most pronounced protective effect, resulting in a marked decline in blood urea (45.7 ± 2.1 mg/dl; 53.3% reduction), serum creatinine (1.1 ± 0.06 mg/dl; 60.7% reduction), blood urea nitrogen (22.8 ± 1.1 mg/dl; 61.2% reduction), and uric acid (2.8 ± 0.09 mg/dl; 49.6% reduction) relative to the diabetic control group ($p<0.01$). Interestingly, these improvements were statistically comparable to those observed in glibenclamide-treated animals ($p > 0.05$), suggesting that WAGE at higher concentrations provides renal protection of similar magnitude to standard pharmacological management. The observed normalization of urea, creatinine, BUN, and uric acid strongly indicates that WAGE not only mitigates hyperglycemia-induced renal dysfunction but also preserves glomerular and tubular integrity, thereby counteracting key pathogenic processes involved in diabetic nephropathy.

Glycated Haemoglobin (HbA1c) Assessment

Analysis of HbA1c levels revealed a significant and dose-dependent improvement in long-term glycemic control following WAGE administration. The highest dose (600 mg/kg) resulted in a 48.63% reduction in HbA1c compared to the diabetic control group (mean 10.8 ± 0.4%), approaching the near-normal values seen in non-diabetic controls (4.2 ± 0.2%). Intermediate doses also showed substantial reductions, with 400 mg/kg and 200 mg/kg lowering HbA1c by 39.64% and 25.78%, respectively. These findings underscore the sustained antihyperglycemic potential of WAGE

Histopathological Analysis

Pancreatic Tissue Regeneration

Histological examination of pancreatic sections demonstrated severe disruption of islet architecture in diabetic controls, characterized by β-cell loss and inflammatory infiltration. In contrast, WAGE treatment induced dose-dependent regenerative changes. Notably, the 600 mg/kg dose nearly restored normal islet morphology with marked recovery of β-cell density and reduced inflammation, surpassing the regenerative effects observed with glibenclamide (Fig. 4). These regenerative effects likely contribute to improved insulin secretion and glycaemic regulation.

Hepatic Histoarchitecture Restoration

Liver histology revealed pronounced structural damage in diabetic rats, including hepatocellular degeneration, fatty infiltration, and sinusoidal congestion. WAGE administration resulted in a dose-dependent progressive hepatoprotective effects, with the 600 mg/kg dose achieving approximately 54.5% restoration of normal hepatic architecture (Fig. 5). This included improved hepatocyte morphology and decreased inflammatory changes, comparable to those seen with glibenclamide treatment.

Renal Tissue Protection

Kidney sections from diabetic animals exhibited significant pathological alterations, including glomerular hypertrophy, tubular damage, and interstitial inflammation, consistent with diabetic nephropathy. WAGE treatment conferred marked renal protection in a dose-dependent manner (Fig. 6). The highest dose (600 mg/kg) restored approximately 80% of healthy glomerular and over 90% of tubular structures, significantly attenuating morphological damage and inflammation ($p<0.001$). The degree of renal protection afforded by WAGE at this dose exceeded that of glibenclamide, which showed partial preservation primarily of tubular structures.

3.1 Tables and Figures

Table 1: Phytochemical screening of wild ash gourd fruit extracts

Phytochemical Class	Testing Method	Aqueous Extract	Petroleum Ether Extract	Ethyl Acetate Extract
Carbohydrates	Molisch's test	+	+	+
	Benedict's test	+	-	+
Amino acids	Ninhydrin test	+	-	-
Alkaloids	Mayer's test	-	-	-
	Wagner's test	-	-	-
	Hager's test	-	-	-
	Dragendorff's test	-	-	-
Tannins	Gelatin test	-	-	-
Glycosides		+	-	+
Saponins	Froth test	-	-	-
	Foam test	-	-	-
Flavonoids	Lead acetate test	-	-	-
	NaOH reaction	+	-	+
Steroids	Salkowski test	+	-	+
	Libermann's test	+	-	+
Phenols	$FeCl_3$ test	-	-	-
	Lead acetate test	+	-	+

Note: (+) indicates presence; (-) indicates absence

Table 2: Effect of WAGE on lipid profile in streptozotocin-induced diabetic rats after 35 days of treatment(mg/dl).

Parameter	Normal Control	Diabetic Control	WAGE 200 mg/kg	WAGE 400 mg/kg	WAGE 600 mg/kg	Glibenclamide (2.5mg/kg)
Total Cholesterol	95.2±4.1	165.8±6.7	111.3±5.2*	108.7±4.8*	101.5±4.3*	106.2±5.1*
HDL Cholesterol	45.8±2.3	32±1.8	38.2±2.1*	41.5±2.4*	44.1±2.2*	40.8±2.3*
LDL Cholesterol	38.2±2.1	98.5±4.2	58.3±3.1*	52.7±2.8*	45.2±2.5*	48.9±2.7*
Triglycerides	86.5 ±3.8	156.2±7.1	118.3±4.9*	108.7±4.2*	98.2±3.7*	105.4±4.5*
VLDL	17.3±0.8	31.2±1.4	23.7±1.0*	21.7±0.8*	19.6±0.7*	21.1±0.9*

Data are presented as mean ± SEM (n = 6). Significant differences compared to the diabetic control group are denoted by *p<0.001.

Table 3: Renal function parameters following treatment with WAGE in diabetic rats after 35 days of treatment.

Parameter	Normal Control	Diabetic Control	WAGE 200 mg/kg	WAGE 400 mg/kg	WAGE 600 mg/kg	Glibenclamide (2.5mg/kg)
Blood Urea (mg/dl)	32.5 ±1.8	125.4 ± 4.2	58.3 ± 2.9*	58.3 ± 2.9*	45.7 ± 2.1*	52.8 ± 2.6*
Serum Creatinine (mg/dl)	0.8 ± 0.05	2.8 ± 0.12	1.4 ± 0.08*	1.4 ± 0.08*	1.1 ± 0.06*	1.3 ± 0.07*
BUN (mg/dl)	15.2 ± 0.9	58.7 ± 2.1	28.5 ± 1.4*	28.5 ± 1.4*	22.8 ± 1.1*	26.1 ± 1.3*
Uric Acid (mg/dl)	2.1 ± 0.08	5.56 ± 0.4	3.46 ± 0.05*	3.46 ± 0.05*	2.8 ± 0.09*	3.2 ± 0.11*

Values are expressed as mean ± SEM (n = 6). Statistical significance compared to diabetic control is indicated as *p<0.01.

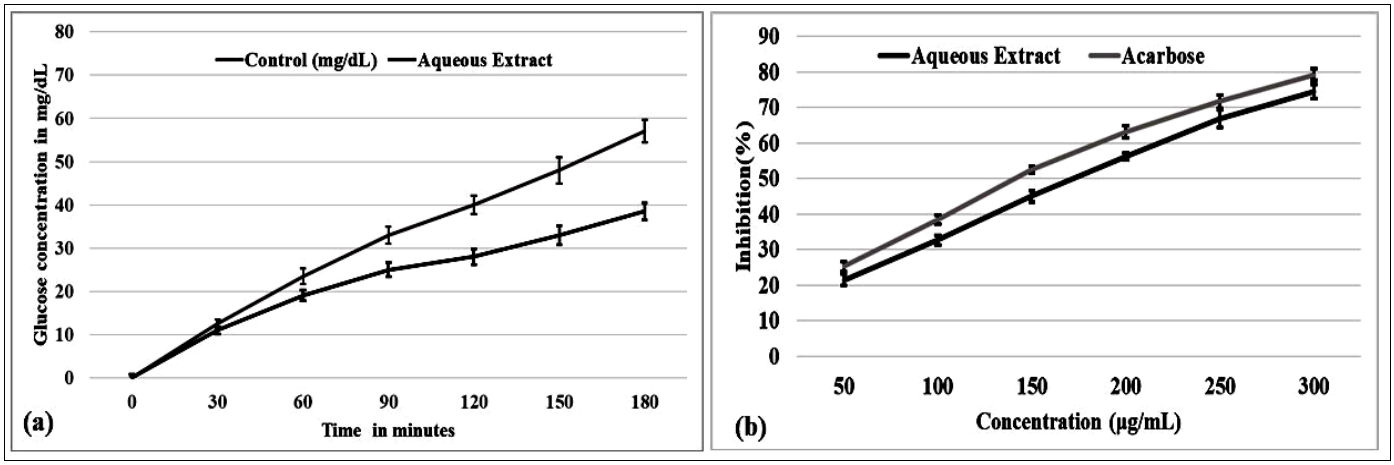

Fig 1: *In vitro* antidiabetic activities of aqueous extract of wild ash gourd (WAGE). (a) Time-dependent inhibitory effect of WAGE on glucose diffusion measured using dialysis tube assay at concentrations of 300 µg/mL. WAGE significantly reduces glucose diffusion compared to control (saline). Data are mean ± SEM (n=3). (b) Dose-dependent α-amylase inhibitory activity of WAGE compared with standard drug, acarbose. WAGE shows substantial inhibitory effect, approaching acarbose response. Values are mean ± SEM (n=3).

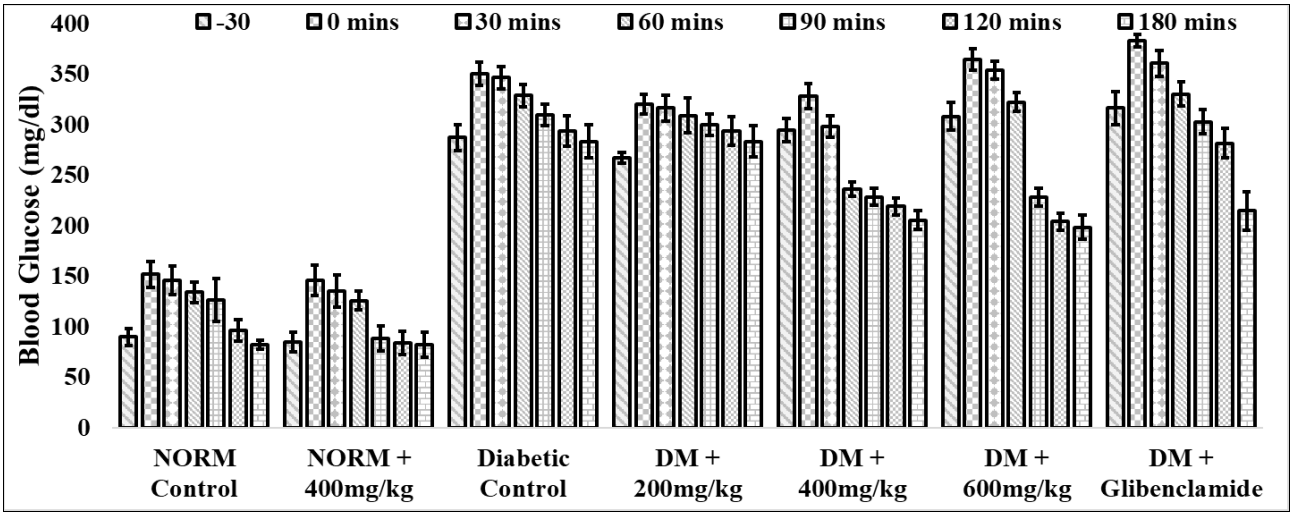

Fig 2: Oral glucose tolerance test (OGTT) curves after 30 days of treatment. WAGE significantly improves glucose tolerance, with 600 mg/kg dose showing comparable efficacy to glibenclamide, with blood glucose measured at multiple time points post-glucose administration. Mean ± SEM (n=6).

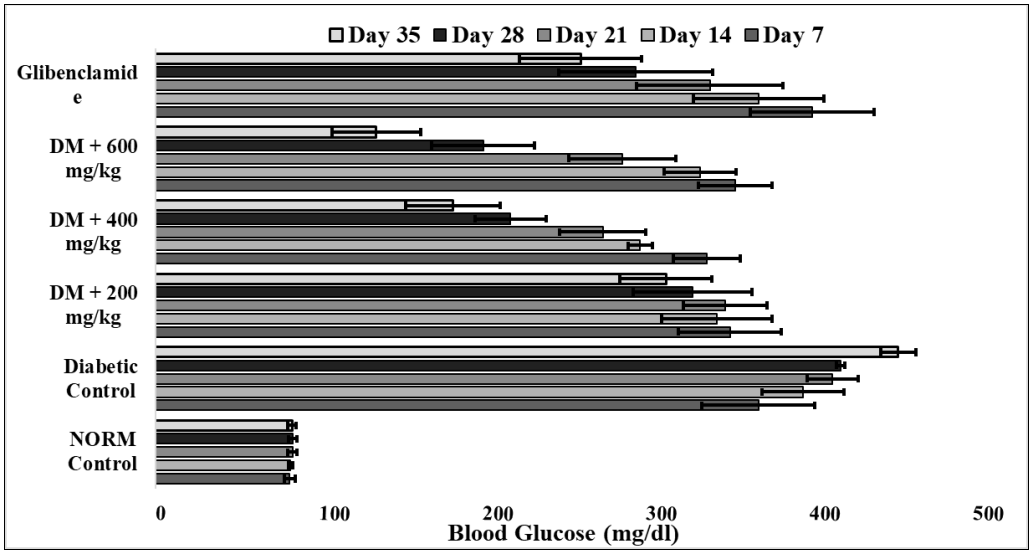

Fig 3: Effect of WAGE on glycemic control in streptozotocin-induced diabetic rats. Longitudinal monitoring of fasting blood glucose (FBG) over 35 days during treatment with WAGE at 200, 400, and 600 mg/kg compared to diabetic control and glibenclamide-treated groups. Values expressed as mean ± SEM (n=6).

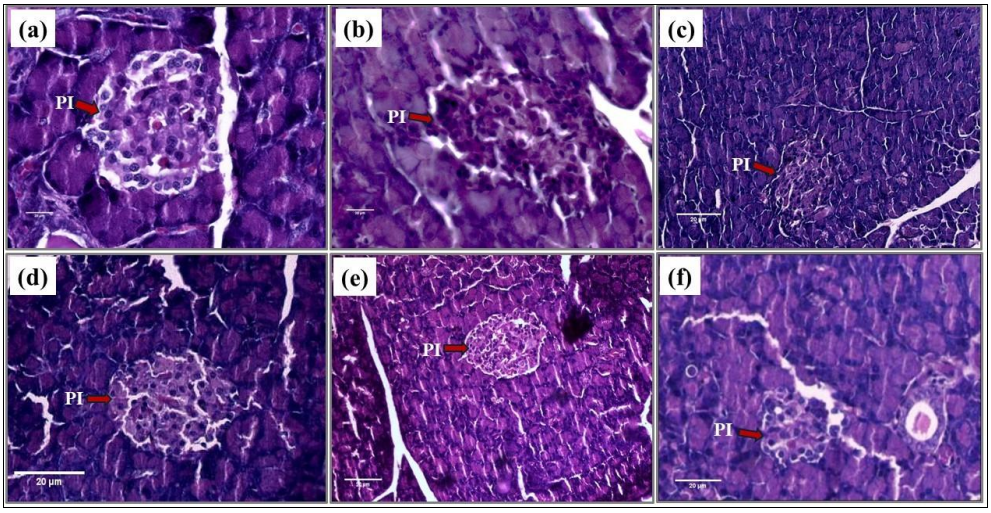

Fig 4: Histopathological evaluation of pancreatic tissue following WAGE treatment. Representative hematoxylin and eosin-stained sections of pancreas (400× magnification): (a) Normal control showing intact islets of Langerhans; (b) Diabetic control illustrating islet degeneration and β-cell loss; (c-e) Progressive regeneration of islet architecture and β-cell density with WAGE treatment at 200, 400, and 600 mg/kg, respectively; (f) Glibenclamide-treated group showing moderate islet preservation. Scale bar: 50 µm. PI-Pancreatic Islet.

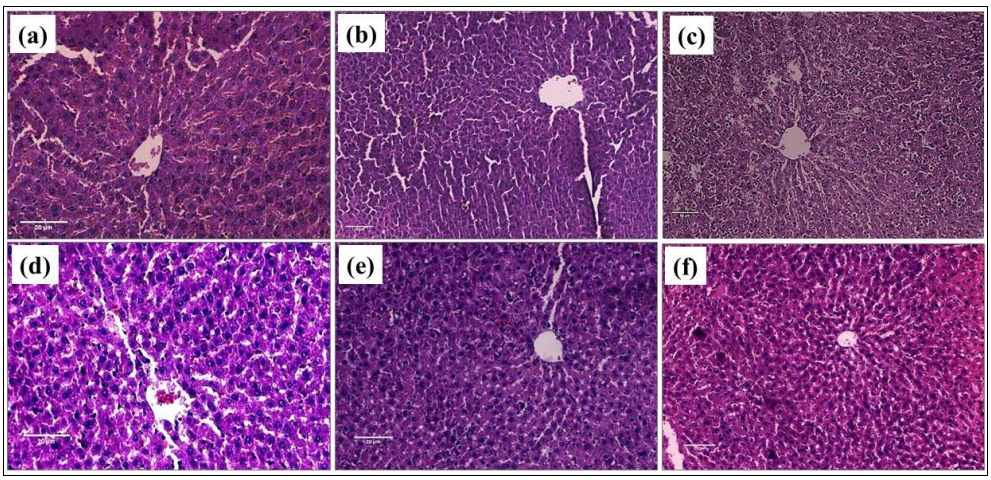

Fig 5: Histopathological analysis of liver tissue after 35 days of treatment. Hematoxylin and eosin staining (400× magnification) displays: (a) Normal control liver with preserved hepatocytes; (b) Diabetic control exhibiting hepatocyte degeneration and fatty infiltration; (c-e) Dose-dependent restoration of hepatic architecture with WAGE at 200, 400, and 600 mg/kg; (f) Glibenclamide-treated rats with partial hepatic recovery. Scale bar: 50 µm.

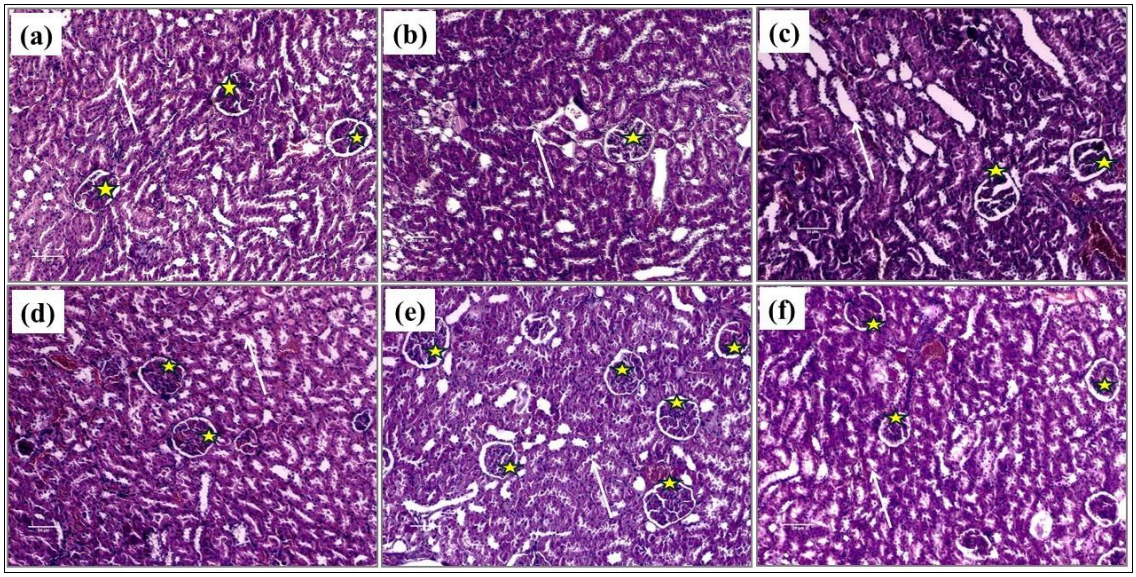

Fig 6: Renal histopathology demonstrating protective effects of WAGE. Representative H&E-stained kidney sections (400× magnification): (a) Normal control showing intact glomeruli and tubules; (b) Diabetic control displaying glomerular hypertrophy, tubular degeneration, and inflammation; (c-e) Progressive renal tissue preservation with WAGE treatment at 200, 400, and 600 mg/kg, respectively; (f) Glibenclamide-treated rats showing moderate renal protection. Scale bar: 50 µm.

4. Discussion

The present study provides compelling preclinical evidence that the aqueous extract of wild ash gourd fruit (*Benincasa hispida;* WAGE) exerts pronounced antidiabetic and multi-organ protective effects in a streptozotocin-induced diabetic rat model. By integrating comprehensive *in vitro* and *in vivo* assessments, we elucidated several converging mechanisms underlying the glycemic control and organ preservation conferred by WAGE, thereby validating ethnomedicinal claims and underscoring its potential as a complementary therapeutic agent.

The rich phenolic (47.3 mg GAE/g) and flavonoid (15.0 mg QE/g) content of WAGE underpins its potent antioxidant capacity, as demonstrated by strong DPPH radical scavenging activity. This antioxidative property aligns with established literature emphasizing the critical role of antioxidative phytochemicals mitigating oxidative stress a major pathogenic factor in β-cell dysfunction and diabetic complications [17]. Consistent with prior findings, the flavonoid constituents likely contribute substantially to the observed antidiabetic effects [18]. By mitigating reactive oxygen species (ROS)-mediated cellular damage, antioxidant phytochemicals protect pancreatic islets as well as hepatic and renal tissues, preserving their functional integrity [19].

Mechanistically, WAGE exhibited significant inhibitory effects on glucose diffusion and pancreatic α-amylase activity *in vitro,* actions that collectively retard intestinal glucose absorption and attenuate carbohydrate breakdown. The alpha-amylase inhibitory activity closely approximated that of acarbose, a clinically approved antidiabetic agent, suggesting comparable therapeutic potential. Previous studies reporting elevated polyphenol and flavonoid levels in ash gourd formulations [20] further corroborate these bioactivities.

In vivo, WAGE administration led to a dose-dependent reduction in fasting blood glucose levels and marked improvement in glucose tolerance tests, indicative of enhanced insulin sensitivity and potential β-cell functional recovery. Notably, a 48.6% reduction in HbA1c after 35 days treatment points to sustained amelioration of glycemic burden, a crucial determinant of long-term diabetes prognosis. Histopathological analyses revealed regenerating pancreatic islets with restored β-cell density and reduced inflammatory infiltration, a significant finding given the rarity of pancreas regenerative effects in existing pharmacotherapies. The complex phytochemical matrix including glycosides, steroids, and polysaccharides present in the WAGE may contribute synergistically to these multifactorial effects, warranting further phytochemical and mechanistic exploration.

Our results resonate with previous investigations, wherein both aqueous and methanolic leaf extracts of *B. hispida* have demonstrated hypoglycemic effects in diabetic rat models [21], while fruit peel extracts showed notable activity in alloxan-induced diabetic rats [22]. Clinical evidence from a recent trial with a powdered *B. hispida* formulation further supports its role in lowering fasting plasma glucose and improving cardiovascular parameters in type 2 diabetic patients [23]. Taken together, these data strengthen the rationale for developing *B. hispida* extracts as functional food components or adjunctive therapies in diabetes management.

5. Conclusion

This comprehensive preclinical investigation provides strong evidence that the aqueous extract of wild ash gourd fruit (*Benincasa hispida*) possesses potent antidiabetic, antioxidant, and multi-organ protective effects in a streptozotocin-induced diabetic rat model. The extract demonstrated dose-dependent glucose-lowering efficacy comparable to the standard antidiabetic agent glibenclamide, while also conferring significant hepatoprotective, renoprotective, and lipid-modulating benefits. Moreover, the observed regeneration of pancreatic β-cells highlights the potential for disease-modifying effects beyond symptomatic glycemic control. The multifaceted therapeutic actions of the extract, combined with its excellent safety profile, validates its traditional use in ethnomedicine and support its further development as a nutraceutical or pharmacological intervention for diabetes management.

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References

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, *et al*. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract. 2019;157:107843.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, *et al*. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183:109119.
3. Pradeepa R, Mohan V. Epidemiology of type 2 diabetes in India. Indian J Ophthalmol. 2021;69(11):2932-2938.
4. Galindo RJ, Trujillo JM, Wang CCL, McCoy RG. Advances in the management of type 2 diabetes in adults. BMJ Medicine. 2023;2(1):e000372.
5. Weinberg Sibony R, Segev O, Dor S, Raz I. Drug therapies for diabetes. Int J Mol Sci. 2023;24(24):17785.
6. Chaudhury A, Duvoor C, Reddy Dendi VS, Kraleti S, Chada A, Ravilla R, *et al*. Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. Front Endocrinol (Lausanne). 2017;8:224539.
7. Nikpour S, Mehrdad N, Sanjari M, Aalaa M, Heshmat R, Mafinejad MK, *et al*. Challenges of type 2 diabetes mellitus management from the perspective of patients: Conventional content analysis. Interact J Med Res. 2022;11(2):e41933.
8. Jugran AK, Rawat S, Devkota HP, Bhatt ID, Rawal RS. Diabetes and plant-derived natural products: From ethnopharmacological approaches to their potential for modern drug discovery and development. Phytother Res. 2021;35(1):223-245.
9. Rahman MM, Dhar PS, Sumaia, Anika F, Ahmed L, Islam MR, *et al*. Exploring the plant-derived bioactive substances as antidiabetic agent: An extensive review. Biomed Pharmacother. 2022;152:113217.
10. Did you know medicinal ash gourd (Vaidya Kumbalanga) can be stored for 3 years - natural farmers Kerala. 2025 Aug 14: https://www.naturalfarmerskerala.com
11. Phumat P, Chaichit S, Potprommanee S, Preedalikit W, Sainakham M, Poomanee W, *et al*. Influence of *Benincasa hispida* peel extracts on antioxidant and anti-aging activities, including molecular docking simulation. Foods. 2023;12(19):3555.
12. Islam MT, Quispe C, El-Kersh DM, Shill MC, Bhardwaj K, Bhardwaj P, *et al*. A literature-based update on *Benincasa hispida* (Thunb.) Cogn.: Traditional uses, nutraceutical, and phytopharmacological profiles. Oxid Med Cell Longev. 2021;2021:6349041.
13. Baghel DS, Mittal A, Singh S, Chaudhary AK, Bhatia A, Chopra S. Formulation, evaluation and assessment of *in vitro* potential of Gokshur Ghan tablet against urolithiasis (Mutrakrichra). Res J Pharm Technol. 2021;14(4):1945-1952.
14. Elbermawi A, Darwish MS, Zaki AA, Abou-Zeid NA, Taher MA, Khojah E, *et al*. *In vitro* antidiabetic, antioxidant, and prebiotic activities of the chemical compounds isolated from *Guizotia abyssinica*. Antioxidants. 2022;11(12):2482.
15. Masaenah E, Elya B, Setiawan H, Fadhilah Z, Wediasari F, Nugroho GA, *et al*. Antidiabetic activity and acute toxicity of combined extract of *Andrographis paniculata*, *Syzygium cumini*, and *Caesalpinia sappan*. Heliyon. 2021;7(12):e08561.
16. Attanayake A, Jayatilaka K, Pathirana C, Mudduwa LKB. Study of antihyperglycaemic activity of medicinal plant extracts in alloxan-induced diabetic rats. Anc Sci Life. 2013;32(4):193-198.
17. Akpoveso OOP, Ubah EE, Obasanmi G. Antioxidant phytochemicals as potential therapy for diabetic complications. Antioxidants. 2023;12(1):123.
18. Obafemi TO, Akinmoladun AC, Olaleye MT, Agboade SO, Onasanya AA. Antidiabetic potential of methanolic and flavonoid-rich leaf extracts of *Synsepalum dulcificum* in type 2 diabetic rats. J Ayurveda Integr Med. 2017;8(4):238-243.
19. Arabshomali A, Bazzazzadehgan S, Mahdi F, Shariat-Madar Z. Potential benefits of antioxidant phytochemicals in type 2 diabetes. Molecules. 2023;28(20):7209.
20. Athira AS, Reshma MV. Ash gourd (*Benincasa hispida* (Thunb.) Cogn. juice as an α-glucosidase inhibitor and its spray-dried juice properties. J Food Meas Charact. 2024;18(4):2598-2608.
21. Uma P, Ramya M, Rani B, Reddy SR, Saraswathi AY, Shreshta R. Phytochemical investigation and antidiabetic activity screening of *Benincasa hispida* (Thunb.). Int J Multidiscip Res Growth Eval. 2023;4(1):395-400.
22. Mishra MK, Bhusan Barik B. Antidiabetic activity of *Benincasa hispida* (Thunb.) Cogn. fruit peels on alloxan-induced diabetic rats. Indian Drugs. 2009;46(10):66-69.
23. Zin CAJCM, Ishak WRW, Khan NAK, Mohamed WMIW. Efficacy of a *Benincasa hispida* powdered drink in improving metabolic control in patients with type 2 diabetes: A placebo-controlled study. Int J Health Sci (Qassim). 2024;18(5):16-25.