



Research Article

Exploratory assessment of mortalin's association with glycemic parameters and its modulation by *Ashwagandha*: *Invitro* and preliminary observational evidence

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Received: 20-04-2026

Accepted: 07-07-2026

Published: 30-09-2026

Abstract

Background: Diabetes mellitus (DM) is a chronic metabolic disorder associated with impaired insulin secretion and/or action, resulting in persistent hyperglycemia and progressive systemic complications. Mortalin protein plays a central role in the intracellular stress response and carcinogenesis. Despite being regulated by glucose, its role in DM remains underexplored in laboratory and clinical contexts. **Objective:** To investigate the association between mortalin and DM, we measured its serum levels in patients with DM and in pancreatic cell differentiation models. **Modulation by *Ashwagandha*** was further investigated through *in vitro* and a preliminary observational study. **Methods:** Serum mortalin levels were measured in diabetic and non-diabetic individuals using antibody-based ELISA. Human pancreatic carcinoma cells were differentiated using retinoic acid and *Ashwagandha* leaf-derived extracts, followed by assessment of mortalin and insulin by immunoblotting and immunostaining. A preliminary observational study included 29 adults with early-stage dysglycaemia receiving *Ashwagandha* leaf powder and was conducted with serial clinical and glycaemic follow-up. **Results:** Mortalin expression analysis of 352 DM serum samples showed significantly lower levels than in non-diabetic controls. *In vitro* differentiation of Panc-1 cells increased mortalin and insulin levels. *Ashwagandha* extracts induced morphological and molecular features consistent with pancreatic differentiation. In a preliminary observational study, a subset of participants demonstrated improvements in glycaemic parameters after receiving *Ashwagandha* leaf powder. **Conclusion:** The study provides insights into the molecular mechanisms underlying mortalin recruitment in diabetes and its modulation by *Ashwagandha* leaf powder, suggesting its therapeutic value for DM and warranting validation through adequately powered clinical trials.

Keywords: Diabetes mellitus, Mortalin, Insulin, *Withania somnifera*, Withanolides, Preventive therapy

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Website:
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DOI: <https://doi.org/10.47552/ijam.v17i2.7083>

Introduction

Diabetes mellitus (DM) is defined as an irreversible, gradual-onset metabolic disorder, characterized by persistent hyperglycemia that gradually progresses to the malfunction of central and peripheral tissues. In DM, cells cannot take up glucose for energy production due to insufficient insulin (1-3). This triggers abnormal cellular signaling of proteins that often progress to cardiac and neurodegenerative diseases, blindness, and hepatic and renal failure. Consistent hyperglycemia, due to inadequate insulin secretion or action, or both, results from destruction or abnormalities in β -pancreatic cells. Hyperglycemia in diabetic

patients is often associated with elevated Hemoglobin A1c (HbA1c) levels, and symptoms including polyuria, polydipsia, malaise, loss of weight and appetite, and blurry vision. 'At risk' or 'pre-diabetics' are clinically diagnosed from blood glucose ranging from 1.1-1.26 mg/ml (fasting), 1.4-2.0 mg/ml (post-prandial), or impaired HbA1c enzyme standards (5.7-6.4%). DM is broadly classified into type 1 (insulin deficiency), type 2 (insulin resistance, > 90%), and other types (genetic defects, obesity, hepatopancreatic injury or rupture, cancer, drug reactions, and/or infections). Intracellularly, both types of DM are directly linked to mitochondrial function (1,2), with Mfn2 (Mitofusins 2), Opa1 (Optic Atrophy 1), and Drp1 (Dynamin-Related Protein 1) among the mitochondrial proteins commonly studied (4).

With more than 85% prevalence of DM in the developing world and a continuously growing global incidence, the diabetic epidemic urges strong and pressing attention. Currently, metformin is the most commonly prescribed oral medication for T2 diabetes. Its mechanism of action involves altering liver glucose output. It was shown to inhibit mitochondrial respiration,

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enhance insulin sensitivity, and upregulate digestive enzymes involved in glucose metabolism (5). An earlier meta-analysis showed that metformin was safe in clinical trials and delayed the conversion of prediabetes to diabetes by about 45% (6). Three randomized controlled trials, collectively involving more than 2500 patients, examined metformin for the prevention or delay of the onset of type 2 diabetes in patients with existing impaired fasting glucose or glucose intolerance, with a follow-up period of at least 6 months (6). This meta-analysis also concluded that metformin at 850 mg twice daily is associated with favorable outcomes. However, patients of South-Asian descent might require a higher dose and may be more prone to adverse reactions. Metformin is recommended only for post-diagnostic patients and helps prevent diabetic complications; it does not affect an individual's likelihood of acquiring the disease. Other compounds that are in practice for treating diabetes include sulfonylureas (close the potassium channels in pancreatic β -cells to increase insulin secretion; indicated in T1 diabetes), exogenous insulin and its analogues (increase insulin concentration; indicated in T1 diabetes), Glucagon-Like Peptide-1 (GLP-1) agonists (enhance insulin secretion; indicated in T1 diabetes), Dipeptidyl Peptidase-4 inhibitor (DDP-4-i; indicated in T1 diabetes), α -glucosidase inhibitors (delay polysaccharides digestion; indicated in T1/T2 diabetes) and thiazolidinediones (Peroxisome Proliferator-Activated Receptor Gamma (PPAR γ)-agonists, enhance insulin response; indicated in T2 diabetes). However, these compounds with varying grades have been associated with serious complications such as hypoglycemic shock, triggering and nurturing neoplastic transformations, delayed gastrointestinal motility, and adverse effects on normal cell proliferation and cell cycle (7). Various adjuvant therapies, such as anti-obesity drugs, antihypertensives, and gastro-surgical interventions, have also been used in practice. However, none have shown the potential to prevent the onset of the primary disease or to cure it. Several medicinal plants have also been reported to be effective in treating diabetes mellitus (8-11). With ongoing patient feedback and laboratory-based research and development, it is possible to discover and nurture new, improved, well-tolerated, broad-spectrum natural compounds as alternatives in medicine.

Many cellular and secreted proteins are used as markers of disease pathogenesis. These include glutamine fructose-6-phosphate amidotransferase (GFAT), tyrosine phosphatases (PTPs), 11 β -hydroxysteroid dehydrogenase, and glucagon and insulin (6, 12-14). Although stress proteins are recognized in the stressed state of cells and tissues, their role in diabetes pathology remains underexplored. Mortalin/mthsp70/Grp75 is an essential protein that regulates cell proliferation (15-18). It is crucial for cellular functions such as ATP generation, mitochondrial genesis and integrity, protein import through the mitochondrial membrane, intracellular trafficking, and chaperoning (19-21). Upregulation of mortalín expression in a wide variety of cancers has been correlated with the unlimited proliferative capacity of cancer cells through multiple molecular mechanisms (20-28). Of note, whereas an upregulation of mortalín has been correlated with cancer, its downregulation has been associated with neurodegenerative diseases (29-34). It has been suggested to play a key role in neuronal mitochondrial dynamics (35). Whereas its downregulation was shown to exacerbate beta-amyloid-mediated mitochondrial fragmentation and dysfunction, its overexpression attenuated beta-amyloid-induced neurotoxicity in SH-SY5Y cells (36,37). Retinoic acid-induced differentiation of neuroblastoma cells was shown to require upregulation and nuclear translocation of mortalín (38-40). Circulating mortalín has been detected in cancer patients and suggested as a risk factor (23, 41, 42). It was

also detected in COVID-19 patients, with its higher levels correlated with severe illnesses and mortality (43). Quantifying blood levels of mortalín has been suggested as a useful prognostic biomarker for cancer and COVID-19, as well as for their treatment and clinical management (41, 43). Recent studies have highlighted the importance of measuring mortalín concentration in blood as a prognostic and diagnostic marker for several diseases (23, 27, 41, 42).

Building on the above premise, we set the current investigation to the following objectives: (i) to determine circulating mortalín levels in diabetic and non-diabetic individuals, (ii) to investigate whether pancreatic β -cell differentiation is associated with changes in mortalín and insulin expression, (iii) to evaluate whether Ashwagandha-derived extracts induce pancreatic differentiation *in vitro* and (iv) to conduct a preliminary observational assessment of glycemic trends after Ashwagandha leaf administration.

Materials and Methods

Mortalín ELISA assay in human blood samples

Human serum samples were obtained after registration, in compliance with the Central Licensing Authority and the Institutional Ethical Committee of KLE University, Belagavi, India, under registration No. ECR/211/inst/KA/2013/RR-19 under Rule 122 DD (Registration of Ethics Committee) of the Drugs and Cosmetics Rules, 1945 (Directorate Medical & Health Service) (44). Samples were stored at -20 °C and thawed on ice for use in the current retrospective cohort study. Aliquots (100 μ L) of diabetic and non-diabetic individuals were screened for mortalín concentration using a specific antibody described earlier (22, 45).

Cell culture and reagents

Human pancreatic duct epithelial carcinoma (Panc-1), epithelial-like lung carcinoma (A549), epithelial breast adenocarcinoma (MDA-MB-231) cell lines, and normal fetal lung fibroblasts (TIG-3) were purchased from the Japanese Collection of Research Bioresources (JCRB) Cell Bank, Japan. These cells/cell lines were cultured in Dulbecco's modified Eagle's medium (Invitrogen)-supplemented with 5% fetal bovine serum and 1% penicillin/streptomycin in a humidified incubator (37°C and 5% CO₂). D (+)-Glucose (049-31165) was purchased from WAKO Japan. Metformin hydrochloride (PHR1084) and retinoic acid (R2625) were purchased from Sigma-Aldrich. Withaferin A (Wi-A)-rich extract (Wi-AE) and Withanone-N (Wi-N)-rich extract (Wi-NE) were prepared from *Ashwagandha* leaves as described earlier (46). Antibodies against mortalín (22), eNOSphospho (ab75639, Abcam), β -actin (ab49900, Abcam), and insulin (8138S, Cell Signaling) were used for molecular assays.

Immunoblotting

Two hundred thousand control/treated/differentiated cells/well were plated in a 6-well plate and allowed to settle overnight, following which they were treated with varying doses of the compounds. Cells were then fixed and carried forward for immunoblotting as described earlier (20, 22). Protein expression was quantified using ImageJ software (NIH) and plotted as a percentage in an Excel sheet with conditional formatting.

Dose titration

Cytotoxicity of the various compounds and reagents in the given cell lines was determined by an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Two thousand cells per well were plated in a 96-well plate and allowed to settle

overnight, following which they were treated with varying doses of the compounds/reagents. Cells were then subjected to an MTT-based cell viability assay as described earlier (47). Cell viability was calculated as a percentage relative to the control to identify the IC₁₀ and IC₅₀ values.

Cell differentiation

Fifty thousand Panc-1 cells/well were plated in a 6-well plate and allowed to settle overnight. Then they were treated with varying doses of the compounds for 7-14 days (until differentiated cell clusters appeared). Control and differentiated cells were subjected to immunostaining.

Immunostaining

Twenty-five thousand control/treated/differentiated cells/well were plated on coverslips in 12-well plates and allowed to settle overnight before being treated with varying compound doses. Cells were then immunostained as described earlier (48). Protein expression was quantified using ImageJ software (NIH) and plotted as a percentage in an Excel sheet with conditional formatting.

Clinical observation study on the effect of Ashwagandha

A small preliminary observational study using Ashwagandha leaf powder (rich in Wi-N and Wi-A) was designed on randomized subjects with/without a history of diabetes. The inclusion criterion was set to Age: 18-60 years, early-stage type 2 diabetes mellitus (fasting blood sugar (FBS) of more than 110 mg%, or post-prandial blood sugar (PPBS) of more than 135 mg%), not undertaking any other diabetic medications, HbA_{1c} levels of 6.5-8.0%, and consent for compliance. The exclusion criterion was set to gestation or feeding mother, age <18 years, clinically unstable neurological, ophthalmological, hepatobiliary, respiratory, hypotension, hypertension, and other complications, and severe or uncontrolled depression. All subjects provided informed consent for inclusion in the study before participating. The study was conducted following The Code of Ethics of the World Medical Association (Declaration of Helsinki of 1975/2013), and the protocol was registered at and approved by the Central Licensing Authority to the Institutional Ethical Committee, KLE University, Belagavi, India (Registration No. ECR/211/inst/KA/2013/RR-19, dated 16th September 2019). Patient anonymity was maintained for privacy rights. This manuscript does not reveal patient names, signatures, images, or medical information. Participants consumed leaf powder (300 mg/capsule/day) and underwent medical follow-up for 1 to 8 months. A vital assessment and a general physical examination were conducted on each visit, along with a blood sugar/HbA_{1c} assessment. Patients who did not return for follow-up or showed irregularity were excluded from the study. A histogram of average fasting blood sugar was plotted for the patients, and the improvements were evaluated for statistical significance.

Statistical analysis

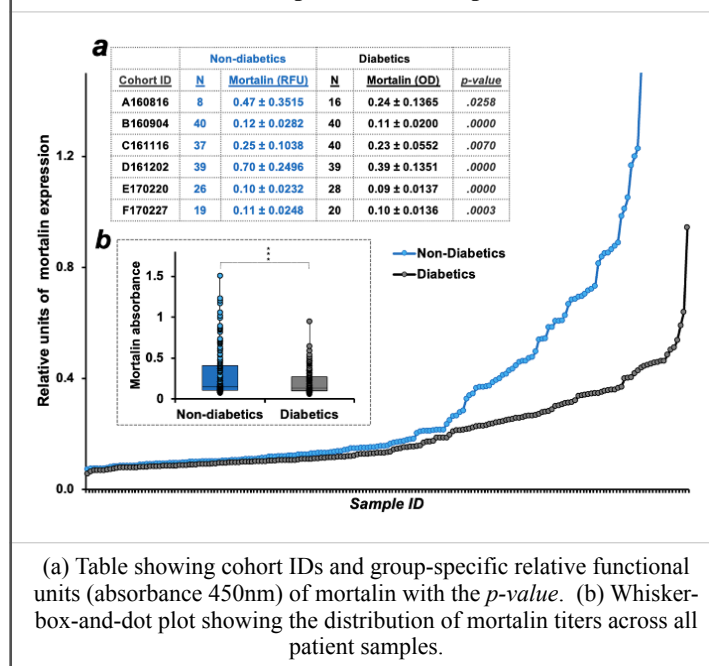
All quantifications were performed using ImageJ (NIH), calculations were performed in Microsoft Office, and results were plotted as percentages. Continuous variables were summarised as mean $\pm$ SD. Comparisons between diabetic and non-diabetic cohorts were performed using an unpaired two-tailed Student's t-test in Microsoft Excel (2016) based on mean and SD from three independent experiments, and are shown as: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact p-values are now shown in Figure 1 and Supplementary Table S1.

Results

Diabetes patients showed lower serum mortalin levels

Blood samples from patients with/without diabetes were subjected to a double-blind analysis of mortalin expression by sandwich ELISA in six unbiased nonoverlapping sets (based on sample acquisition date). As shown in Figure 1, overall mortalin expression was significantly lower in serum samples from diabetic patients than in those from healthy cohorts. Of note, this difference was consistently significant across all sets (Figure 1a); collectively, the results were statistically highly significant (Figure 1b).

Figure 1. Quantitative analysis of mortalin by ELISA in serum samples of diabetic patients



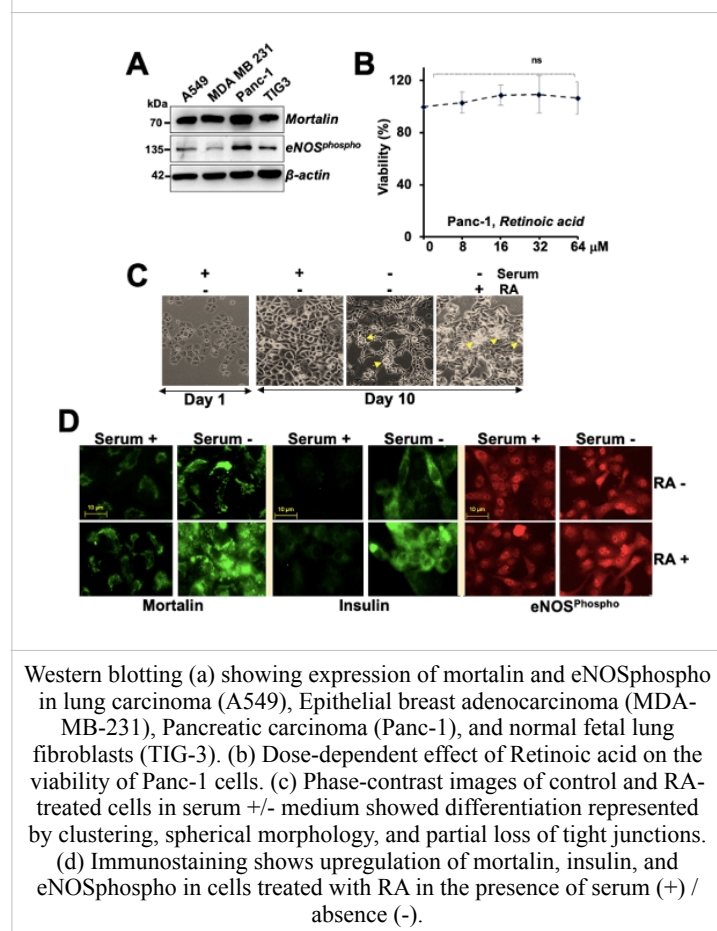
Mortalin expression in pancreas-derived cells and their differentiated derivatives

Western blotting using a mortalin-specific antibody on cell lines (A549; non-small cell lung cancer, MDA MB 231; triple-negative breast cancer, and TIG3; normal lung fibroblasts) that have been extensively used for earlier mortalin studies, and Panc-1 (Human pancreatic duct epithelial carcinoma that shows undifferentiated characteristics) showed that the latter possess a high level of mortalin expression (Figure 2a). Furthermore, as expected, endothelial nitric oxide synthase (eNOS; a constitutive calcium-calmodulin-dependent enzyme that catalyzes the synthesis of soluble nitric oxide gas from the amino acid L-arginine in endothelial cells) was also highly expressed in Panc-1 (Figure 2a). Nitric oxide is an important cellular signaling molecule with many biological properties. Its downregulation dysregulates local cell growth, vascular homeostasis, and insulin secretion, and has been associated with DM, cardiac dysfunction, atherosclerosis, hypertension, and hypercholesterolemia (12, 49).

Next, we investigated the molecular association between mortalin and insulin in diabetic pathology by using retinoic acid (RA)-induced differentiation of Panc-1 cells. El-Metwally *et al.* (50) reported RA-induced differentiation of pancreatic ductal adenocarcinoma (HPAF), resulting in upregulation of insulin. We first treated Panc-1 cells with RA and assessed their response

using the MTT assay (Figure 2b). Cells were then cultured in RA-supplemented medium (10 μ M; nontoxic concentration) for 5-10 days. As shown in Figure 2c, Panc-1 cells exhibited cuboidal morphology with complete tight junctions (strong cell-cell adhesion), as reported earlier (51). Whereas the cell morphology was maintained in the control culture (Serum+ and RA-), the RA-treated cells exhibited islet-like clustering and a spherical morphology (Figure 2c) resembling that of differentiated Panc-1 cells, as reported earlier (52, 53). As expected, RA treatment under serum-free conditions resulted in stronger differentiation than in cells cultured in the presence of serum. We next subjected control and treated cells to Mortalin, insulin, and eNOS immunostaining. As shown in Figure 2d, differentiation was marked by a higher level of mortalin, insulin, and eNOS expression. These data suggested an association of mortalin expression with the functionally differentiated state of pancreatic cells.

Figure 2: The relative level of Mortalin expression in human cancer cells

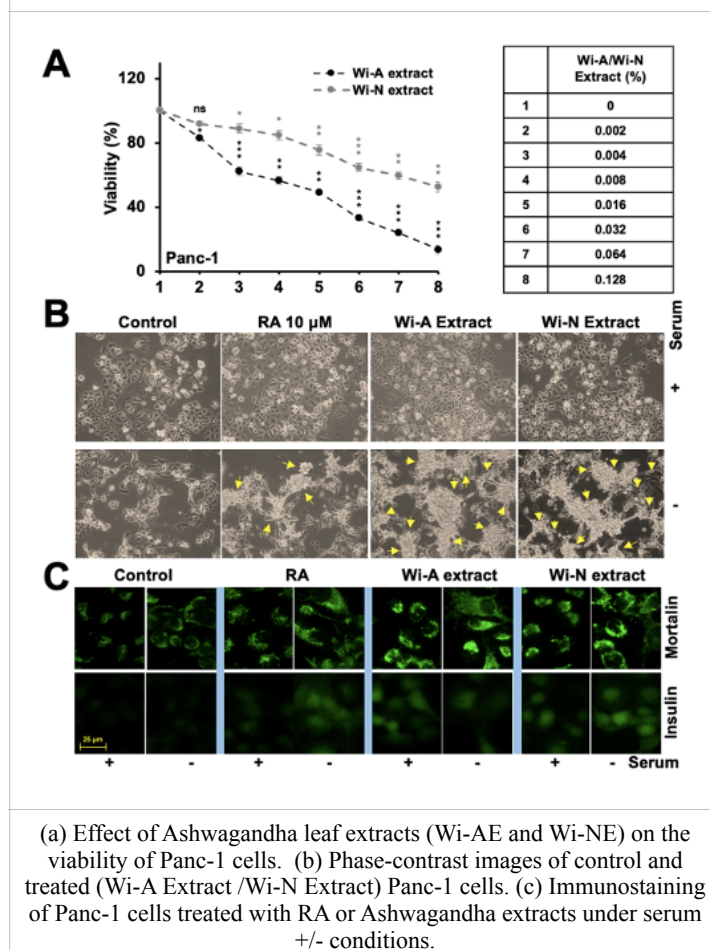


Ashwagandha leaf extract caused functional differentiation of Panc-1 cells

Given the existing literature on the anti-stress and differentiation-inducing properties of Ashwagandha-derived withanolides (Withaferin A, Wi-A, and Withanone, Wi-N) and extracts in brain and muscle-derived cells (40,54-56), we predicted that Ashwagandha-derived withanolides and extracts may induce differentiation of Panc-1 cells, similar to RA. Cells were treated with non-toxic concentrations (as determined by an MTT assay, Figure 3a) of extracts (Wi-A-rich extract (Wi-AE: 0.001%) or Wi-N-rich extract (Wi-NE: 0.002%) (46) for 9 days. As shown in

Figure 3b, cells treated with either Wi-AE or Wi-NE exhibited clustering and differentiation morphology (as indicated by yellow arrows) similar to those treated with RA. Furthermore, as with RA, the extracts induced stronger differentiation of Panc-1 cells in serum-free culture medium (Figure 3b).

Figure 3: Ashwagandha extracts caused differentiation of Panc-1 cells *in vitro*.



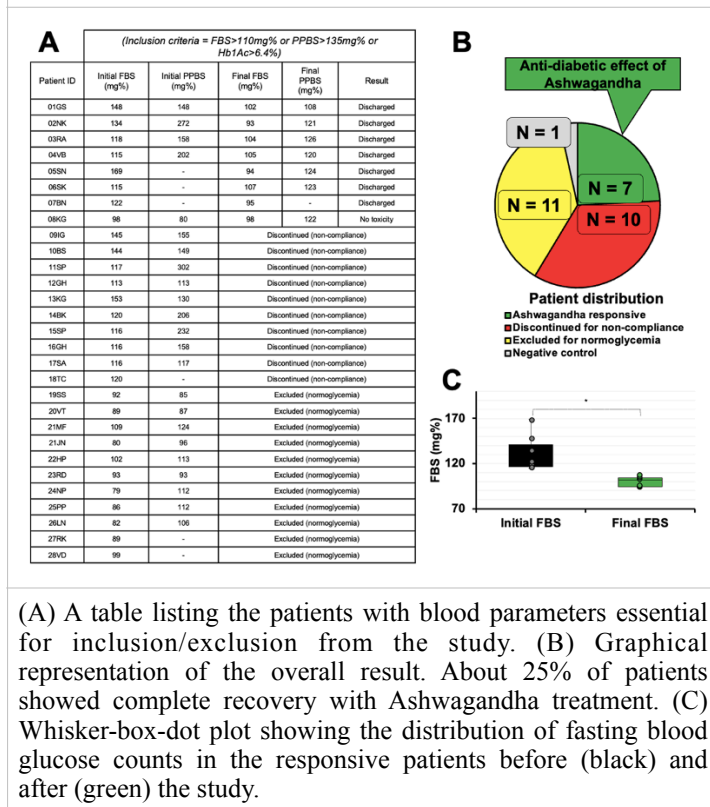
Consistent with the upregulation of differentiation in treated cells, we found that mortalin and insulin expression was increased in treated cells compared with controls (Figure 3c). These data suggested functional differentiation of Panc-1 cells was induced by treatment with Ashwagandha extracts.

Consumption of Ashwagandha leaf powder caused remission of diabetes

Based on our *in vitro* results showing differentiation of Panc-1 cells with Wi-AE and Wi-NE, we recruited Ashwagandha leaf powder, which was tested to possess high Wi-N and Wi-A (46). We conducted a preliminary observational study on 29 human subjects, using the inclusion criteria of Fasting Plasma Glucose (FPG) >110 mg/dL, Postprandial Plasma Glucose (PPG) >135 mg/dL, or Glycated Hemoglobin (HbA1c) >6.4%. One normoglycemic subject was taken as the control.

Ashwagandha leaf powder was preferred over roots for (i) the leaves contain comparatively higher levels of the bioactive withanolides (such as Wi-A and Wi-N) (46), offering the possibility of using lower doses; (ii) leaf harvesting offers a more sustainable and renewable approach, allowing repeated collection without uprooting and destroying the plant. The

Figure 4: Representative data of a randomized observational clinical study involving diabetic and non-diabetic patients and response to Ashwagandha treatment



(A) A table listing the patients with blood parameters essential for inclusion/exclusion from the study. (B) Graphical representation of the overall result. About 25% of patients showed complete recovery with Ashwagandha treatment. (C) Whisker-box-dot plot showing the distribution of fasting blood glucose counts in the responsive patients before (black) and after (green) the study.

exploratory dose of 300 mg/day was selected based on evidence from human clinical studies demonstrating favorable tolerability and efficacy across stress-related and functional outcomes. Published clinical trials have most commonly employed doses ranging from 250 to 600 mg twice daily (BID), with benefits observed in stress reduction, sleep quality, and overall well-being during treatment periods of 2–3 months (57–60). Some studies have reported clinically significant effects at lower doses (125-250 mg twice daily), suggesting that higher dosing may not always be necessary to achieve biological responses (61, 62). Notably, a randomized, placebo-controlled study using 200 mg BID demonstrated improvements in fatigue and stress-related parameters with good tolerability, supporting the feasibility of this intermediate dose (61). In light of these reports, we considered 300 mg/day as an appropriate balance between efficacy and safety, enabling evaluation of therapeutic benefit at a level that remains within the clinically investigated range and may facilitate future translation into long-term use settings. Ten subjects were discontinued due to unavoidable irregularities and/or a lack of improvement. As shown in Figures 4a and 4b, seven subjects (~25%) responded positively to the treatment. Patients in both diabetic and pre-diabetic subcategories showed improvement, with fasting and postprandial blood sugar returning to normoglycemic levels. This improvement was statistically significant (Figure 4c). Of note, the negative control did not show any signs of toxicity or hypoglycemia.

Discussion

Mortalin performs various cellular functions, including stress response, energy production, mitochondrial genesis, membrane transport, intracellular trafficking, and chaperoning (17, 38, 39). It plays a significant role in carcinogenesis (overexpressed) (16, 27,

63, 64) and in several neurodegenerative diseases (underexpressed) (34, 38, 39). As a glucose-regulated protein 75, mortalin is strongly implicated in diabetes mellitus, a chronic blood glucose-dependent disease. The uncontrolled over-synthesis or secretion of the protein has been associated with carcinogenesis, suggesting it as a diagnostic, prognostic, and therapeutic target (64, 65). We report, for the first time, that diabetes is associated with downregulation of serum mortalin, suggesting a role in glucose metabolism. *In vitro* experiments showed functional differentiation of Panc-1 cells upon RA treatment, similar to that observed in pancreatic ductal adenocarcinoma (HPAF) (50, 66). The differentiated Panc-1 cells showed upregulation of mortalin and insulin. Furthermore, Panc-1 cells treated with Ashwagandha leaf-derived extracts exhibited differentiation similar to that induced by RA, as evidenced by increased levels of mortalin and insulin.

Diabetes is a state of persistent hyperglycemia leading to peripheral degenerative diseases and cancer (67, 68). The global diabetic toll is aggressively rising as an epidemic. T2 diabetes is the most common, with 537 million people recorded in 2025 and expected to increase to 643 million by 2030. Metformin is a widely accepted first-line therapy. It has also been shown to possess anticancer properties (7, 69), strengthening an association between diabetes and cancer. Studies have shown that prolonged hyperglycemia contributes to carcinogenesis, which may subsequently result in increased glucose consumption and depletion of glucose reserves (68), excessive ROS production, and ROS-mediated oxidative stress and DNA damage (well-established oncogenic factors) (70). The relevance of Panc-1 cells to functional differentiation into pancreatic cells has been previously supported (51, 52). Islet cell functions in pancreatic cancer have been shown to depend on and directly reflect mechanisms within cancer cells (52). Szablewski reported that the current drugs used to treat cancer might cause diabetes or even worsen pre-existing diabetes (71). More than 75% of patients who have pancreatic cancer were found to have diabetic symptoms or at least a severe grade of glucose intolerance. One of the most commonly used therapies for T1 diabetes includes sulphonylureas (which increase fasting insulin secretion) and insulin analogs, thereby lowering blood sugar levels immediately. Since this mechanism could easily take an abnormally exaggerated course (overt hypoglycemia), it could even take a pathological course. Therefore, slow, gradual, comprehensive, and preventive therapy could be more suitable and encourage the use of natural compounds.

Withanolides and their aglycones have been known for their anti-diabetic and protective activities against oxidative stress (55, 72-75). In one of the clinical studies designed to evaluate dose-related safety, tolerability, and effectiveness against diabetes-associated vascular abnormalities in young, healthy adults, an increasing number of capsules (equivalent to 6, 8, and 10 grams of crude pulverized Ashwagandha roots, minimum dose = 750 mg/day) were well tolerated by 17 of 18 volunteers (76). A volunteer excluded from the study presented with polyphagia, increased libido, and hallucinogenic effects. Six reported improved sleep. In another randomized, double-blind study of Type-2 diabetic patients maintained on 1500-2500 mg/day metformin (76), 250-500 mg/day Ashwagandha root extract administered for 12 weeks led to significant improvements in endothelial function and in biomarkers of oxidative stress, systemic inflammation, and HbA1c, with good tolerability. Previous studies have reported anticancer and antistress activities of Wi-A and Wi-N at high and low doses in cell culture-based assays (77-80). Extracts from

Ashwagandha leaves have previously been shown to induce differentiation in glioblastoma and neuroblastoma cells and to protect them against oxidative and glutamatergic stress (40, 73).

Li et al. have reported anti-steatosis and antioxidative-stress properties of extracts from Ashwagandha leaves (80). Here, we found upregulation of mortalin and insulin in Panc-1 cells treated with Ashwagandha leaf-derived extracts, similar to that caused by RA (81). In a preliminary observational clinical trial of patients with diabetes taking Ashwagandha leaf powder, no adverse effects were observed in the study cohort. 7/29 patients (~25%) benefited significantly from Ashwagandha intake.

Conclusion

Diabetic patients exhibited reduced serum mortalin levels. Panc-1 cells treated with low, non-toxic concentrations of Ashwagandha leaf extracts showed differentiation, similar to the ones treated with RA. The differentiated cells were characterized by increased expression of mortalin and insulin, suggesting that diabetic patients may benefit from consuming Ashwagandha leaf. Indeed, 25% of individuals in an observational clinical trial demonstrated favorable glycemic trends in both fasting and postprandial blood sugar, returning to normoglycemic levels. Taken together, the present study suggested an association between reduced circulating mortalin levels and diabetes and demonstrated that Ashwagandha-leaf derived preparations could modulate pancreatic differentiation markers *in vitro*. Preliminary observations of our study indicated glycemic improvement in a subset of participants; however, well-designed clinical trials are required before clinical endorsement can be made.

Future research is warranted to

Elucidate molecular insights and gene expression profiling of the effects of Ashwagandha withanolides and extracts in other diabetes disease models and advance clinical trials. Integrative approaches that combine Ashwagandha with conventional diabetes management may offer new, effective, and safe interventions for this rapidly rising disease.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

The Authors thank Sukant Garg and Priyanshu Bhargava for technical support.

Declaration of degenerative ai in scientific writing

None.

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