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Original Article

Characterization and Evaluation of Antidiabetic Potentials of *Plectranthus amboinicous*-Derived Nanoparticles

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ABSTRACT

Background: *Plectranthus amboinicus* (Lour.) Spreng, a member of the Lamiaceae family, is commonly utilized in both traditional medicine and culinary applications. This research focuses on optimizing the pharmacodynamic and pharmacokinetic characteristics of *Plectranthus amboinicus* by developing and assessing a nanoparticle formulation combined with gelatin. The objective was to evaluate the effectiveness of this nanoformulation in enhancing glucose metabolism and its potential as an antidiabetic agent. **Materials and Methods:** The nanoparticle formulation of *Plectranthus amboinicus* was developed using gelatin as the carrier via the solvent evaporation method. The resulting nanoparticles were roughly 250 nm in diameter and exhibited a spherical morphology. Toxicity tests were conducted at concentrations up to 1000 µg/mL. The potential antidiabetic effects were evaluated by measuring glucose uptake in 3T3-L1 adipocytes and L6 myoblast cells. **Results:** The gelatin-conjugated nanoparticles of *Plectranthus amboinicus* were non-toxic at concentrations up to 1000 µg/mL. They significantly improved glucose uptake in L6 myoblast cells, indicating better glucose metabolism. Additionally, the formulation showed promise in decreasing fat accumulation and carbohydrate digestion, implying significant antidiabetic effects. **Conclusion:** The Gelatin-conjugated *Plectranthus amboinicus* Nanoparticles (GPAN) show promise for enhancing glucose metabolism and offering antidiabetic benefits. This study highlights GPAN as a promising candidate for further investigation and development due to its effectiveness in improving glucose uptake and its favorable safety profile. This positions GPAN as a potential therapeutic option for diabetes and other related metabolic disorders.

Keywords: Spherical, Glucose, Preadipocytes, Nanotechnology, Therapies, Solvent Evaporation.

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INTRODUCTION

Diabetes mellitus, a group of metabolic disorders, is characterized by elevated blood glucose levels and inadequate pancreatic insulin production. This condition primarily results from insulin resistance and is associated with metabolic syndrome and obesity. Type 2 Diabetes (T2DM) involves a gradual decline in β-cell insulin secretion, leading to insulin resistance. The core pathophysiological mechanism behind T2DM involves insulin resistance affecting insulin-sensitive tissues such as the liver, skeletal muscle and adipose tissue. This resistance leads to increased hepatic glucose production; diminished glucose uptake in insulin-sensitive organs and accumulation of Free Fatty Acids (FFAs), resulting in impaired glucose metabolism, persistently high blood glucose levels and a heightened risk of complications like cardiovascular disease, neuropathy, retinopathy and nephropathy.[1-4] Furthermore, insulin inhibits lipolysis by reducing hormone-sensitive lipase activity. Under basal circumstances in 3T3-L1 cells, 70%-90% of GLUT4 was detected in GLUT4 Storage Vesicles (GSV).[5] Furthermore, 3T3-L1 cells can be used *in vitro* to develop drugs that target pathways involved in adipocyte insulin resistance development. Although traditional antidiabetic medications like metformin and sulfonylureas are effective, they are often associated with significant side effects and limitations in long-term effectiveness. This has spurred interest in alternative therapies, particularly those involving natural products and nanotechnology, to improve glycemic control with fewer adverse effects. Long-term malnutrition and lack of physical activity contribute to insulin resistance and excessive adipose tissue accumulation, which are major factors in the development of type 2 diabetes and related cardiovascular conditions. Insulin resistance also inhibits lipolysis by decreasing hormone-sensitive lipase activity. In 3T3-L1 cells under basal conditions, 70%-90% of GLUT4 is localized in GLUT4 Storage Vesicles (GSV). These cells can be utilized *in vitro* to develop drugs targeting pathways involved in adipocytes insulin resistance, though appropriate differentiation media and maturation times are critical. However, 3T3-L1 must utilize the correct differentiation medium and the amount of time it takes for the cells to mature. Skeletal muscle

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is the most insulin-sensitive tissue and is involved in about 75% of the insulin-induced glucose utilization process, particularly during the postprandial phase. Skeletal muscle, being highly insulin-sensitive, plays a significant role in insulin-mediated glucose utilization, especially postprandially. Insulin resistance in skeletal muscle is a key factor influencing the development of systemic insulin resistance.[6] Herbal treatments are increasingly popular for their potential to address various disorders with reduced toxicity compared to conventional drugs. However, challenges such as high first-pass metabolism and instability at very acidic pH can limit their therapeutic effectiveness. To overcome these issues, herbal drugs are often formulated with new carriers to reduce degradation and minimize adverse side effects, which could result in blood drug concentrations below the therapeutic concentration, which would have a minimal or no therapeutic impact.[7] Herbal drugs are put onto new carriers to decrease drug breakdown and harmful side effects produced by pharmaceutical accumulation in the non-targeted area to eliminate such effects.[8,9]

Plectranthus amboinicus (Lour.) Spreng, also known as *Coleus amboinicus* Benth., is a perennial plant from the Lamiaceae family with a rich tradition of use and various bioactive phytochemicals. This study aims to evaluate the bioactivity of this plant in the context of diabetes and the development of nanoparticle formulations.

MATERIALS AND METHODS

Plectranthus amboinicus Lour. Spreng leaves were gathered from Mysore, Karnataka, India, it was identified and authenticated by Dr. Madhava Chetty plant taxonomist. All solvents and chemicals were used of analytical grade.

Extraction of *Plectranthus amboinicus*

After being cleaned, the fresh leaves were dried. The herb was dried and ground into a fine powder. Following maceration, the powdered plant sample was extracted with ethanol using a cold maceration extraction method. Following filtering, the material was concentrated using a rotatory evaporator. For later usage, the extracted material was kept in a desiccator.

Preparation of Gelatin Nanoparticles Loaded with Extract

Plectranthus amboinicus (PA) nanoparticles were created utilizing the solvent evaporation method.[10] The organic phase was created by sonicating 10 mL of ethanol with the required PA dry extract for 60 sec at 20 watts. The aqueous phase containing Tween 80 and gelatin was then gradually mixed with this organic phase using a syringe, drop by drop while being constantly agitated magnetically at 1000 rpm. A 0.01% glutaraldehyde solution was applied to crosslink the gelatin after an hour. To aid in the evaporation of the solvent and the formation of nanoparticles, the mixture was then constantly agitated for 7 hr at room temperature. Additionally, blank nanoparticles were created using only the polymer and surfactant, without the extract, for use in comparative studies. After 30 min of centrifugation, the nanoparticles were resuspended in Milli-Q water and washed three times. After drying with a lyophilizer, the suspension was stored at 4°C until needed again.

Optimization

The formulation was improved by adjusting the polymer and formulation concentration from 1% to 3%. They were assigned the codes PA1 through PA9 and the Blank Nanoparticles (BNP) were also included for comparative purposes.[11] The optimized formulation was then evaluated for polydispersity index, zeta potential, particle size and surface morphology as described in Tables 1 and 2.

Characterisation

Polydispersity index and Particle size

A Malvern Zeta sizer (2000, UK) and the dynamic light scattering technique were used to determine the formulation's average Particle Size (PS), Polydispersity Index (PDI) and electrokinetic potential. To avoid particle obstruction, the prescribed amount of formulation was re-dispersed in a large volume of Milli-Q water and vortexed for 5 min.[12] Following that, the final sample was tested in triplicate at 25°C for 1 min.

Analysis of Zeta potential

A zeta potential investigation was performed to evaluate the nanoparticles' stability. The main forces that cause repulsion between nearby particles are electrostatic charges and the zeta potential measures their impact. Depending on the strength of the forces involved, either attraction or repulsion will occur. When positive and negative charges collide, they attract each other, resulting in a force that pulls them together. The link between zeta potential values and the nanoparticle reactions is described by the thumb rule. The surface charge was determined using Dynamic Light Scattering (DLS) from Malvern Instruments. Electrophoretic light scattering was conducted at 150 V and 25°C to collect the data.

Scanning Electron Microscopy (SEM analysis)

The morphology of the formulation was examined using a Scanning Electron Microscope (SEM) (Philips XL 30 microscope, Hillsboro, USA). After adding a 30 nm layer of gold to the powder sample using double-sided tape, it was placed under a 2 min vacuum (10-6 Pa) and SEM observations were performed at a 15 kV accelerating voltage with the magnification of 5 μm.[13] The image was captured using a soft imaging viewer.

FTIR analysis

The drug's compatibility FTIR spectra were collected in the 4000-400 cm^{-1} range using a spectrophotometer. A pellet of potassium bromide was utilized. Five tons of pressure were applied for 5 min after mixing the drug sample with dry potassium bromide.[14] IR spectrophotometer (Perkin Elmer Instruments, North Billerica, MA, USA) was used to observe the Fourier Transform Infrared (FTIR) spectra at room temperature. Qualitative measurements were taken to compare and analyze the peak patterns.

Phytochemical identification of nanoformulation

The prepared nanoformulation of the plant extract was screened for identifying metabolites like flavonoids, steroids, saponins, tannins, phenolic compounds, alkaloids, etc., using standard protocols.

Cell culture

L6 myotubes and 3T3-L1 preadipocytes were procured from the National Centre for Cell Science, Pune. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and antibiotics: 120 units/mL penicillin, 75 µg/mL streptomycin, 160 µg/mL gentamycin and 3 µg/mL amphotericin B. The culture conditions included incubation at 37°C in a 5% carbon dioxide atmosphere. For differentiation, L6 myoblasts were transferred to DMEM with 2% FBS for four days post-confluence, with differentiation assessed by the presence of multinucleated cells. 3T3-L1 preadipocytes were grown in 24-well plates until two days post-confluence and then induced to differentiate using a medium containing 0.5 mM IBMX, 0.25 µM DEX and 1 mg/mL insulin in DMEM with 10% FBS. Three days after induction, the differentiation medium was replaced with a medium containing 1 mg/mL insulin alone. After an additional two days, the medium was replaced again with fresh DMEM containing 10% FBS and differentiation was confirmed by observing the formation of multinucleated cells.

Cytotoxicity assay

A colorimetric method for determining cell metabolic activity is the MTT assay,[15] colorimetric technique called the MTT assay by assessing the functionality of the mitochondrial succinate dehydrogenase enzyme is used to determine cellular metabolic activity. This technique, initially described by T. Mosmann in

Table 1: Physicochemical properties of prepared nano-formulations.

Sl. No.	Formulation	Particle Size (nm)	Zeta Potential (mV)	PDI
1.	PA-1	100	-10.5	0.45
2.	PA-2	154	-12.8	0.56
3.	PA-3	124	-14.6	1.4
4.	PA-4	224	-15.8	1.6
5.	PA-5*	252	- 40.5	0.04
6.	PA-6	485	-22.8	1.1
7.	PA-7	1318	-15.9	2.2
8.	PA-8	1254	-18.2	1.8
9.	PA-9	1485	-19.5	1.6
10.	Blank Nanoparticles	--	-32.5	2.2

Table 2: Optimization.

Sl. No.	Formulation	PA extract concentration	Gelatin Concentration
1	PA-1	1%	1%
2	PA-2	1.5 %	1%
3	PA-3	2 %	1%
4	PA-4	1%	2%
5	PA-5	1.5 %	2%
6	PA-6	2 %	2%
7	PA-7	1%	3%
8	PA-8	1.5 %	3%
9	PA-9	2 %	3%
10	BNP (Blank Nanoparticles)	--	2%

1983, involves the process by which the enzyme mitochondrial succinate dehydrogenase transforms a yellow tetrazole molecule soluble in water into an insoluble violet formazan crystal. The cytotoxicity study relies on the premise that cells exposed to toxic substances undergo cell death, with the extent of cell death reliant on the test compound's toxicity level. *In vitro*, cell viability and cytotoxicity assays using cultured cells are commonly employed to test the toxicity of chemicals and screen drugs. The use of these assays has been growing in popularity in recent years.[16] In simpler terms, a more poisonous test compound will result in a higher rate of cell death. To measure and understand cell death, certain chemical compounds are used to distinguish between living and dead cells effectively. One such compound is Tetrazolium bromide (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl). Here, tetrazolium bromide salt is used to measure cytotoxicity. The activity is founded on the action of the mitochondria in cells. Succinate dehydrogenase, an enzyme found in the mitochondria of living cells, transforms the tetrazolium salt into formazan, a blue-colored substance. Live cells contain mitochondrial succinate dehydrogenase enzymes that catalyze the conversion of MTT, which means the number of viable cells is directly correlated with the amount of formazan produced. Conversely, the MTT assay measures the number of dead cells or their products that do not reduce tetrazolium. In the current study, the cytotoxicity of *Plectranthus amboinicus* nanoparticles was evaluated against 3T3-L1 and L6 myoblast cells. Results indicated that as the dose of nanoparticles increased, cell viability declined. The IC_{50} value was determined to be greater than 1000 µg/mL, indicating a relatively low level of cytotoxicity at higher concentrations. Comparatively, previous studies have reported IC_{50} values for various nanoparticle formulations and other compounds affecting 3T3-L1 and L6 myoblast cells. For instance, certain plant-derived nanoparticles demonstrated IC_{50} values ranging from 500 to 800 µg/mL, indicating stronger cytotoxic effects at lower concentrations. In contrast, the *Plectranthus amboinicus* nanoparticles displayed a safer profile, with no significant detrimental effects observed at concentrations below 500 µg/mL. This suggests that the nanoparticles may be less toxic than other formulations studied, making them a promising candidate for further research in therapeutic applications.

Glucose uptake

A non-radioactive technique was used to measure the glucose absorption activity in differentiated L6 cells, as reported by Pareek *et al*. in 2009. The capacity of the plant extract to promote glucose uptake was evaluated under two different circumstances: insulin-free glucose uptake (extract alone) and insulin-free glucose uptake (extract plus insulin). Differentiated cells were subjected to a 5 hr serum starvation period for this experiment. Following that, they were treated for 30 min at 37°C with different non-toxic quantities of the extract, nanoparticles, a reference medication and extra glucose (1 M). After that, the cells were either given 10 nM insulin to activate them or left untreated for 20 min. The cells were washed three times with cold water. KRP buffer solution stopped the glucose absorption process. After that, three cycles of freezing and thawing were used to lyse the cells. To ascertain the glucose content linked to the cells, an aliquot of the cell lysates was utilized. The most widely used oral anti-diabetic drugs on the market today hardly ever offer sustained glycaemic control. To bridge the gap, some medicinal plant extracts are used as anti-diabetic medications since they are thought to be beneficial in lowering blood glucose levels.[16]

Antiadipogenic analysis

The process of changing different cell types into adipocytes-specialized fat cells that store triglycerides as lipid droplets-is known as adipogenesis. Oil Red O staining can be used to assess the amount of lipid droplets. 3T3-L1 preadipocytes were cultured in DMEM with 10% FBS and antibiotics to promote adipogenesis. On day 0, the 3T3-L1 preadipocytes were treated with a mixture of IBMX, DEX and insulin to initiate differentiation. On day five, the differentiation medium was changed to 10% FBS-DMEM with 1 mg/l insulin after a 72 hr induction phase. The fresh culture material was then added and left for 48 hr (Day 7). Different doses of PA and GPAN were added starting on day 0 and continued throughout the induction and post-induction stages to evaluate the degree of cellular differentiation. The concentrations ranged from 15 µg/mL to 250 µg/mL. On the other hand, some preadipocytes were kept alive for the duration of the induction phase in fresh FBS-DMEM. To assess the level of differentiation, an Oil Red O staining experiment was conducted after the induction period. In addition, a comparison of the triglyceride buildup under various treatment settings was done using a photo-microscopic analysis. The GOD-POD method was used to calculate the difference between the initial and final glucose levels in the incubated media to evaluate glucose uptake. To be precise, 10 µL of the sample was mixed with 1 mL of reagent and incubated for 10 min at 37°C. The absorbance of the sample and standard was measured at 510 nm for 60 min, with a reagent blank as a reference.[17] For accurate comparisons, the standard, control and sample must all have the same time between sample addition and measurement.

Oil-red-O staining

The 3T3-L1 adipocytes were rinsed twice with phosphate-buffered saline (pH 7.4). The cells were then fixed for 30 min in a 10% formalin solution before being washed with deionized water. The cells were then stained with Oil Red O, a 0.25% w/v solution in 60% isopropanol and left at room temperature for 30 min. The dye was subsequently removed using isopropanol and the amount of dye retained in the 3T3-L1 cells was determined by measuring absorbance at 540 nm with a microplate reader, as Huang (2006) stated.

Statistical analysis

The sample findings were provided as ±SEM (Standard Error Mean) and statistical significance was assessed using an ANNOVA test Dunnett's test and $p<0.0001$.

RESULTS AND DISCUSSION

Since they are more versatile and perform better than their parent materials, Nanoparticles (NPs) are essential to technological advancements. Traditionally, the synthesis of NPs involves the use of hazardous reducing agents to reduce metal ions into uncharged nanoparticles. But lately, the emphasis has been on creating green technology, which produces NP using natural resources rather than hazardous chemicals. Because biological processes are safe, economical, clean, eco-friendly and extremely productive, they are used in green synthesis to synthesize natural polymers. Gelatin has long been utilized as an encapsulant in the pharmaceutical and biomedical industries because of its low cost, wide availability, biocompatibility and degradability. *Plectranthus amboinicous* nanoparticles were easily, quickly and affordably manufactured. They were also tested for toxicity and shown to have anti-diabetic properties.

Characterisation

The PA-5 formulation was the most optimized of all the formulations and demonstrated the required PDI, zeta potential and particle size. The PA-5 formulation was further investigated for a variety of evaluation parameters. The highest particle size determined may be due to the increased viscosity of the solution of the polymer. The authors have reported that due to the high concentration of the polymer, the particles can aggregate. The zeta potential lies between -40.5 as shown in Figure 1 indicating the stability of nanoparticles. Particle size 252 nm, combined with a high zeta potential (-40.5 mV), suggests good colloidal stability.[9]

SEM analysis: Surface morphology analysis of nanoparticles involves examining the shape, size, texture and surface features of the particles. The gelatin-conjugated drug showed a spherical shape as shown in Figure 2.

FTIR: The FT-IR spectra of PA leaf extract's and PA 5 formulation, revealed distinct bands at 3448 cm^{-1} (attributed to -NH stretching of secondary amide or O-H stretching of alcohol or phenol groups), 2093 cm-1 (attributed to O-H stretching of carboxylic groups, H-bonded), 1638 cm^{-1}(attributed to C=O stretching vibration of aromatic ketones or carboxyl groups) and 1080 cm^{-1}

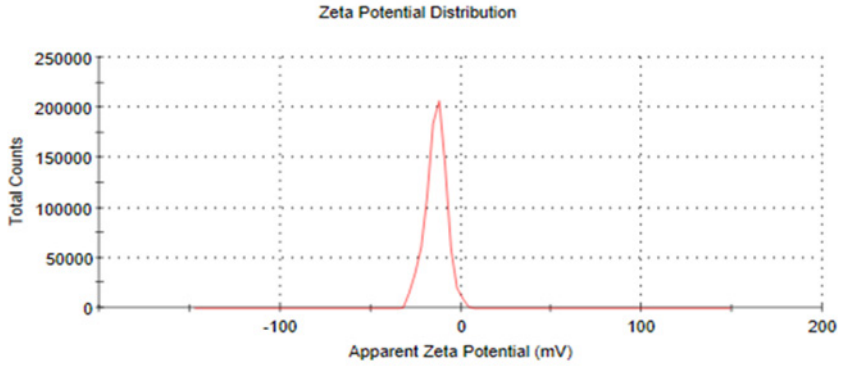

Figure 1: Zeta potential of *Plectranthus amboinicous* nanoparticles.

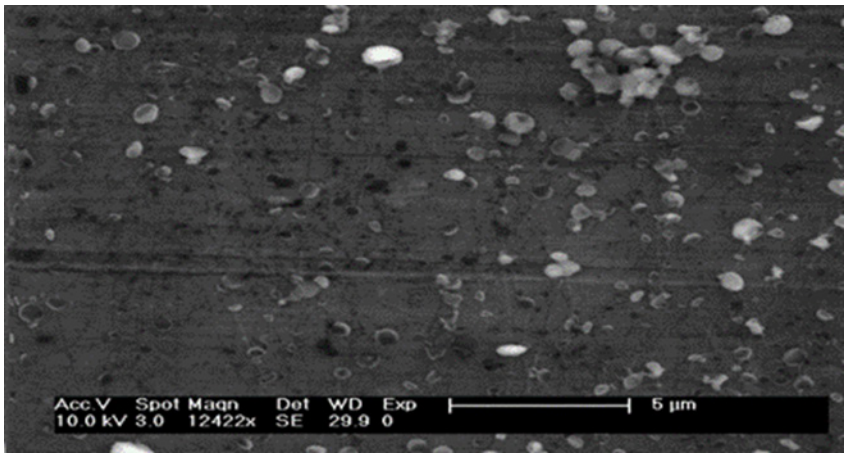

Figure 2: Scanning electron micrograph of GPAN.

(attributed to C-OH stretching of secondary alcohols or primary aliphatic amines) as shown in the Figures 3 and 4.

Phytochemical evaluation of *Plectranthus amboinicous* nanoparticles: The results indicated the constituents present in the extract are not degraded during the preparation of nanoformulation.

Cytotoxicity

The MTT assay relies on the number of dead cells or their products that do not reduce tetrazolium. The current work investigated the cytotoxicity of *Plectranthus amboinicous* nanoparticles against 3T3L1 and L6 myoblast cells. As the dose increased, cell viability decreased, resulting in an IC_{50} value greater than 1000 µg/mL as shown in the Figure 5. The ideal concentration was less than 500 µg/mL, as there were no detrimental effects.

Antidiabetic activity

Chronic overnutrition and a lack of physical exercise led to increased adipose tissue deposition and insulin resistance, both of which play important roles in the pathogenesis of type 2

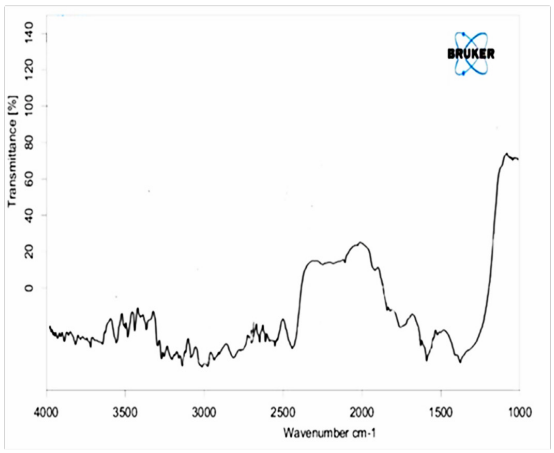

Figure 3: FTIR spectra of PA extract.

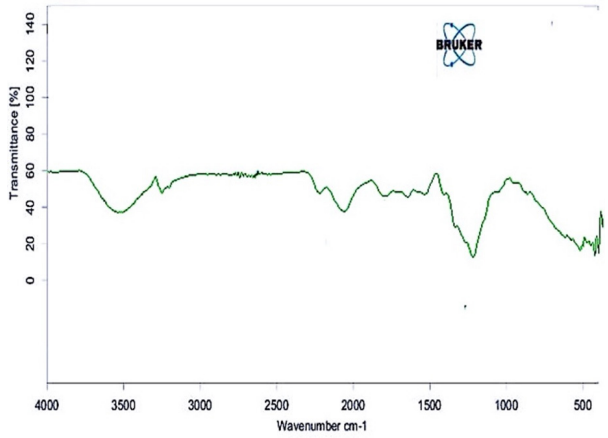

Figure 4: FTIR spectra of PA-5 formulation.

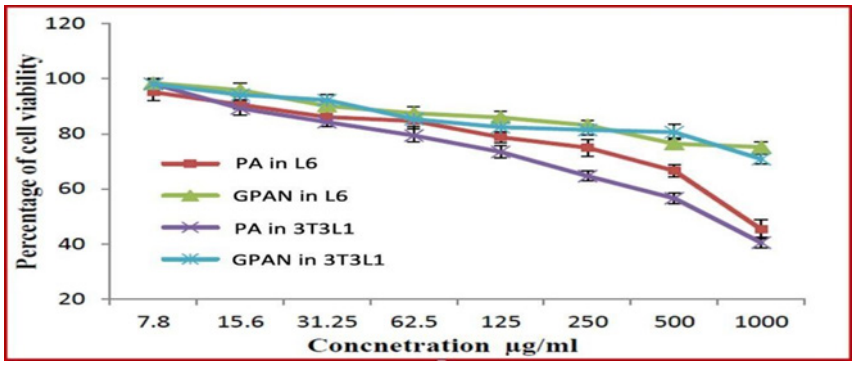

Figure 5: MTT assay results of PA and GPAN in L6 Myotubes and 3T3 L1 preadipocytes.

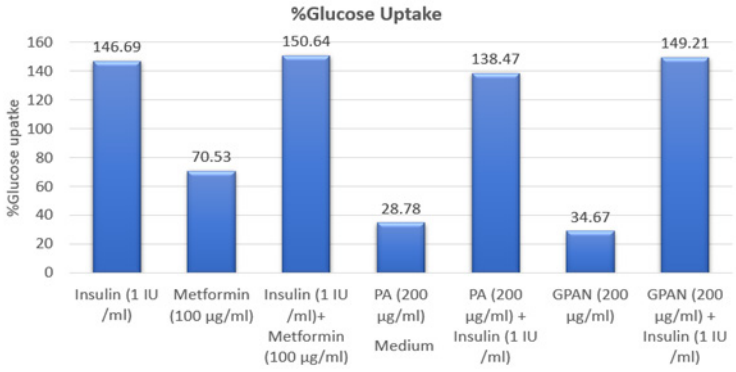

Figure 6: Basal Glucose uptake assay of PA and GPAN in L6 myoblasts.

diabetes and cardiovascular diseases.[9] In the current study, the plant extract and produced nanoparticles exhibit an insulin-like effect, which plays a crucial role in glucose homeostasis. After incubation, the nanoparticles greatly improved glucose absorption in a dose-dependent manner compared to normal insulin and Metformin. The effect is caused by different receptors found on L6 skeletal muscles. The accumulation of lipids and acquired metabolic activity promotes glucose uptake in response to fatty acid production, insulin and triglyceride accumulation. Phytochemicals are secondary chemical metabolites that occur naturally in fruits, vegetables and herbs. They have various structures and have been shown to improve human health.[13] *Plectranthus amboinicus* extract and produced nanoparticles can minimize fat buildup as shown in the Figure 6. The presence of polyphenols, which influence endogenous antioxidant systems via multiple mechanisms, may explain the anti-diabetic effect of *Plectranthus amboinicous* nanoparticles.

CONCLUSION

The present study investigated the anti-adipogenic and glucose-lowering abilities of the herbal extract. The extract's various phytochemicals possess antioxidant, anti-inflammatory and anti-hyperglycemic properties, which can potentially prevent diabetic complications. The extract and gelatin-conjugated nanoparticles exhibit their diabetic properties. The significant enhancement of glucose uptake by PA and GPAN in L6 myoblasts suggests their potential for amelioration. GPAN's powerful inhibition of key enzyme activities involved in carbohydrate digestion offers a mechanism for their antihyperglycemic actions, which could be investigated further in future research. Our data support the idea that PA and GPAN have potential as supplementary or alternative therapies for diabetic control. More research is needed to better understand the underlying mechanisms of action, optimize formulation and delivery techniques and evaluate efficacy and safety in clinical settings. With further research, PA and GPAN may make essential contributions to the arsenal of antidiabetic drugs, opening up new paths for enhancing glycemic control and lowering the burden of diabetes-related comorbidities.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this research.

ABBREVIATIONS

T2DM: Type 2 Diabetes Mellitus; **FFAs:** Free Fatty Acids; **GLUT4:** Glucose Transporter Type 4; **GSV:** GLUT4 Storage Vesicles; **PA:** *Plectranthus amboinicus*; **GPAN:** *Gelatin-Plectranthus amboinicus* Nanoparticles; **FTIR:** Fourier Transform Infrared Spectroscopy; **nm:** Nanometres; **µg/mL:** Micrograms per Milliliter; **mV:** Millivolts.

SUMMARY

The research explores the antidiabetic effects of nanoparticles made from *Plectranthus amboinicus* (PA) and conjugated with Gelatin (GPAN). *Plectranthus amboinicus*, a member of the Lamiaceae family, is used in traditional medicine and cooking. The GPAN nanoparticles were synthesized through a solvent evaporation technique, resulting in particles approximately 250 nm in diameter with a spherical shape. Toxicity tests indicated that the nanoparticles were non-toxic up to a concentration of 1000 µg/mL.

The GPAN formulation was assessed for its impact on glucose metabolism using L6 myoblast cells and 3T3-L1 adipocytes. The findings revealed that GPAN significantly increased glucose uptake in L6 cells and reduced fat accumulation in 3T3-L1 cells, suggesting enhanced glucose metabolism and potential antidiabetic effects. The formulation exhibited a high zeta potential of -40.5 mV, indicating good stability and FTIR analysis primarily provides information about the functional groups and chemical bonds present in a sample, which helps assess the compatibility of compounds within a formulation. FTIR analysis confirmed the retention of these bioactive compounds in the nanoparticle formulation. The study concluded that GPAN holds potential as a therapeutic agent for diabetes, demonstrating effective glucose uptake enhancement and a favorable safety profile. Further research is needed to refine the formulation and assess its clinical efficacy.

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