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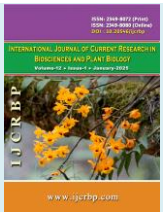

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Green Synthesized Calcium Nanoparticles of *Momordica cymbalaria* to Enhance Antidiabetic and Antioxidant Activity in *Drosophila melanogaster*

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Abstract

The global prevalence of Type 2 diabetes mellitus (T2DM) necessitates the exploration of safer, more effective therapies. This study investigates the antidiabetic potential of *Momordica cymbalaria* Hook. F. by leveraging green nanotechnology and an in vivo *Drosophila melanogaster* model. Calcium nanoparticles (CaNPs) were synthesized using methanolic extracts of *M. cymbalaria* fruit and root via an eco-friendly ionic precipitation method. EDAX analysis confirmed the elemental composition of Ca, O, and C, validating nanoparticle formation. Biochemical and behavioural assays were conducted on wild-type and vestigial mutant *Drosophila* strains subjected to a high-sucrose diet to induce diabetic phenotypes. Treated groups received either crude plant extracts or CaNP formulations. Results showed a significant improvement in locomotor performance, climbing assay, and marked restoration of antioxidant defenses, catalase activity. Diabetic flies exhibited elevated nitric oxide and glucose levels, which were significantly reduced upon treatment, particularly with seed crude extracts and CaNPs. Among treatments, seed-derived CaNPs demonstrated the most consistent efficacy across physical and biochemical parameters. The study highlights the therapeutic synergy between phytochemicals and nanotechnology. It also validates *Drosophila* as a robust model for screening traditional plant-based antidiabetic interventions. Findings support the integration of green-engineered plant nanoparticles in managing metabolic disorders and promote *M. cymbalaria* as a promising plant resource for future nanomedicine development.

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Introduction

The global rise in non-communicable diseases is a direct consequence of modern lifestyle transformations. A shift toward sedentary routines, nutrient-poor diets, reduced physical activity, and heightened psychological stress has fueled the prevalence of metabolic syndromes, with diabetes mellitus at the forefront (Whiting *et al.*, 2011). Alarmingly, this metabolic disorder is no longer confined to older adults but is increasingly affecting youth, suggesting a multifactorial etiology rooted in environmental and behavioral patterns (Zimmet *et al.*, 2001). Among the types of diabetes, Type 2 diabetes mellitus (T2DM) dominates in

incidence, attributed to insulin resistance and impaired pancreatic β-cell function. Its chronic nature predisposes individuals to complications involving the cardiovascular, renal, and nervous systems. Although pharmacotherapies including metformin, sulfonylureas, and insulin analogs have improved disease management, they often entail adverse effects, reduced patient compliance, and limited efficacy over time (Nathan, 2015). This has encouraged exploration into alternative and complementary approaches that are safer and more sustainable in the long term.

Experimental models are vital in understanding disease mechanisms and evaluating new therapeutics. While rodent models remain the cornerstone for metabolic disease research, invertebrate systems, such as *Drosophila melanogaster*, have emerged as valuable platforms due to their genetic tractability, conserved metabolic pathways, and cost-effectiveness (Galikova & Klepsatel, 2018). Approximately 75% of human disease-related genes have orthologs in *Drosophila*, and the insulin-like signaling pathways regulating carbohydrate metabolism are functionally conserved (Teleman, 2009). Moreover, their short life cycle and high fecundity allow for rapid, large-scale screening of candidate compounds.

The fruit fly has been successfully employed in various diabetic phenotypes, including hyperglycaemia, obesity, insulin resistance, and oxidative stress (Musselman *et al.,* 2011). This positions *Drosophila* as an effective tool not only in molecular genetic studies but also in pharmacodynamic testing of bioactive compounds, including those derived from traditional medicines.

Traditional systems of medicine, particularly Ayurveda, offer a holistic perspective in managing chronic disorders like diabetes. Ayurvedic formulations often employ polyherbal mixtures rich in bioactive phytochemicals that modulate metabolism, insulin sensitivity, and oxidative stress (Sharma *et al.,* 2020). However, the scientific validation of these formulations and their mechanisms remains insufficiently explored. Bridging this gap, *Drosophila* provides an efficient, scalable system for validating Ayurvedic treatments in a genetically controlled environment, offering insights into molecular pathways and therapeutic efficacy (Jayaraj *et al.,* 2018).

One such promising medicinal plant is *Momordica cymbalaria* Hook. f., a member of the Cucurbitaceae family and native to India. Traditionally used in South Indian folk medicine, it is recognized for its applications in treating metabolic disorders, including diabetes, constipation, and liver ailments. While it shares taxonomic similarities with the well-studied *Momordica charantia*, *M. cymbalaria* boasts a unique phytochemical profile. It is particularly rich in flavonoids, saponins, phenolic acids, and triterpenoid compounds, which are known to exert hypoglycaemic and antioxidant effects (Rani *et al.,* 2010; Venkatesh *et al.,* 2012).

Pharmacognostic studies have revealed that the roots and fruits of *M. cymbalaria* possess a higher concentration of bioactive agents than their aerial parts. These include charantin, gallic acid, quercetin, and linolenic acid—all of which are associated with blood glucose regulation, lipid metabolism, and free radical scavenging (Bhat *et al.,* 2018). Furthermore, it contains essential nutrients such as calcium, iron, potassium, and vitamin C, which contribute to its therapeutic value. Compared to *M. charantia*, *M. cymbalaria* exhibits superior mineral content, particularly in calcium and trace elements such as zinc and manganese, which are crucial in insulin signaling pathways (Basalingappa *et al.,* 2019).

In recent years, nanotechnology has gained momentum in enhancing the pharmacological performance of plant-based therapeutics. Green synthesis of nanoparticles using plant extracts is not only eco-friendly but also enhances drug bioavailability, stability, and targeted delivery (Iravani, 2011). Incorporating *M. cymbalaria* extract into calcium nanoparticle formulations represents a novel approach to improve its therapeutic efficacy. This study thus aims to assess the antidiabetic efficacy of *Momordica cymbalaria*-derived calcium nanoparticles using *Drosophila melanogaster* as a model system. It seeks to correlate phytochemical richness with therapeutic outcomes while leveraging the genetic power of fruit flies to uncover conserved molecular mechanisms of action.

Biosynthesis of Calcium Nanoparticles from *Momordica cymbalaria* Extract

The application of green nanotechnology in synthesizing metal nanoparticles has gained considerable traction due to its environmentally sustainable and biologically compatible nature. Unlike conventional physical or chemical methods, which often

require toxic solvents and high energy inputs, green synthesis employs plant-derived phytochemicals that serve as both reducing and capping agents (Ahmed *et al.,* 2016).

These secondary metabolites—such as flavonoids, phenols, terpenoids, and alkaloids—facilitate the reduction of metal ions into stable nanoparticles while enhancing their biological activity (Mittal *et al.,* 2013). In this study, *Momordica cymbalaria*, a medicinally important plant known for its antidiabetic and antioxidant properties, was used as a biological template for the synthesis of calcium nanoparticles (CaNPs).

Materials and Methods

Collection and Authentication of Plant Material

Whole plants of *Momordica cymbalaria* Hook. F. was collected during the early flowering stage from agricultural zones near Raketla village, Uravakonda Mandal, Anantapur district, Andhra Pradesh, India. A certified taxonomist authenticated the botanical identity of the specimen, and a voucher specimen was deposited in the institutional herbarium for future reference (Specimen Code: MC-2025-RA01).

All parts of the plant (leaves, fruits, and roots) were washed under running tap water and then rinsed with distilled water. The material was shade-dried at room temperature (28 ± 2°C) for 10–12 days and ground into a fine powder using a high-speed pulverizer. The powder was stored in air-tight amber glass containers at 4°C until further use (Nayak *et al.,* 2010).

Preparation of Plant Extracts

Two solvent-based extraction methods were employed to isolate phytoconstituents with reducing potential.

Soxhlet Extraction

Approximately 50 g of dried plant powder (fruit/root) was extracted using 500 mL of ethanol (95%) in a Soxhlet apparatus for 8 hours. The extract was concentrated under reduced pressure using a rotary vacuum evaporator at 45°C.

The resulting residue was saponified using 0.1 N alcoholic KOH for 30 minutes. The saponified mass was partitioned twice with diethyl ether to isolate the organic phase. This layer was subjected to centrifugation at 4000 rpm for 5 minutes, and the supernatant was collected as the ethanolic extract (Patel *et al.,* 2012).

Cold Maceration Method

In a parallel extraction, 10 g of plant powder was macerated with 100 mL of methanol in a sterile conical flask.

The mixture was agitated intermittently for 6 hours at room temperature, followed by 24 hours of static maceration.

The solvent layer was filtered through Whatman No.1 filter paper. The filtrate was used directly in nanoparticle synthesis (Sasidharan *et al.,* 2011).

Green Synthesis of Calcium Nanoparticles (CaNPs)

Aqueous plant extracts were used for the green synthesis of calcium nanoparticles following a modified ionic precipitation method (Anandalakshmi *et al.,* 2016). Briefly presented the methodology that includes, A total of 50 mL of 0.05 M calcium chloride ($CaCl_2$) solution was added dropwise to 25 mL of either the fruit or root extract under constant stirring. The pH of the resulting mixture was adjusted to 8.5 using 1 M ammonium hydroxide (NH_4OH), and the solution was continuously stirred for 2 hours at room temperature using magnetic stirrers. A visible color change was observed, indicating the formation of calcium nanoparticles (CaNPs). The reaction mixture was then centrifuged at 10,000 rpm for 20 minutes, and the resulting pellet containing CaNPs was washed three times with deionized water and dried at 60 °C in a hot-air oven.

The dried nanoparticles were stored in a desiccator until further use (Nawaz *et al.,* 2020).

Characterization of Synthesized Nanoparticles

The synthesized calcium nanoparticles were subjected to preliminary characterization:

Centrifugation and Recovery: The reaction mixtures were centrifuged at 8000 rpm for 30 minutes to isolate the nanoparticle pellets.

Elemental Composition Analysis Using Energy Dispersive X-ray Spectroscopy (EDS):

In the context of calcium nanoparticle synthesis using plant extracts, EDS can validate the presence of major components, such as calcium (Ca), oxygen (O), and carbon (C), and ensure the absence of unwanted metallic or environmental contaminants (Balasubramanian *et al.,* 2022). This technique thus serves as a powerful, non-destructive tool for confirming the successful synthesis and purity of green-engineered nanoparticles.

The dried CaNPs were analyzed using Energy Dispersive X-ray Analysis (EDAX) to confirm the presence and purity of calcium. This technique enabled elemental mapping and determination of atomic and weight percentages of Ca, O, and C in the samples (Pandey *et al.,* 2018).

Confirmatory In Vivo Studies on Animal Models

Drosophila melanogaster as a Model Organism

The fruit fly, *Drosophila melanogaster*, has emerged as a powerful model for studying human diseases, including metabolic disorders such as diabetes and obesity. Its utility stems from its well-characterized genome, short generation time (~10 days), ease of genetic manipulation, and conservation of major signaling pathways, including the insulin/IGF signaling (IIS) pathway (Baker & Thummel, 2007). Notably, approximately 75% of human disease-related genes have orthologs in *Drosophila*, making it a translationally relevant system for functional genomics and pharmacological studies (Pandey & Nichols, 2011).In the present study, two *D. melanogaster* strains were employed:

Canton-S (CS) Wild-type strain, representing healthy baseline physiology.

Vestigial (vg) mutant strain, commonly used in metabolic and oxidative stress studies due to their genetic variability and morphological traits.

Both strains were kindly provided by Dr. Gurudatta Baraka's laboratory at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India. The flies were maintained under controlled laboratory conditions to ensure reproducibility and minimize environmental stressors.

Experimental Setup

All fly stocks were cultured on a standard Drosophila diet comprising cornmeal, agar, yeast, and sucrose. To prevent microbial contamination, the medium was supplemented with an ethanolic solution of *p*-hydroxybenzoic acid methyl ester (methyl paraben) and propionic acid. Flies were reared under ambient laboratory conditions (20–25°C) with a relative humidity of 60–70%, maintained on a 12-h light/dark cycle (Shen *et al.,* 2010).

Before experimentation, adult flies (3–5 days old) were separated by sex, anesthetized briefly using CO_2, and starved for 22–24 hours (provided access to water only) to normalize their feeding behaviour. This starvation protocol is a standard pre-treatment in food intake and metabolic assays to enhance experimental sensitivity (Wong *et al.,* 2009).

Experimental Group Design

To evaluate the therapeutic efficacy of *Momordica cymbalaria* extracts and its calcium nanoparticle (CaNPs) formulation under diabetic conditions, flies were divided into four experimental groups:

Normal Control (NC)

Flies were fed with standard Drosophila diet to represent baseline metabolic activity.

Diabetic Control (DC)

Flies were administered a high-sugar diet (HSD) containing 30% sucrose to induce hyperglycemic conditions, which mimic Type 2 diabetes-like phenotypes such as insulin resistance, increased lipid storage, and oxidative stress (Musselman *et al.,* 2011).

Extract-Treated Group (ET)

Diabetic flies were fed a high-sugar diet supplemented with methanolic extract of *M. cymbalaria* at a concentration standardized from preliminary toxicity assays.

CaNPs-Treated Group (NT)

Diabetic flies were fed with a high-sugar diet supplemented with green-synthesized calcium nanoparticles derived from *M. cymbalaria* extracts.

All treatments were administered for 7–10 days, during which survival, behavioural responses, and metabolic parameters (glucose levels, catalase activity, lipid peroxidation) were evaluated in subsequent assays.

Parameters Assessed

Climbing Assay (Negative Geotaxis Test)

The negative geotaxis assay is a standard behavioural test used to assess locomotor performance and neuromuscular integrity in *Drosophila melanogaster*. Healthy flies exhibit an innate tendency to climb against gravity (negative geotaxis) when startled, while those with metabolic or neurological impairments show diminished climbing ability. This assay is particularly useful for evaluating the progression of diabetes-induced physical deterioration and for assessing the therapeutic effects of pharmacological interventions (Gargano *et al.*, 2005).

To investigate the effects of dietary interventions, including standard, high-sucrose, and treatment-enriched diets, flies were subjected to a 15-day exposure period, after which their climbing performance was measured. Ten adult flies from each group were gently transferred into an empty, transparent climbing vial (length: 25 cm). The vial was gently tapped three times against a flat surface to bring all flies to the bottom.

After 12 seconds, a photograph was captured to document the vertical position of the flies. This procedure was repeated 10 times per group to ensure accuracy and account for behavioural variability.

A rest interval of 120 seconds was provided between each trial to minimize fatigue-related bias.

To quantify locomotor performance, the vial was visually divided into five equal horizontal sections (each 5 cm in height), numbered from 0 (bottom) to 4 (top). Each fly's position in the tube was scored based on the section it occupied.

$$\mathrm{CI} = \frac{\sum_{i=0}^{4}(n_i \times i)}{N}$$

The Climbing Index (CI) was calculated using the formula:

Where:

n_i = number of flies in section *i*

i = score assigned to that section (0 to 4)

N = total number of flies (usually 10)

A higher CI reflects better climbing ability and neuromuscular performance. Comparisons of CI across experimental groups allowed us to assess the degree of locomotor recovery in flies treated with *Momordica cymbalaria* extracts and calcium nanoparticles compared to diabetic controls.

Biochemical Assays

Biochemical assays are analytical techniques used to detect, quantify, and characterize biological molecules and their activities within cells, tissues, or organisms. These assays are essential tools in biomedical research, enabling scientists to evaluate metabolic functions, enzyme kinetics, oxidative stress responses, and cellular signaling pathways. In the context of diabetes research, biochemical assays play a pivotal role in assessing biomarkers of glucose metabolism, antioxidant defense, and inflammation, thereby providing insight into the pathophysiology and therapeutic responses to antidiabetic treatments.

In *Drosophila melanogaster*, which shares a high degree of genetic homology with humans, biochemical assays allow for the rapid, cost-effective, and reproducible analysis of physiological changes associated with experimental diabetes and its treatment. These assays can be adapted for whole-fly homogenates, specific tissues (e.g., gut, brain, fat body), or haemolymph samples.

Each biochemical assay operates based on a specific chemical or enzymatic reaction that produces a detectable signal, often a color change, fluorescence, or absorbance, which correlates with the concentration or activity of the target molecule. The principle underlying each assay is tailored to the molecule of interest:

Enzyme Activity Assays

Rely on monitoring the conversion of substrates (such as hydrogen peroxide) into products. The rate of

substrate degradation or product formation is measured spectrophotometrically to determine enzyme activity.

Catalase (CAT) Activity Assay

Catalase is a key antioxidant enzyme that decomposes hydrogen peroxide (H_2O_2) into water and oxygen, protecting cells from oxidative damage.

The assay was performed according to the method described by Aebi (1984), with minor modifications. In the catalase assay, a decrease in absorbance at 240 nm indicates the breakdown of H_2O_2 by catalase, reflecting the antioxidant capacity.

Buffer Preparation: A 50 mM phosphate buffer (pH 7.4) was prepared by dissolving 10.105 g of Na_2HPO_4 and 6.80 g of KH_2PO_4 in 800 mL of MilliQ water. The pH was adjusted to 7.4 using HCl or NaOH, and the volume was made up to 1 L.

H_2O_2 Substrate Solution: 0.75 mL of 30% hydrogen peroxide was diluted in 100 mL of phosphate buffer. This solution was freshly prepared and stored at 4°C until use. 2.0 mL of sample extract was transferred to a quartz cuvette (1-cm path length) and incubated at 25°C for 5 minutes.

Following this, 1.0 mL of the prepared H_2O_2 solution was added. Two blanks were prepared: one with only phosphate buffer, and the other with buffer + H_2O_2 (no enzyme). The decrease in absorbance was recorded at 240 nm using a UV-Vis spectrophotometer.

Calculation

Catalase activity (U/mL) was calculated using the formula:

$$\text{CAT}\ (\text{U/mL}) = \Delta A \times 34.4 \times \text{DF}$$

Where:

ΔA = change in absorbance per minute

34.4 = derived from the molar extinction coefficient of H_2O_2 (43.6 $M^{-1}cm^{-1}$ × dilution factor 0.79)

DF = dilution factor

Metabolite Quantification Assays

Utilize coupled enzymatic reactions. For glucose, glucose oxidase oxidizes glucose to gluconic acid and hydrogen peroxide, which then reacts with a chromogen via peroxidase, forming a coloured product proportional to glucose concentration. Absorbance is typically measured at wavelengths between 505 and 540 nm.

Glucose Oxidase–Peroxidase (GOD–POD) Assay

The GOD-POD method is a reliable enzymatic assay used to quantify glucose concentration. The reaction is based on the oxidation of glucose by glucose oxidase, resulting in the formation of gluconic acid and hydrogen peroxide. The peroxidase enzyme then catalyzes the reaction of H_2O_2 with a chromogen to produce a colored compound, which can be measured by spectrophotometry.

To perform the GOD-POD assay using a standard kit, first prepare the working reagent according to the kit instructions. Label test tubes or microplate wells for the blank, glucose standard, and test samples. Add 1.0 mL of GOD-POD reagent to each test tube (or 200 µL per well in a microplate), followed by 10 µL of the sample, glucose standard, or distilled water (for the blank). Mix well and incubate at 37°C for 10–15 minutes or at room temperature for 20–30 minutes. After incubation, measure the absorbance at 505 nm or 540 nm using a spectrophotometer, ensuring the blank is used for baseline correction. The glucose concentration is calculated using the formula:

$$\text{Glucose}\ (\text{mg/dL}) = \left(\frac{\text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Standard}}}\right) \times \text{Standard Concentration}$$

Nitric Oxide (NO) AssayDetection

Nitric Oxide (NO) Nitric oxide (NO) levels, indirectly measured as nitrite, were estimated using the Griess reagent method, a standard colorimetric assay (Green *et al.*, 1982).

Reagent Preparation

The Griess reagent was prepared as a 1:1 mixture of1% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride (NED).1.0

mL of sample supernatant was mixed with 1.0 mL of Griess reagent and incubated at room temperature (25°C) for 20 minutes. The Griess reaction forms a diazonium compound that reacts with NED to form a pink azo dye. The resulting pink color intensity was measured at 540 nm using a UV-visible spectrophotometer. The intensity of the color correlates with NO production, an indicator of inflammation or oxidative stress.

Results and Discussion

This investigation explored the antidiabetic benefits of *Momordica cymbalaria* by using an eco-friendly approach to create Calcium (CaNPs) nanoparticles. It has been carefully characterized that these nanoparticles and their effects on Drosophila have been thoroughly investigated, yielding insightful results.

Extraction of Plant Material

The bioactive constituents of *Momordica cymbalaria* were extracted using two conventional phytochemical extraction techniques: Soxhlet extraction and cold maceration. Initially, Soxhlet extraction was performed using methanol as the solvent, targeting the phytochemical-rich leaves, roots, and fruits of the plant. However, as the Soxhlet process involves sustained high temperatures, partial degradation of thermolabile constituents such as flavonoids and phenolic compounds was observed (Azwanida, 2015).

To preserve the integrity and biological activity of these sensitive compounds, a subsequent extraction was conducted using cold maceration at ambient temperature. In this method, dried and powdered plant material was immersed in methanol with intermittent agitation, allowing efficient diffusion of active molecules into the solvent without thermal degradation. Methanol was selected for its high polarity and efficiency in solubilizing a wide range of phytoconstituents, as reported by Khan *et al.*, (2014), who noted enhanced extraction yields of flavonoids, terpenoids, and phenolics in methanol over other solvents. Qualitative phytochemical screening of the resulting extracts revealed the presence of major secondary metabolites, including flavonoids, phenolic compounds, steroids, and terpenoids, across all parts of the plant. These compounds are known for their antioxidant, anti-inflammatory, and potential antidiabetic properties. The results are in accordance with (Alam *et al.*, 2017).

Nanoparticle Synthesis

Synthesis of Calcium Nanoparticles (CaNPs)

The green synthesis of calcium nanoparticles (CaNPs) was carried out using the methanolic extracts of *M. cymbalaria* as both reducing and stabilizing agents. A 50 mL aqueous solution of calcium chloride ($CaCl_2$) (5×10^{-2} M) was gradually added to 25 mL of the plant extract under constant stirring. The pH of the solution was adjusted to 8.5 using 1 M ammonium hydroxide to facilitate alkaline-mediated precipitation. The mixture was stirred continuously at room temperature (27 ± 2°C) for 2–3 hours.

Day 1

Day 3

Fig.1 A& B: Visual observation of the Calcium nanoparticle formation. At the beginning of the reaction (A) and after 3 Days (B).

The formation of calcium carbonate nanoparticles was visually confirmed by a change in solution color to milky white, indicating nanoparticle nucleation and it was shown in Fig:1. The precipitate was separated by centrifugation at 8000 rpm for 30 minutes, washed multiple times with distilled water and ethanol to remove unbound phytochemicals, and dried in a hot air oven to obtain CaNPs in powder form (Rajakumar *et al.*, 2013; Singh *et al.*, 2021). Calcium Nanoparticles (CaNPs): Observed as a cloudy precipitate after reaction with $CaCl_2$ and Na_2CO_3.

This green synthesis method is environmentally friendly, as it avoids the use of toxic chemicals and aligns with the principles of green nanotechnology. Plant-based synthesis offers biocompatibility, cost-effectiveness, and improved therapeutic potential due to the presence of surface-bound phytoconstituents on the nanoparticles, which may enhance their bioavailability and biological effects (Iravani, 2011).

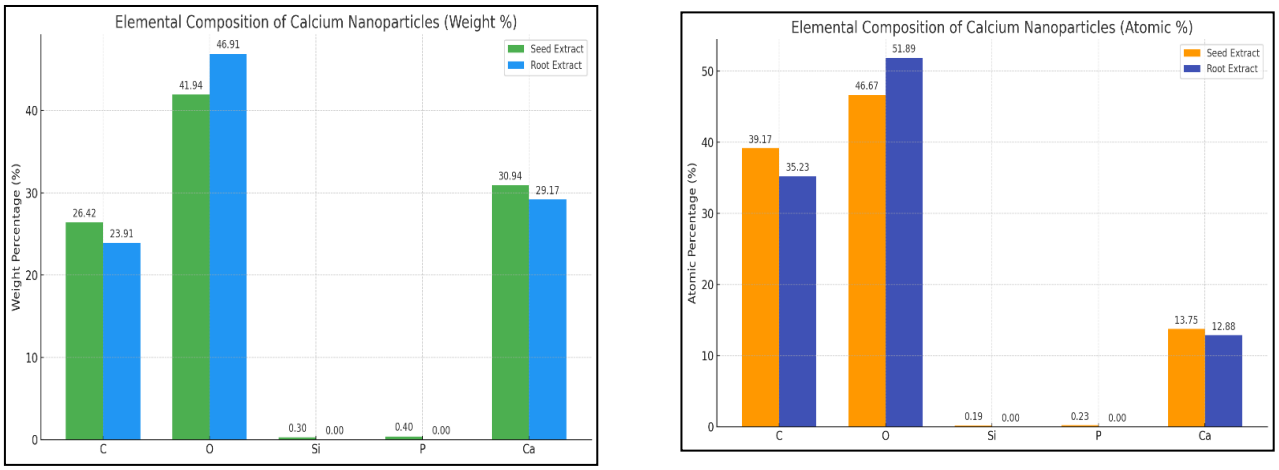
Characterization of Nanoparticles: Energy Dispersive X-ray Spectroscopy Analysis of CaNPs

The elemental composition of the synthesized calcium nanoparticles (CaNPs) was determined using Energy-Dispersive X-ray Spectroscopy (EDAX).

The analysis revealed the presence of carbon (C), oxygen (O), and calcium (Ca) as the major elements, with traces of silicon (Si) and phosphorus (P)also present in the seed-derived nanoparticles. The results are summarized in Table 1.

Table.1 Elemental Composition (%) of CaNPs Synthesized from *M. cymbalaria* Seed and Root Extracts

Element	Atomic% (Seed)	Atomic% (Root)	Weight% (Seed)	Weight% (Root)
C (Carbon)	39.17	35.23	26.42	23.91
O (Oxygen)	46.67	51.89	41.94	46.91
Si (Silicon)	0.19	—	0.30	—
P (Phosphorus)	0.23	—	0.40	—
Ca (Calcium)	13.75	12.88	30.94	29.17



"**Fig.2** Bar chart showing comparative elemental composition (by weight% & Atomic%) of calcium nanoparticles synthesized using seed and root extracts of *Momordica cymbalaria*, based on EDAX analysis."

Calcium (Ca) was confirmed as a major constituent in both samples, affirming the successful synthesis of calcium-based nanoparticles. The high oxygen content supports the formation of calcium oxide or calcium carbonate nanoparticles, often stabilized by oxygen-containing phytochemicals in plant extracts. The carbon content reflects the presence of organic stabilizing agents, likely flavonoids, phenolics, and other phytoconstituents from *the M. cymbalaria extract*, which bind to the nanoparticle surface.

Minor elements (Si and P) were detected only in the seed-derived nanoparticles, possibly due to the presence of trace minerals inherent in seed tissues or contamination during sample preparation.

These results align with previous findings where plant-mediated nanoparticle synthesis typically yields CaNPs with organic surface capping, as shown in Fig. 2, which enhances stability and biocompatibility (Kumar *et al.*, 2015; Choudhury *et al.*, 2020).

In Vivo Antidiabetic Activity Using *Drosophila melanogaster*

Physical Assay

Climbing Assay (Negative Geotaxis Test)

The negative geotaxis (climbing) assay is a widely accepted test to evaluate locomotor activity, neuromuscular coordination, and the overall physiological status of *Drosophila melanogaster*. It serves as a functional readout for aging, neurodegeneration, and metabolic impairments associated with diabetes and oxidative stress (Gargano *et al.*, 2005; Piazza *et al.*, 2009).

In this study, flies were subjected to the climbing assay under ambient laboratory conditions. Briefly, groups of adult flies were gently tapped to the bottom of a clean, vertical plastic cylinder (25 cm height × 2.5 cm diameter), ensuring they began from a uniform starting point. The number of flies that successfully climbed past a 17.5 cm mark within a 2-minute interval was recorded. Each group was tested in triplicate, and the average response was calculated to ensure reproducibility.

Table.2 Climbing Performance of Wild-Type (WT) and Vestigial (VS) Mutant Groups Under Different Dietary Conditions

Climbing Ability	WT Group	VS Group
Normal	4	6
Intermediate	5	4
Diabetic	1	2

The data presented in Table 2 indicate a decline in locomotor function in both WT and VS groups exposed to a high-sucrose diet (Diabetic condition). Under normal dietary conditions, the majority of flies showed robust climbing performance, suggesting preserved neuromuscular function. However, when subjected to a hyperglycemic environment, a marked reduction in climbing ability was observed, reflecting the physiological stress induced by diabetes.

Interestingly, the intermediate (starvation-induced) group displayed moderate performance, lower than usual but better than the diabetic group, as shown in Fig. 3. This pattern suggests that metabolic stress from overnutrition (high sugar) has a more profound impact on fly mobility than nutrient restriction, aligning with previously reported findings (Siddique *et al.*, 2021). Statistical analysis using ANOVA followed by post hoc Tukey's test confirmed that the differences in climbing performance across the three conditions were statistically significant ($p < 0.05$). The consistency of these trends across both WT and VS genotypes reinforces the robustness of the model.

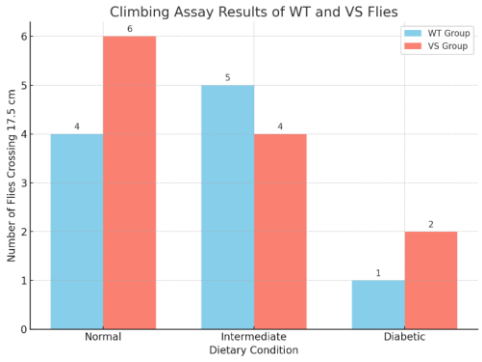


Fig.3 Showing the climbing performance of wild-type (WT) and vestigial (VS) mutant *Drosophila* under normal, intermediate (starved), and diabetic (high-sugar diet) conditions. The graph visually confirms that both genotypes show a significant reduction in locomotor activity under diabetic conditions.

Biochemical Assays

Catalase Assay

The catalase activity (U/mL) was assessed in both wild-type (WT) and mutant (Vestigial, VS)*Drosophila melanogaster* groups across different experimental conditions, including control, diabetic, and treatment groups with seed and root-derived calcium nanoparticles (CaNPs) and crude extracts. As shown in Figure X, diabetic flies exhibited a notable decrease in catalase activity in both WT and VS groups when compared to their respective controls, indicating oxidative stress induced by the high-sugar diet. In the VS mutant group, catalase activity significantly improved in the CaNP-treated groups, with seed-derived CaNPs (yellow) showing activity levels nearly identical to the control group (red), whereas root-derived CaNPs (green) displayed moderate recovery. The crude seed and root extracts (purple and gray, respectively) showed lesser improvement, with root crude extract having the lowest activity level among all treatments.

In the WT group, a similar trend was observed: seed-derived CaNPs restored catalase levels close to normal, while root-derived CaNPs also improved activity but to a lesser extent. Interestingly, the seed crude extract in WT flies exhibited the highest catalase activity overall, surpassing even the control, suggesting a potent antioxidant effect. Root crude extract again showed the lowest performance.

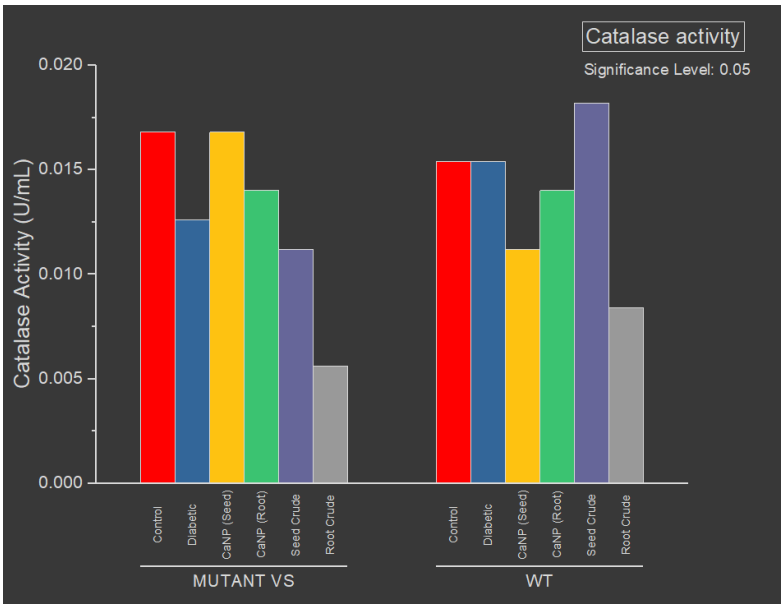


Fig.4 Catalase activity (U/mL) in *Drosophila melanogaster* under diabetic and treatment conditions.

The bar graph (Fig.4) compares catalase enzyme activity in mutant (Vestigial, VS) and wild-type (WT) flies across six experimental groups: Control, Diabetic, CaNP (Seed), CaNP (Root), Seed Crude, and Root Crude. Diabetic groups showed a reduction in catalase activity, indicating oxidative stress.

Treatment with seed-derived calcium nanoparticles (CaNPs) significantly restored catalase levels in both strains, comparable to those of the controls. The WT group treated with seed crude extract exhibited the highest catalase activity overall, suggesting strong antioxidant potential. All differences were statistically significant at $p < 0.05$.

Nitric Oxide (NO) Assay

Nitric oxide levels were assessed in *Drosophila melanogaster* using the Griess reagent method to evaluate oxidative stress in both mutant (VS) and wild-type (WT) strains under control, diabetic, and treatment conditions. As shown in Figure X, diabetic groups in both VS and WT strains exhibited a marked increase in NO levels, with mutant diabetic flies showing the

highest nitric oxide accumulation (~3.4 AU), significantly higher than their respective controls (~0.6 AU), indicating elevated oxidative stress due to hyperglycemia. WT diabetic flies also demonstrated a significant rise (~2.8 AU) compared to controls (~0.8 AU).

Treatment with Calcium Nanoparticles (CaNPs) synthesized from both seed and root extracts of *Momordica cymbalaria* resulted in a notable reduction in NO levels, approaching control values. Among treatments, root crude extracts exhibited the most effective NO suppression in both strains (~1.2–1.3 AU), followed by seed crude and CaNPs.

The results demonstrate that treatment with *M. cymbalaria*-derived extracts and nanoparticles effectively attenuates nitric oxide overproduction induced by diabetic stress. The mutant strain appeared slightly more responsive to treatment than the WT. All results were statistically significant at $p < 0.05$, as indicated in the graph as shown in Fig. 5.

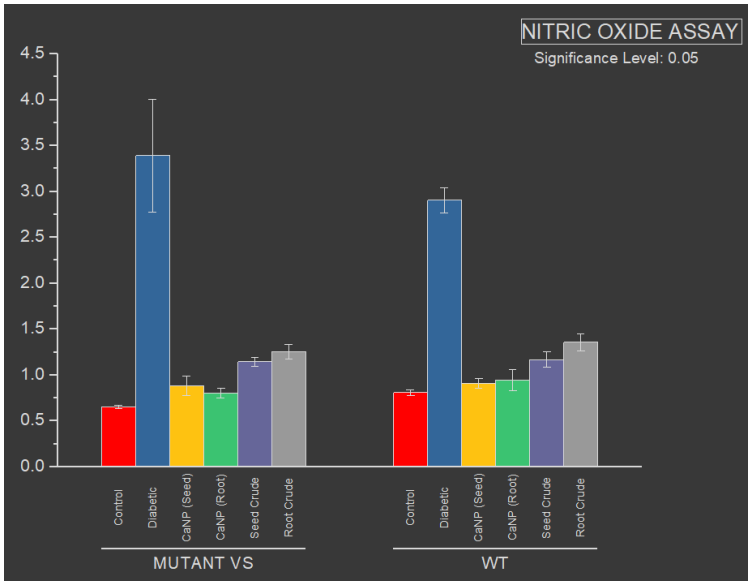


Fig.5 Nitric oxide levels in mutant (VS) and wild-type (WT) *Drosophila melanogaster* across control, diabetic, and treatment groups.

Glucose Oxidase–Peroxidase (GOD-POD) Assay

The GOD-POD assay was employed to quantify blood glucose levels in *Drosophila melanogaster* under control, diabetic, and treatment conditions in both mutant (VS) and wild-type (WT) strains.

As illustrated in Figure X, diabetic groups in both strains exhibited a dramatic elevation in glucose levels, with the mutant diabetic group reaching the highest value (~190 mg/dL) and the WT diabetic group reaching ~155 mg/dL, indicating successful induction of hyperglycemia. In contrast, control groups maintained relatively low glucose levels (~30–55 mg/dL).Treatment with Calcium Nanoparticles (CaNPs) and crude extracts of *Momordica cymbalaria* significantly reduced glucose levels in diabetic flies, as shown in Fig. 6. Among the treatment groups:

- In the mutant strain, seed crude extract was the most effective in reducing glucose levels (~75 mg/dL).
- In the WT strain, seed crude extract again showed strong hypoglycemic potential (~85 mg/dL).

The data suggest that crude extracts, particularly from seeds, are more effective than CaNPs in lowering blood glucose levels, and their efficacy is evident in both genetic backgrounds of flies. These results further reinforce the potential of *M. cymbalaria* extracts and green-synthesized CaNPs in the management of diabetes.

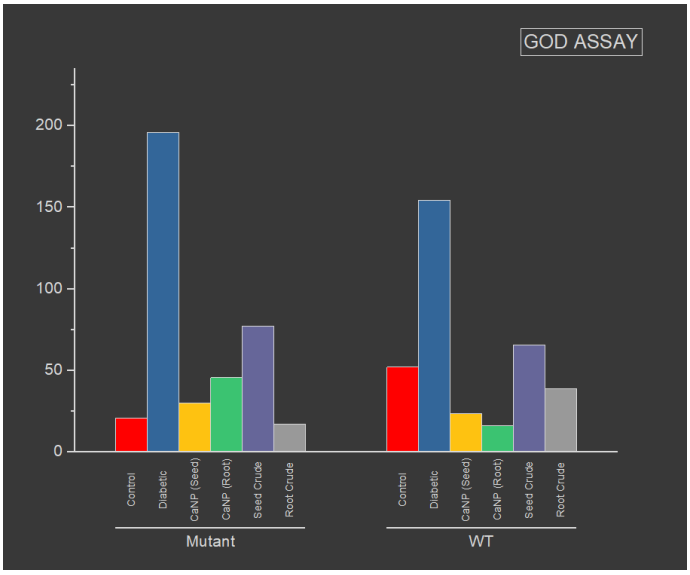


Fig.6 Glucose levels in mutant and wild-type *Drosophila melanogaster* measured by GOD-POD assay.

Conclusion

This study provides compelling evidence for the antidiabetic efficacy of *Momordica cymbalaria*, attributed to its green-synthesized calcium nanoparticles. The combination of traditional phytomedicine and nanotechnology not only enhanced bioavailability but also amplified therapeutic outcomes in diabetic Drosophila models.

Notably, seed-derived CaNPs and crude extracts demonstrated superior ability to restore oxidative balance and regulate glucose metabolism, as indicated by improved catalase activity, reduced nitric oxide levels, and normalized glucose concentrations. These findings pave the way for deeper molecular investigations and potential translational studies in mammalian systems. Incorporating such integrative approaches could revolutionize diabetes management by offering safer, plant-based, and nanotechnology-enhanced alternatives to synthetic drugs.

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