

SPECIMEN FORMAT FOR THESES OF MONTH

Faculty : School of Biosciences

Department : Biochemistry, Biotechnology and Bioinformatics

Branch/ Area: : Biotechnology

Sub Subject Heading: : Biotechnology

Candidate's Name : A.Reena Joy

Candidate's Address with email : 15, Bommanapalayam, Vadavalli, Thondamuthur road, Coimbatore - 641041
reenajoy62@gmail.com

Title of the thesis : Therapeutic Effects of Silver Nanoparticles Synthesized Ethanolic Extracts of *Boerhavia diffusa* L. on Experimentally Induced Diabetes and Obesity in Rats

(i) In Roman Script -

(ii) In roman Script -

Nomenclature of Degree: : Ph.D

Month & Year of Enrolment: : July 2018

Month & Year of Registration: : July 2018

Month &Year of Submission: : December 2025

Month &Year of Award : June 2026

Name of Supervisor : Dr.S.Gayathri Devi

Designation of Supervisor : Professor, Department of Biochemistry, Biotechnology and Bioinformatics

Centre/department/school in which research was conducted : Department of Biochemistry, Biotechnology and Bioinformatics

University's Name & Address : Avianshilingam Institute for Home Science and Higher Education for Women

Abstract within 300 words:

The rising prevalence of diabetes, obesity and their interconnected metabolic complications has heightened the need for therapeutic agents with diverse and synergistic mechanisms of action. This study synthesized silver nanoparticles using the ethanolic extract of *Boerhavia diffusa* L. (EBdAgNPs) and its antidiabetic and antiobesity efficacy through both *in vitro* and *in vivo* experimental models. FTIR, XRD, SEM-EDX, zeta potential analysis and UV-visible spectroscopy were used to analyze the EBdAgNPs. These results verified that the EBdAgNPs exhibit stability, a spherical morphology, a crystalline structure and reducing and capping properties. The *in vitro* assays revealed strong antioxidant potential and significant inhibition of α -amylase, α -glucosidase, protein glycation, glucose diffusion and pancreatic lipase activities. The *in vivo* studies conducted in albino rat models of diabetic, obese and obesity-associated diabetes treated with EBdAgNPs demonstrated improvements in fasting blood glucose, lipid profile, liver and kidney function markers and hematological parameters. Antioxidant enzyme activities were restored, and weight gain was attenuated in obesity models. Gene expression analysis by RT-qPCR demonstrated the normalization of PPAR- α and PPAR- γ levels, alongside a significant reduction in RBP4 expression, suggesting enhanced lipid oxidation, suppression of adipogenesis and improved insulin sensitivity. The synergistic interplay between the phytochemical constituents of *B. diffusa* and the nanoscale delivery of silver nanoparticles contributed to alleviating oxidative stress, modulating key metabolic enzymes and restoring both glucose and lipid homeostasis. Collectively, these results position EBdAgNPs as an efficient multifunctional therapeutic approach for the management of diabetes, obesity and associated metabolic disorders.

i) Major objectives :

- To synthesize and characterize of silver nanoparticles from ethanolic extract of *Boerhavia diffusa* L. (EBdAgNPs)
- To investigate the *in vitro* antioxidant and antidiabetic activities of silver nanoparticles synthesized from ethanolic extract of *Boerhavia diffusa* L. (EBdAgNPs)
- To examine the *in vivo* antidiabetic and antiobesity effects of silver nanoparticles synthesized from ethanolic extract of *Boerhavia diffusa* L. (EBdAgNPs) in rats
- To quantify the relative expression of PPAR- α , PPAR- γ and RBP4 genes using the RT-qPCR method

ii) Methodology :

The present study was carried out in four phases to evaluate the therapeutic potential of green synthesized silver nanoparticles prepared from the ethanolic extract of *Boerhavia diffusa* (EBdAgNPs) against diabetes and obesity.

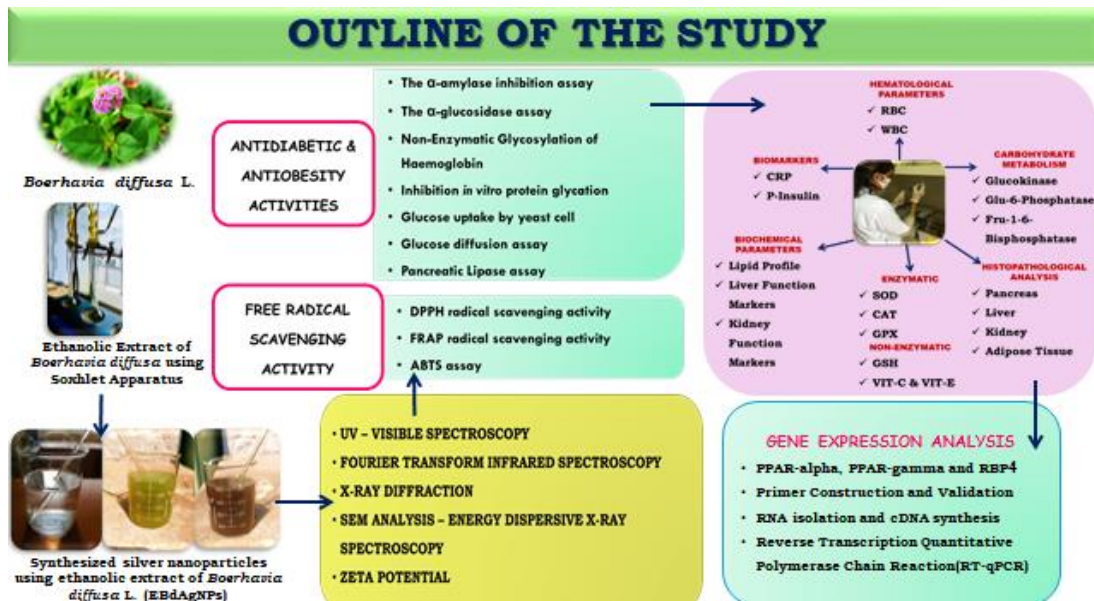
Phase I – Synthesis and Characterization: Collected and authenticated *Boerhavia diffusa* L., prepared the ethanolic extract, synthesized silver nanoparticles (EBdAgNPs) using a green synthesis method and characterized them using UV-Vis, XRD, FTIR, SEM, EDAX and Zeta Potential analysis.

Phase II – *in vitro* Evaluation: Assessed the antioxidant, antidiabetic and antiobesity potential of EBdAgNPs using antioxidant assays (DPPH, ABTS and FRAP), antidiabetic assays (α -amylase, α -glucosidase, protein glycation, glucose uptake and glucose diffusion) and pancreatic lipase inhibition assay.

Phase III – *in vivo* Animal Study: Evaluated the therapeutic efficacy of EBdAgNPs in STZ–nicotinamide-induced diabetic, high-fat diet-induced obese and diabetic-obese rat models by monitoring body weight, blood glucose, hematological parameters, lipid profile, liver and kidney function markers, plasma insulin, CRP, carbohydrate metabolic enzymes, antioxidant status and histopathological changes in the pancreas, liver, kidney and adipose tissue.

Phase IV – Molecular Analysis: Investigated the molecular mechanism by analyzing the expression of PPAR- α , PPAR- γ and RBP4 genes using RT-qPCR to understand the effect of EBdAgNPs on glucose metabolism, lipid metabolism and adipogenesis.

Statistical Analysis: All experimental data were expressed as mean \pm SD and analyzed using one-way ANOVA followed by Dunnett's post hoc test, with $p < 0.05$ considered statistically significant.



iii) Findings:

The current research was conducted with the primary objective of exploring the synthesis, characterization and therapeutic potential of silver nanoparticles synthesized using the ethanolic extract of *Boerhavia diffusa* L. (EBdAgNPs), particularly for its efficacy in treating diabetes mellitus, obesity and their comorbid condition commonly referred to as obese-diabetic. These conditions, deeply interlinked by shared pathophysiological mechanisms such as chronic inflammation, oxidative stress and metabolic dysregulation, are emerging as global epidemics. Despite advances in modern pharmacotherapy, limitations related to side effects, resistance and target specificity have driven the scientific community to investigate natural alternatives with enhanced biocompatibility and multi-target actions.

In the first phase of the study, the green synthesis of silver nanoparticles was achieved using the phytochemically rich ethanolic extract of *Boerhavia diffusa* L. The color change from pale yellow to deep brown visually indicated nanoparticle formation, which was further confirmed through UV–Visible spectroscopy with a distinct surface plasmon resonance peak around 410 nm. The structural characterization via X-ray diffraction revealed a face-centered cubic crystalline structure with prominent diffraction peaks, indicating the formation of well-defined silver crystals. The average crystallite size was estimated at approximately 24 nm, aligning with the results obtained from scanning electron microscopy (SEM), which showed spherical particles with a smooth morphology. Energy-dispersive X-ray spectroscopy (EDAX) confirmed silver as the major constituent, while functional group analysis through FTIR spectroscopy which revealed the presence of hydroxyl, carbonyl and other bioactive groups that facilitated both reduction and stabilization of nanoparticles. Zeta potential

analysis indicated a value of -20.2 mV, confirming moderate colloidal stability due to the negative surface charge imparted by capping phytochemicals.

The second phase focused on evaluating the *in vitro* antioxidant and antidiabetic properties of EBdAgNPs through a battery of biochemical assays. The nanoparticles demonstrated significant inhibitory effects on α -amylase and α -glucosidase enzymes, which are responsible for carbohydrate hydrolysis and glucose absorption. Notably, EBdAgNPs outperformed standard drugs in α -amylase inhibition, indicating its potential to reduce postprandial hyperglycemia. It also inhibited non-enzymatic glycation of hemoglobin and total protein glycation, both of which contribute to the development of long-term diabetic complications. Furthermore, the nanoparticles enhanced glucose uptake in yeast cells and effectively restricted glucose diffusion, mimicking insulin-like activity and retarding glucose absorption. The antioxidant assays, including DPPH, ABTS and FRAP, revealed potent free radical scavenging and reducing capabilities, suggesting the formulation's role in combating oxidative stress, a key contributor to both diabetes and obesity.

The third phase of the study extended the findings to *in vivo* models involving experimental albino rats, categorized into control, diabetic, obese and diabetes groups. Treatment with EBdAgNPs significantly influenced physiological and biochemical parameters in these models. In terms of body weight, the formulation mitigated weight loss in diabetic rats and controlled weight gain in high-fat diet-fed obese rats, showcasing its bidirectional regulatory capacity. In the combined obese-diabetes group, EBdAgNPs treatment led to a pronounced reduction in body weight, indicating its therapeutic efficacy in complex metabolic disorders. Fasting blood glucose levels, which were significantly elevated in diabetic and obese-diabetes groups, were notably reduced after EBdAgNPs administration, restoring near-normal glycemic status. The results were comparable and in several cases superior, to standard drugs like glibenclamide and sibutramine.

The hematological analysis provided further evidence of the nanoparticles' protective role. In the disease groups, there was a noticeable decrease in red blood cell count and an increase in white blood cells, reflecting anemia and systemic inflammation. EBdAgNPs treatment reversed these alterations, improving erythrocyte counts and reducing leukocytosis, thereby suggesting erythropoietic support and anti-inflammatory activity. Lipid profile assessment showed marked dyslipidemia in diabetic, obese and obese-diabetes groups, characterized by elevated total cholesterol, triglycerides and low-density lipoproteins, along with reduced high-density lipoprotein levels. EBdAgNPs treatment successfully corrected these imbalances, improving lipid metabolism and reducing cardiovascular risk.

Liver enzyme levels, including SGOT, SGPT and ALP, which were significantly elevated in pathological groups due to hepatic stress and damage, were brought back to near-normal values by EBdAgNPs. The data confirmed the hepatoprotective nature of the formulation. Similarly, renal biomarkers such as creatinine, urea and uric acid were elevated under diabetic and obese conditions, suggesting nephrotoxicity. Treatment with EBdAgNPs effectively reduced these markers, indicating preservation of renal function and structural integrity.

Plasma insulin levels, which were reduced in diabetic and obese rats, were significantly restored upon EBdAgNPs treatment. Concurrently, C-reactive protein (CRP), a marker of systemic inflammation, was elevated in disease groups and was substantially reduced by the formulation, reinforcing its anti-inflammatory and insulinsensitizing properties. The hepatic carbohydrate-metabolizing enzymes also revealed substantial normalization following treatment. Elevated activities of glucose-6-phosphatase and fructose-1-6-bisphosphatase, indicating enhanced gluconeogenesis, were decreased, while glucokinase activity, suppressed under diabetic and obese conditions, was upregulated. These shifts suggested improved hepatic glucose metabolism and reduced glucose output, contributing to glycemic control.

The atherogenic index, an indicator of cardiovascular risk derived from lipid parameters, was significantly elevated in disease models but markedly reduced following EBdAgNPs treatment. This finding, supported by improvements in cholesterol fractions, triglycerides and LDL, HDL ratios emphasized the cardioprotective efficacy of the formulation. The antioxidant status of hepatic tissue further affirmed the formulation's efficacy. Enzymic antioxidants such as catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx), which were depleted in diabetic and obese rats, were significantly restored by EBdAgNPs. Non-enzymic antioxidants like vitamin C, vitamin E and glutathione also followed similar trends, confirming enhanced hepatic antioxidant defense.

Histopathological studies supported these biochemical findings by revealing tissue-level recovery across multiple organs. In the pancreas, islet damage and β -cell degeneration in diabetic and obese-diabetes models were alleviated by EBdAgNPs, with evident preservation of islet architecture. In liver tissues, necrosis, inflammation and

steatosis induced by metabolic stress were reduced and normal hepatic architecture was restored in EBdAgNPs-treated rats. Renal histology showed that glomerular and tubular alterations observed in disease groups were reversed following treatment. Likewise, in adipose tissues, inflammation and adipocyte hypertrophy were minimized, indicating restoration of tissue function and metabolic homeostasis.

The final phase explored the molecular mechanisms underlying the observed therapeutic benefits. Gene expression profiling using RT-qPCR focused on PPAR- α , PPAR- γ and RBP4 in pancreas and adipose tissue. In the pancreas, diabetes and obesity led to downregulation of PPAR- α and PPAR- γ and upregulation of RBP4, reflecting impaired lipid metabolism and insulin resistance. EBdAgNPs treatment reversed these changes, enhancing PPAR- α and PPAR- γ expression while reducing RBP4 levels, thereby improving insulin sensitivity and glucose regulation. In adipose tissue, where PPAR- γ was upregulated in response to fat accumulation and RBP4 expression was markedly elevated, EBdAgNPs treatment normalized these expressions, indicating reduced adipogenesis and improved adipokine balance. The modulation of these gene expressions reflected the nanoparticle's ability to influence metabolic pathways at the transcriptional level, adding a molecular dimension to its therapeutic profile.

Collectively, the study demonstrated that EBdAgNPs synthesized through an eco-friendly, green chemistry approach using *Boerhavia diffusa* L. not only retained the biological activity of the plant's phytochemicals but also amplified their effects via nanoparticle delivery. The formulation exhibited multifaceted therapeutic potential encompassing antidiabetic, antiobesity, antioxidant, hepatoprotective, nephroprotective and anti-inflammatory actions across multiple experimental models. Unlike conventional drugs that typically target a single pathway, EBdAgNPs showed holistic modulation of multiple biochemical and molecular markers, establishing their value as a comprehensive metabolic regulator.

These findings are significant because they highlight a safe, effective and natural alternative for the treatment of complex metabolic disorders. The green synthesis process ensures environmental sustainability, while the dual action of silver ions and phytochemicals provides a potent pharmacological synergy. By restoring physiological

homeostasis at the cellular, tissue, organ and gene expression levels, EBdAgNPs emerge as a promising nanomedicine platform for future therapeutic development in managing diabetes, obesity and associated complications.

In conclusion, the research successfully achieved its objectives by providing a comprehensive analysis of the synthesis, characterization and biological evaluation of EBdAgNPs. The results consistently demonstrated their superior efficacy compared to standard treatments across a wide range of pathological parameters. This study not only contributes to the growing field of plant-based nanomedicine but also offers a viable therapeutic strategy for managing obese-diabetes through multi-target intervention. Further investigations involving clinical trials, pharmacokinetics and long-term toxicity assessments are recommended to establish translational applicability. Nonetheless, the present findings provide a strong scientific foundation for the continued development of EBdAgNPs as a novel and integrative therapeutic approach.

Examiners

Internal Examiner : Dr.Gireesh Babu K
Principal
NIMS Institute of Applied & Life Sciences,
NIMS University, Rajasthan.
Jaipur- 303121

External Examiner :
Dr. Sri Renukadevi Balusamy
Associate Professor
Department of Food Science and Biotechnology
Sejong University
Korea.