5

SUMMARY AND CONCLUSION

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 diabesity 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.

Future Recommendations

1. To explore the metabolic gene regulation by EBdAgNPs of genes other than PPAR-α, PPAR-γ and RBP4.
2. To develop EBdAgNPs as standardized herbal drug formulations that may help to create novel treatments for metabolic disorders.