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Effect of biologically synthesised silver nanoparticles from ethanolic extract of *Boerhavia diffusa* on High fat diet and streptozotocin Nicotinamide induced obese and diabetic rats

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Abstract

Boerhavia diffusa (B. diffusa), renowned in Ayurveda and traditional Chinese medicine, was the focal point of our study. Despite lacking scientific validation for its traditional use in synthesizing silver nanoparticles (AgNPs) within the whole plant ethanolic extract of B. diffusa (EBdAgNPs), our investigation was focused on exploring the effects of EBdAgNPs on serum lipid profiles, which play a crucial role in various cardiovascular diseases. The findings suggest that EBdAgNPs holds promise as an intervention for conditions associated with obesity and diabetes, demonstrating its potential to curb weight gain, diminish body fat and reduce blood glucose levels. Male albino rats were exposed to a high-fat diet (HFD) and streptozotocin-nicotinamide (STZ-NI) to induce obesity and diabetes. They were subsequently treated with EBdAgNPs and a standard drug from day 0 to day 42 to assess the response. This indicated a significant reduction in final body weight, triglycerides (TG), total cholesterol, low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) concentrations in rats treated with EBdAgNPs under both HFD and STZ-NI conditions, compared to obese and diabetic rats. Additionally, there was a significant increase in high-density lipoprotein (HDL) levels in the treated groups. The findings of our study suggest that EBdAgNPs has the potential to be utilized for the effective treatment and prevention of diseases associated with obesity and diabetes. This can be accomplished by diminishing blood glucose levels, body fat and restricting the acquisition of weight.

Keywords: *Boerhavia diffusa*, diabetes, Obesity, silver nanoparticle, lipid profile.

1. Introduction

An increased intake of dietary fats, particularly saturated and/or trans fats, making up a substantial fraction of total caloric intake, is indicative of a high-fat diet (HFD). This dietary approach is associated with diverse health impacts, encompassing both positive and negative effects, and has become a focal point of discussions related to metabolic health, obesity and associated conditions [1]. The incidence of obesity, coupled with hyperlipidemia, has risen across various age and socioeconomic groups due to changes in lifestyle and dietary patterns in recent times. Obesity has a detrimental effect on every body system, elevating the risk of conditions such as insulin resistance, arthritis and coronary artery disease [2]. Unless the current trend is reversed, it is projected that by 2030, approximately 60% of the global population will be obese. This highlights obesity as a more serious global health issue, as stated by the World Health Organization (WHO) [3].

Numerous pharmacological substances serve as anti-obesity drugs, but they often come with harmful side effects. Consequently, the utilization of natural derivatives for obesity treatment has seen a rise, particularly in many Asian countries [4]. Numerous organic resources have been employed to minimize diet and combat obesity, including isolated pollutants and unpolished plant extracts.

Over time, the quest for novel medications with the ability to lower and regulate blood levels of lipids and triglycerides has intensified, leading to a plethora of studies on the noteworthy effects of natural agents [5]. Plant-based goods are often thought to be less harmful and to have fewer adverse effects than synthetic ones. This characteristic has spurred the discovery of novel therapeutic agents, including antioxidants, hypoglycemics and hypolipidemics. The administration of natural therapies for curing obesity-related illnesses has surged tremendously in the past few decades [6].

The relationship between diabetes and obesity is intricate, with both conditions often co-existing and influencing each other. Diabetes, characterized by elevated blood sugar levels, can contribute to the development of obesity, marked by excess body weight and adipose tissue. The onset of diabetes, on the other hand, is significantly increased by fat. Complex mechanisms such as insulin resistance, inflammation, and hormonal imbalances play a role in this interconnected relationship. Managing both diabetes and obesity requires comprehensive approaches to promote overall health and well-being [7].

Boerhavia diffusa (B. diffusa), frequently referred to as the herb Punarnava or Red Spiderling, is an herbaceous perennial that has been utilized throughout several centuries in traditional medical systems, notably traditional Chinese and Ayurvedic medical practices [8].

Originating from tropical and subtropical areas, this plant is widely recognized for its numerous therapeutic applications. *B. diffusa* is said to have liver-protective, anti-inflammatory and diuretic qualities in traditional medicine. It is commonly employed to enhance kidney function, alleviate inflammation and contribute to overall well-being [9]. *B. diffusa* is rich in various bioactive compounds such as alkaloids, flavonoids, steroids, lignans, and more [10]. Traditional uses and some modern research suggest potential benefits, but it is essential to seek advice from a healthcare professional before incorporating this medicinal plant into a health routine. This precaution is especially crucial for individuals with pre-existing medical conditions or those taking medications.

This study seeks to harness the full potential of the *B. diffusa* plant in synthesizing silver nanoparticles (AgNPs) using its ethanolic extract (EBdAgNPs), aiming to address obesity and diabetes. The unique properties of these nanoparticles and their interactions with biological systems are under scrutiny to comprehend their impact on factors related to obesity and diabetes. The research delves into both molecular and physiological aspects, striving to offer a comprehensive understanding of how these nanoparticles could provide promising avenues for the effective management of obesity and diabetes. Focussing on blood overall cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL) and very-low-density lipoprotein (VLDL) the lipid profile, is crucially utilized in the context of obesity and diabetes research. Monitoring these lipid markers provides valuable insights into the intricate interplay between lipid metabolism and the development of obesity and diabetes.

The synthesis of AgNPs in medicinal plants, like *B. diffusa*, for obesity and diabetes research is noteworthy for multiple reasons. Initially, medicinal plants often contain bioactive compounds with potential health benefit and integrating these into nanoparticles could amplify their therapeutic properties. The AgNPs possess unique physicochemical properties that can interact innovatively with biological systems, making them a subject of interest for diverse biomedical applications. Lastly, the individual's anti-obesity potential of *B. diffusa* and AgNPs has been investigated and synthesizing AgNPs from this plant extract may result in a synergistic effect, enhancing anti-obesity properties. This research aims to unveil the mechanisms and impacts of such nanoparticles on obesity and diabetes-related factors, offering valuable insights for future therapeutic approaches.

2. Materials and Methods

2.1 Collecting and procuring plant material from *B. diffusa*

The discovery of *B. diffusa* in Coimbatore was verified by the Botanical Survey of India (BSI), TNAU, Coimbatore, under validation BSI/SRC/5/23/2013-14/Tech/1041 [11]. Initial processing involved thorough washing, air-drying and crushing to obtain a fine powder. The Soxhlet extraction of 10g fine powder with 100ml ethanol ensures clean and integral plant material for analysis [12].

2.2 Synthesis of Silver Nanoparticles from *B. diffusa*

To synthesize AgNPs, 500 mg of *B. diffusa* ethanolic extract (EBdAgNPs) was mixed with 100ml of deionized water and combined with a 90ml 0.1Mm silver nitrate solution. The solution turned brown within 10-20 minutes of daylight exposure, indicating nanoparticle synthesis. Refrigerated centrifugation at 13,000 rpm for 20 minutes and subsequent washing yielded the collected pellet containing the synthesized AgNPs [13].

2.3 Animal Testing

Male albino adult animals weighing between 150–200g were procured from the Animal House, Coimbatore, India. The Animal Ethics Committee granted Institutional approval for the study (Ref: 623/02502CBEA19/02/2019). The animals underwent a 15-day acclimatization period having unlimited access to food and water during a 12-hour cycle of light and dark [14]. After acclimatization, the high-fat diet (HFD) and Streptozotocin-Nicotinamide (STZ-NI) regimen was initiated.

2.4 Induction of HFD and STZ-NI induced in rats

The animals were distributed into eight groups, with four groups allocated to both HFD and diabetic rats (Table 1A and Table 1B).

2.5 The composition of a diet rich in fat

The High Fat Diet (HFD) formulation involved combining specified quantities of Cholesterol (2%), Cholic acid (1%), Dalda (20%) and Coconut oil (6%) [15].

2.6 A 30-day HFD induction period

HFD groups, animals were exposed to a high-cholesterol diet for 30 days and on the 30th day, The retroorbital plexus was numbed with moderate ether anaesthesia to obtain blood

samples. to confirm the induction of hyperlipidemia. The assessment of total cholesterol concentration was performed using standard diagnostic kits [16].

2.7 Development of STZ-NI in rats

In the STZ-NI groups, initial fasting blood glucose levels were measured after an overnight fast, utilizing the rat tail vein tip. A single intraperitoneal injection of 60 mg/kg streptozotocin and an intraperitoneal dosage of 120 mg/kg nicotinamide were used to produce non-insulin-dependent diabetic mellitus (NIDDM). Animals with blood glucose levels higher than 250 mg/dl were selected for the investigation, and hyperglycemia was confirmed 72 hours after injection [17].

2.8 Grouping and collection of blood in different given period

Fasting body weight and blood glucose levels were assessed at days 0, 21st and 42nd in both HFD and STZ-NI groups, with blood samples collected from the rat tail vein tip.

Table 1A Experimental Design (HFD)

Group 1H	Control rats + 0.05% Carboxymethyl cellulose**.**
Group 2H	HFD (High Fat Diet) Cholesterol – 2 %, Cholic acid – 1 %, Dalda –20 % and Coconut oil – 6%
Group 3H	HFD + (10 mg/kg b.w) EBdAgNPs
Group 4H	HFD + (10 mg/kg b.w) Sibutramine

Table 1B Experimental Design (Diabetes)

Group 1D	Control rats + 0.05% Carboxymethyl cellulose**.**
Group 2D	Streptozotocin-Nicotinamide (STZ-NI) - induced rats (60 mg/kg-120mg/kg b.w)
Group 3D	STZ-NI + (10 mg/kg b.w) EBdAgNPs
Group 4D	STZ-NI + (10 mg/kg b.w) Glibenclamide

2.9 Estimation of serum lipid parameters

After the experiment came to an end, blood samples were taken under light ether anaesthesia from the rat's retro-orbital plexus. The plasma was separated through centrifugation to obtain serum for subsequent biochemical estimation. Enzymatic colorimetric methods, involving commercially available kits, were employed to assess various lipid parameters, including Total Cholesterol (TC) [18], Triglycerides (TG) [19], High-Density Lipoproteins (HDL) [20], Low-Density Lipoproteins (LDL) [21] and Very Low-Density Lipoproteins (VLDL) [22]. This methodology facilitated a comprehensive assessment of the rat's lipid profiles and metabolic indicators.

2.10 Statistical analysis

The data gathering technique used GraphPad Prism 5 for statistical analysis. The standard error of the mean, or mean ± SEM, is displayed with the results of a one-way analysis of variance (ANOVA) and Dunnett's test. Statistical significance was declared for values with $p < 0.05$. [23].

3. Results and Discussion

3.1 Lipid profile

Obesity has been identified as a substantial syndrome linked to diabetes, especially in cases of non-insulin-dependent diabetes (Type 2 diabetes) [24]. The prevention of the progression of these disorders is crucial for effective management and control. Various animal models have been developed to simulate human obesity and diabetic conditions [25]. In this study, rat model was utilised to expose to a high-fat diet (HFD) consisting of cholesterol (2%), cholic acid (1%), dalda (20%) and coconut oil (6%) to induce obesity, along with the injection of streptozotocin-nicotinamide (STZ-NI) to induce Type 2 diabetes-like conditions in rats.

3.1.1 Lipid profile of EBdAgNPs in treated obese rat

The impact of EBdAgNPs on serum lipids in HFD-fed rats is depicted in Table 2. On 42nd day HFD group G2H showed lower HDL levels compared to the control group G1H. However, treated groups G3H and G4H (HFD + EBdAgNPs and HFD + Sibutramine) exhibited significantly increased ($p < 0.0001$) compared to the HFD group, while the HFD group had higher LDL, TC, TG and VLDL levels than the control group, the differences were significant. Conversely, all treated groups, particularly G3H and G4H (HFD +

EBdAgNPs and HFD + Sibutramine), demonstrated significantly decreased levels ($p < 0.0001$) of LDL, TC, TG and VLDL compared to the HFD group. The elevated levels of LDL, TC, TG, and VLDL, coupled with reduced HDL levels observed in obese rats (G2H), pose an increased risk of cardiovascular diseases such as atherosclerosis, heart attacks and strokes. As a result, EBdAgNPs may play a pivotal role in monitoring and regulating lipid profiles to mitigate adverse health consequences.

Table 2 Effects of EBdAgNPs on Serum Lipid Profiles in obese rats

Groups	**Total Cholesterol (TC)**		
	0th Day	**21stDay**	**42ndDay**
Control - G1H	77±0.6	75±1	78±0.6
Only HFD - G2H	148±1.9	257±16.8	298±11.1###
HFD + EBdAgNPs - G3H	78.7±0.3	81.7±0.9	83.7±4.4***
HFD + Sibutramine - G4H	79±0.0	83.7±0.3	88.7±0.3***
	Triglycerides (TG)		
Control - G1H	96.3±2.6	97.7±2.7	97.3±1.9
Only HFD - G2H	118±0.9	152±5.8	159±7.1###
HFD + EBdAgNPs - G3H	98.7±1.8	104±2.3	102±3.2***
HFD + Sibutramine - G4H	102±3.8	109±0.9	108±2.2***
	High-Density Lipoprotein (HDL)		
Control - G1H	48.3±0.9	49.7±1.4	49.7±0.9
Only HFD - G2H	26.3±0.9	19.3±0.3	16±1.6###
HFD + EBdAgNPs - G3H	49.0±0.6	45.3±0.3	48.7±0.9***

HFD + Sibutramine - G4H	44.7±1.8	51.3±1.9	52.3±0.3***
	Low-Density Lipoprotein (LDL)		
Control - G1	42.0±1.1	44.0±0.6	42.0±1.5
Only HFD - G2H	178±5.2	181±4.1	254±2.2###
HFD + EBdAgNPs - G3H	46.7±0.9	50.7±0.3	53.7±0.3***
HFD + Sibutramine - G4H	48.3±2.3	54.3±0.3	56.7±0.3***
	Very-Low-Density Lipoprotein (VLDL)		
Control - G1H	18.3±0.3	19.3±0.3	19±0.6
Only HFD - G2H	69.3±0.3	78.3±0.7	91.7±2.2###
HFD + EBdAgNPs - G3H	18.3±0.3	20±0	22±1***
HFD + Sibutramine - G4H	19.3±0.3	22.3±1.2	24±0***

The impact of EBdAgNPs on serum lipid profiles in HFD-induced obese rats is depicted in Table 2. The various lipid components, including (A) high-density lipoprotein (HDL), (B) low-density lipoprotein (LDL), (C) total cholesterol (TC), (D) very low-density lipoprotein (VLDL) and (E) triglyceride (TG), were assessed. Data, presented as means ± SEM (n = 6). a: Significant compared to different groups with control. b: Significant compared different group in HDF + EBdAgNPs to obese. c: Significant compared to standard drug. The $p < 0.0001$ which is significant in all the groups compared with the HFD G2H.

The primary aim of this study was to assess the impact of AgNPs synthesized from the ethanolic extract of *B. diffusa* (EBdAgNPs) on serum lipid profile levels in both high-fat diet (HFD) and streptozotocin-nicotinamide (STZ-NI) induced diabetic rats. Additionally, EBdAgNPs treatment exhibited positive effects on lipid profiles, including elevated high-density lipoprotein (HDL) and decreased total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL) levels. These findings suggest a beneficial impact on lipid profile in obese and diabetic rats.

Consistent monitoring and efficient management of the lipid profile are critical in reducing cardiovascular risks and related complications in individuals with diabetes and

obesity. A holistic approach involves lifestyle adjustments, including maintaining a balanced diet, engaging in regular physical activity, and managing weight. Depending on individuals with health profiles, medical interventions like medications targeting lipid levels may be considered. Ensuring regular monitoring and personalized care is essential to enhance health outcomes for those dealing with diabetes and obesity. A comparable study has documented the antidiabetic and anti-obesity effects of *T. erecta* [26].

3.1.2 Lipid profile of EBdAgNPs in treated diabetic rat

In STZ-NI-induced diabetic rats, there were notable changes in various lipid profile parameters, including TC, LDL, HDL, VLDL, and TG, as illustrated in Table 3. Diabetic rats exhibited significantly higher levels of LDL, VLDL, TG, and TC compared to the healthy rat group, while HDL levels were slightly lower in diabetic rats compared to the healthy group. Similar patterns were observed in the treated groups G3D and G4D (STZ-NI + EBdAgNPs and STZ-NI + Glibenclamide) across all lipid profile parameters, including LDL, TC, TG, VLDL, and HDL.

Table 3: Effects of EBdAgNPs on Serum Lipid Profiles Diabetic rats

Groups	**Total Cholesterol (TC)**		
	0th Day	**21stDay**	**42ndDay**
Control - G1	77±0.6	75±1	78±0.6
STZ-NI - G2D	173±9.3	262±12.3	263±21.9$^{###}$
STZ-NI + EBdAgNPs - G3D	80.7±2.2	82±1.1	89.7±2.3***
STZ-NI + Glibenclamide - G4D	81±0.6	86±1	95.3±3.7***
	Triglycerides (TG)		
Control - G1	96.3±2.6	97.7±2.7	97.3±1.9
STZ-NI - G2D	126±1.4	187±0.9	205±1.8$^{###}$

STZ-NI + EBdAgNPs - G3D	98.7±0.3	102±0.9	110±0.7***
STZ-NI + Glibenclamide - G4D	101±1.2	106±3.3	108±3***
	High-Density Lipoprotein (HDL)		
Control - G1	48.3±0.9	49.7±1.4	49.7±0.9
STZ-NI - G2D	28±0.6	20.3±0.9	17±1.6#
STZ-NI + EBdAgNPs - G3D	42.3±12.6	47±2.6	48±0.6*
STZ-NI + Glibenclamide - G4D	46±1.1	51±4.5	51±2*
	Low-Density Lipoprotein (LDL)		
Control - G1	42±1.1	44±0.6	42±0.9
STZ-NI - G2D	163±2.5	180±5.8	228±5.4##
STZ-NI + EBdAgNPs - G3D	45±0	48.3±0.3	50.7±0.7*
STZ-NI + Glibenclamide - G4D	47±0	49.3±0.3	52.7±0.9*
	Very-Low-Density Lipoprotein (VLDL)		
Control - G1	18.3±0.2	19.3±0.2	19±0.6
STZ-NI - G2D	68±0.6	77±1.5	89±0.6###
STZ-NI + EBdAgNPs - G3D	16.3±1.2	20.7±0.9	22.3±0.7**

STZ-NI + Glibenclamide - G4D	17±1	21.7±0.9	24±2.1**

The impact of EBdAgNPs on the serum lipids of STZ-NI-induced diabetic rats was investigated at the end of the study. Diabetic rat groups were subjected to treatment with EBdAgNPs and Glibenclamide for a duration of 0 to 42 days after being induced with STZ-NI Table 5. The Data, presented as means ± SEM (n = 6), indicate no significant differences ($p < 0.05$) among values sharing a common superscript in the same row. G1D to G4D – Group 1 Diabetic to Group 2 Diabetic.

Obesity and diabetes are associated with elevated blood levels of total cholesterol (TC), LDL-cholesterol, very-low-density lipoprotein (VLDL)-cholesterol and triglycerides (TG), along with decreased levels of high-density lipoprotein (HDL)-cholesterol. Numerous studies have demonstrated these associations, and various treatments involving medicinal plants, such as *P. oleracea* powder [27], aqueous extracts of *Foeniculum vulgare* [28], *Cinnamomum cassia* [29] and *Trigonella foenum graecum L* [30] have been explored as potential measures to prevent cardiovascular diseases. These conditions increase the risk of cardiovascular issues like hyperlipidemia, hypercholesterolemia and atherosclerosis. Chronic excess fat intake is also known to impact vascular health through oxidative and inflammatory reactions induced by metabolic stress. In uncontrolled diabetes, lipid profiling becomes a crucial parameter, as high lipid levels contribute to the severity of complications, particularly cardiovascular diseases. Diabetes disrupts lipid metabolism by enhancing the release of fats from adipose tissue into circulation [31].

Given the well-established changes in serum lipid profiles associated with diabetes and high-fat diet (HFD), which elevate the risk of coronary heart disease, reducing serum lipids, especially LDL and VLDL fractions, and triglyceride levels is considered advantageous for the long-term well-being of individuals with these conditions [32]. The compounds present in the EBdAgNPs extract are believed to contribute, either wholly or partially, to its antihyperlipidemic activity. Thus, the findings suggested that the increased levels of total serum cholesterol, triglycerides, total lipids, VLDL, and LDL-cholesterol seen in diabetes can be alleviated by the EBdAgNPs extract. Additionally, the antihyperlipidemic impact of the extract may act as a protective mechanism against the development of atherosclerosis [33]. Hence, the EBdAgNPs extract holds potential for managing the progression of hyperlipidemia and atherosclerosis in diabetic individuals, emphasizing the importance of adopting a healthier lifestyle to address obesity [34]. This study lays the groundwork for further investigations in this area.

4. Conclusion

EBdAgNPs demonstrated efficacy in reducing glucose, body weight and lipid profile levels compared to diabetic and obese rats induced by STZ-NI and HFD. Ongoing research highlights its potent anti-diabetic and anti-obesity capabilities. These initial findings suggest that the AgNPs-mediated *B. diffusa* could offer a novel medicinal treatment approach for managing diabetes and obesity. This investigation into its effects on HFD-induced obesity and STZ-NI induced diabetes revealed positive outcomes in biochemical parameters, including lipid profile (TG, TC, HDL, LDL and VLDL). In conclusion, our results indicate the beneficial effects of EBdAgNPs, making it a potential candidate for further development as a new drug in the treatment of obesity and diabetes, deserving further exploration.

Authorship contribution statement

Reena Joy: Writing – review & editing, Writing – original draft, Validation, Visualization, Supervision, Software, Resources, Project administration, Methodology, Investigation, Analysis, Data collection, Conceptualization. Dr. Gayathri Devi: Writing – review & editing. Dr. Vasundhara: Writing – review & editing. Priyadharshini: Writing – review.

Declaration of competing interest

The authors declare no competing financial interest.

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Data availability

Data will be made available on request.

Ethics approval

The animal study protocol was approved by the Institutional Animal Ethical Committee under reference number 623/02502CBEA19/02/2019. All methods were carried out in accordance with relevant guidelines and regulations.

Consent to publication

All the authors consent to the publication of this article.

Consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

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