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Green Synthesis, Physicochemical Characterization and in Vivo Anti Diabetic Evaluation of Eugenia Jambolana Mediated Silver Nanoparticles

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ABSTRACT: Objective: Current study is designed to synthesize silver nanoparticles (AgNPs) using the ethanolic seed extract of Eugenia Jambolana through an eco-friendly green synthesis. Characterize the synthesized AgNPs using physicochemical methods, and investigate there in vivo antidiabetic potential in alloxan induced diabetic wistar rats.

Methods: The silver nanoparticles were successfully synthesized using ethanolic seed extract of Eugenia Jambolana by using green synthesis and characterized by particle size, zeta potential, SEM and XRD. Moreover acute oral toxicity was evaluated. In vivo antidiabetic activity was evaluated using an alloxan induced diabetic Wistar rat model. The assessment included monitoring changes in body weight, fasting blood glucose, triglycerides, total cholesterol, serum creatinine, and key indicators of oxidative stress markers such as reduced glutathione, superoxide dismutase, and. malondialdehyde.

Result: Physicochemical characterization confirmed the successful synthesis of stable AgNPs with crystalline nature, nanoscale particle size and good surface morphology. Acute toxicity studies shown that no mortality at limit dose. Animal studies demonstrated that the oral administration of AgNPs for 21 days significantly reduced FBG levels, total cholesterol, triglycerides, and serum creatinine levels compared with the diabetic control group along with restoration of body weight and improvement in lipid profile, Furthermore, oxidative stress markers showed significant improvement indicating improved antioxidant status. The antidiabetic efficacy of synthesized AgNPs was comparable to that of standard drug metformin.

Conclusion: The synthesized AgNPs demonstrated potent antihyperglycemic, antioxidant and renoprotective effects, suggesting its potential as an environmentally sustainable nanotherapeutic approach for diabetes management.

1. INTRODUCTION:

Diabetes mellitus (DM) is a long time metabolic condition defined by consistently raised blood glucose levels arising from defects in insulin discharge and reduced insulin action, or a combination of both. It has turn in to one of the most important global health challenges due to its rapidly growing prevalence, high morbidity and considerable socioeconomic impact. The illness condition affects people across all age groups and is recognized as a major contributing factor to cardiovascular (CV) disease, long lasting kidney disease, neuropathy, vision loss, lower limb amputation, and quick death the increasing rate of obesity inactive lifestyle unhealthy dietary routines, urbanization and an aging population have considerably accelerated the

occurrence of type second DM creating it as one of the foremost non communicable disease globally. Diabetes mellitus has become one of the most serious public health issues of the twenty first century. Its global footprint is growing in both size and complexity, and there is an urgent need for more prevention, detection, and therapeutic innovation [1, 2].

Recent population estimates show that the number of adults (aged 20 79) living with diabetes worldwide is now about 589 million **(International Diabetes Federation, 2024).** If current trends continue, this number will rise to 853 million by 2050 **(International Diabetes Federation, 2024).** Regardless of remarkable advances in diagnosis and therapeutic interventions diabetes continues to present significant challenges to healthcare system due to its chronic condition and the progressive complications it produces.

About 90–95% of all confirmed or diagnosed cases of diabetes are type 2 diabetes mellitus, which is typified by insulin resistance alongside a gradual decline in pancreatic beta cell function. Persistent hyperglycemia induces excessive production of reactive oxygen species (ROS) which disrupt the cellular antioxidant defense system against oxidative damage and promotes oxidative stress [3, 4].

Several allopathic medicines and pharmacological agents such as sulfonylureas, biguanides, sodium glucose transporter 2 inhibitors, thiazolidinedione's, dipeptidyl peptidase 4 inhibitors, glucagon like peptide 1 receptor agonists, and insulin preparations are commonly prescribed for the management of diabetes mellitus [5]. While these agents have profoundly enhanced glycemic control and reduced diabetes related complications, prolonged administration is frequently associated with adverse effects such as gastrointestinal discomfort, hypoglycemia, weight gain edema hepatotoxicity renal complications, liver toxicity, and kidney related complications and elevated treatment cost. These shortcomings have motivated researchers to explore safer, more effective, and cost efficient therapeutic alternatives obtained from natural sources [6].

Medicinal plants have served as significant sources of therapeutic compounds for long periods and continue to hold a central place in modern drug discovery efforts. Plants contain phytochemicals such as alkaloids, glycosides, phenolic compounds, flavonoids compounds, tannins, terpenoids, anthocyanin's, display a wide variety of biological activities, including antimicrobial, antioxidants, anti-inflammatory, anti-diabetic and anti-cancer effects.

Several experimental studies have revealed that these bioactive compounds regulate glucose balance through multiple mechanism including the inhibition of carbohydrates digesting enzymes. (α amylase and α glucosidase), stimulation of insulin secretion, improvement of insulin sensitivity, suppression of glucose production in the liver, promotion of glucose uptake in peripheral tissues, and protection of pancreatic β cells from oxidative damage [7, 8].

Among the medicinal plants that have been extensively studied for antidiabetic properties, *Eugenia jambolana* commonly known as Jamun, Indian blackberry, S. Cumini, black plum Java plum, is a widely used medicinal plant known for its hypoglycemic, antioxidant, and anti-inflammatory properties. The plant has been traditionally used in Ayurveda, Unani, and Siddha systems of medicine for managing diabetes mellitus, dysentery, and diarrhea. The key bioactive component of *E. jambolana* is Jambosine, an alkaloid responsible for its potent antidiabetic activity. Jambosine acts by modulating glucose metabolism and enhancing insulin sensitivity. Besides jambosine, the seeds also contain gallic acid, ellagic acid, and anthocyanins, which contribute to antioxidant and hepatoprotective activities. Several studies have confirmed the antidiabetic effect of *E. jambolana* seed extract through inhibition of α glucosidase and α amylase enzymes. In addition to metabolic regulation, extracts of *E. jambolana* exhibit antimicrobial, anticancer, and cardio protective activities, making it a multifunctional therapeutic plant. The presence of jambosine serves as a reliable phytochemical marker for quality assessment in standardization studies. These pharmacological characteristics make *S. cumini* one of the most promising medicinal plants for the development of new antidiabetic therapeutics [9-11].

In recent years nanotechnology has established itself as a transformative field in biomedical research contribute new approaches to increase the therapeutic performance of natural products. Silver nanoparticles possess distinctive physicochemical characteristics such as nano scale dimensions, a high surface area to volume ratio, adjustable optical properties, improve surface reactivity, and enhanced interaction with biological systems. Silver nanoparticles frequently demonstrate greater stability, improved bioavailability, and stronger biological activity, as the phytochemicals present on the nanoparticle surface serve simultaneously as reducing, capping, and stabilizing agents [12-14].

Green synthesis has emerged as an environmentally sustainable and economically attractive approach for the fabrication of metallic nanoparticles. Unlike conventional chemical and physical methods, which frequently require hazardous reducing agents, toxic solvents, high energy input, and sophisticated instrumentation, green synthesis utilizes naturally occurring biomolecules from plants, microorganisms, algae, or fungi as reducing and stabilizing agents [15-19].

The biosynthesis of AgNPs using herbal extracts has drawn a lot of interest because it is rapid, economical, environmentally friendly, and nonpathogenic. This green synthesis technique provides a straightforward, one step way for producing nanoparticles. In order to reduce silver ions and stabilize the resulting nanoparticles, plant derived phtoconstituents such as enzymes, polysaccharides, proteins, amino acids, alkaloids, tannins, phenolic compounds, saponins, terpenoids and vitamins are essential. Despite their chemical complexity, these naturally occurring phytochemicals which are well known for their therapeutic qualities offer an environmentally friendly substitute [20].

Comprehensive physicochemical characterization essential to confirm successful nanoparticles synthesis & to establish the relationship between nanoparticles and biological activity. X ray diffraction (XRD) confirms the crystalline nature and phase purity of AgNPs, whereas scanning electron microscopy (SEM) provides information regarding particle morphology, size, and surface architecture, while zeta potential analysis evaluates surface charge and colloidal stability [21-24].

Despite these encourage findings, no studies have comprehensively investigated ethanolic extract of *Eugenia jambolana* seed mediated silver nanoparticles using extensive physicochemical characterization together with systematic in vivo antidiabetic evaluation. Moreover, establishing correlations between nanoparticle characteristics and their therapeutic efficacy remains an important area of investigation [25-26].

Hence, the present study was designed to synthesize silver nanoparticles using the ethanolic seed extract of *Eugenia jambolana* through an environmentally friendly green synthesis approach, characterize the synthesized nanoparticles using particle size, XRD, SEM, and zeta potential analysis, and evaluate their antidiabetic efficacy in alloxan induced diabetic Wistar rats by assessing fasting blood glucose, body weight, lipid profile, renal function parameters, and oxidative stress markers. The findings of this study are expected to contribute to the development of a safe, ecofriendly, and effective phytopharmaceutical nanotherapeutic for the management of diabetes mellitus.

2. MATERIALS AND METHODS:

2.1. Collection of Plant Materials

Eugenia jambolana was collected from forested areas in Western Maharashtra during the summer season when the fruits are ripe and seeds are mature.

2.2. Preparation and Preservation of Plant Samples

After collection, plant materials were washed with distilled water to remove adhering soil and other contaminants. The materials were shade dried to preserve the phytochemical integrity and then coarsely powdered using mechanical grinders. The dried plant powder was stored in airtight containers under protection from light and moisture to maintain its quality prior to extraction and analytical studies.

2.3. Extraction Procedure

As per **Awolu et al., 2013** with slightly modification. The extraction process is crucial for isolating phytoconstituents from plant materials and serves as a prerequisite for further phytochemical and pharmacological evaluations. In the present study, the Soxhlet extraction technique was employed for exhaustive extraction of phytochemicals from the dried and coarsely powdered plant samples. Ethanol was chosen as the solvent due to its efficiency in extracting a wide range of polar and semi polar constituents such as alkaloids, flavonoids, glycosides, saponins, tannins, and phenolic compounds [27].

2.4. Green Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles were made utilizing a green synthesis method that used plant extract a reducing & stabilizing agents. In brief, 10 ml of plant extract was combined with 90 ml of aqueous silver nitrate solution (1mM AgNO3) under continuous stirring at room temperature. The formation of AgNPs was confirm by a noticeable color change from pale yellow to dark brown caused by surface plasmon resonance. The reaction mixture was incubated for 24 hours to guarantee that the silver

ions were completely reduced. Centrifugation at 10,000 rpm for 20 minutes was used to extract the produced nanoparticles, which were then washed repeatedly with distilled water to remove unreacted components. The cleaned nanoparticles were then dried and kept for future examination and formulation [28-30].

2.5. Characterization of Synthesized AgNPs

The particle size measurement of silver nanoparticles generated from *Eugenia jambolana* extract revealed the establishment of a wide nanoscale population SEM scanning of synthesized AgNPs showed the spherical morphology and a smooth surface and XRD showed that the nanoparticles were crystalline by showing characteristic diffraction peaks at the face centered cubic (fcc) structure of silver. Zeta potential analysis showed high values of surface charge (greater than ±30 mV) which implies that the particles have good colloidal stability because of repulsion between particles owing to electrostatic repulsive forces [31-34].

2.6. Acute Oral Toxicity Study

In accordance with OECD Guideline 423, acute oral toxicity was assessed. A single oral dose of 2000 mg/kg of the silver nanoparticles was given to Wistar rats (n=3). For the first 24 hrs. And every day for the next 14 days, animal were monitored for symptoms of poisoning, altered behavior, and death [35].

2.7. In Vivo Antidiabetic Study

2.7.1. Ethical Approval

The animal tests were only conducted after receiving consent from the Institutional Animal Ethics Committee (IAEC). (Approval No.: GCP/IAEC/25/08). All procedures were performed in full compliance with the regulations and ethical guidelines established by the Committee for the Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.7.2. Experimental Animals

Healthy adult Wistar albino rats of either sex, weighing between 200 and 250 g, were obtained from a CPCSEA registered animal breeding center. Prior to the initiation of the experiment, all animals were allowed to acclimatize for seven days under controlled laboratory conditions maintained at a temperature of 25 ± 2°C, relative humidity of 50 ± 10%, and a 12 h light/12 h dark photoperiod. The rats were housed in polypropylene cages, with three animals accommodated per cage on sterile rice husk bedding. During the acclimatization period, they were fed a standard laboratory pellet diet and had unrestricted access to potable drinking water.

2.7.3. Preparation of Test and Standard Drug Solutions

To ensure uniform dispersion before oral administration, the optimized silver nanoparticles was coarsely powdered and suspended in distilled water using 0.5% w/v carboxymethyl cellulose (CMC) as a suspending agent. The standard Metformin hydrochloride, was produced in distilled water at a dose of 150mg/kg body weight. All formulations were freshly prepared prior to dosing.

2.7.4. Induction of Experimental Diabetes

Diabetes mellitus was induced by a single intraperitoneal administration of freshly prepared alloxan monohydrate at a dose of 150 mg/kg body weight, dissolved in sterile normal saline. The animals were deprived of food for 12 h before alloxan injection, although water was available ad libitum throughout the fasting period. To prevent the occurrence of alloxan induced transient hypoglycemia, the rats received 5% glucose solution as drinking fluid for the subsequent 24 h. After 72 h of alloxan administration, fasting blood glucose (FBG) concentrations were measured using a glucometer. Rats with FBG values of 250 mg/dL or higher were considered diabetic and enrolled in the experimental study.

2.7.5. Experimental Design and Treatment Protocol

A total of 24 diabetic and non-diabetic rats were randomly divided into four groups (n = 6 per group) as follows:

Group I (Normal Control): Received normal saline (1 mL/kg, p.o.)

Group II (Diabetic Control): Received alloxan only

Group III (Standard): Received metformin (150 mg/kg, p.o.)

Group IV (Test): *Eugenia Jamolana* AgNPs (200 mg/kg, p.o.)

All treatments were administered orally once daily for 21 consecutive days using an oral gavage. Body weight and fasting blood glucose levels were monitored at predetermined intervals throughout the study.

2.7.6. Measurement of Fasting Blood Glucose (FBG)

On Days 0, 7, 14, and 21, fasting blood glucose levels were assessed. After an overnight fasting blood samples were taken from the tail vein. The glucose oxidase peroxidase (GOD POD) enzymatic technique was used to assess glucose levels [36].

2.7.7. Body Weight Monitoring

Each animal's body weight was measured on days 0, 7, 14, and 21 using a digital weighing balance. Changes in the body weight were utilized to assess metabolic status and therapy response. The prevention of weight loss in treated groups was thought to indicate improved glycemic control and metabolic recovery [37].

2.7.8. Biochemical Analysis

At the end of the experiment (day 21), the animals were fasted overnight and anaesthetized with light ether, after which blood samples were taken via retro orbital puncture using capillary tubes. Blood samples were first allowed to clot at ambient temperature and subsequently centrifuged at 3000 rpm for 10 min to obtain serum. The separated serum was carefully collected and preserved at −20°C until further biochemical analysis. Serum biochemical investigations included lipid profile parameters, namely total cholesterol (TC) and triglycerides (TG), along with oxidative stress biomarkers such as malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD). All estimations were carried out according to the manufacturer's instructions using commercially available diagnostic assay kits (Elabscience) to evaluate the antihyperlipidemic and antioxidant activities of the synthesized silver nanoparticles [38-41].

2.7.9. Statistical Analysis

Experimental data were summarized as mean ± SEM, with six animals included in each group (n = 6). Comparisons among groups were analyzed by one way or two way ANOVA, as appropriate, followed by Bonferroni's multiple comparison post hoc test using GraphPad Prism software (version 10.0). A value of $p < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Collection, Authentication, preparation of Plant Materials

Collection of the plant materials was performed during their peak phytochemical periods to ensure maximum yield and potency. Standard pharmacogenetic protocols were followed to maintain consistency and botanical accuracy. Post-harvest handling of the plant materials was carefully managed to prevent degradation of sensitive phytoconstituents.

All collected parts were washed thoroughly with distilled water to remove extraneous material such as soil and microbes. The plant materials were then shade dried at ambient temperature to avoid loss of heat sensitive secondary metabolites. Grinding was performed using a mechanical grinder, and the powdered materials were stored in clean, airtight, light protective containers under desiccated conditions until further analysis. The selected plants were authenticated by "Government of India, Ministry of Environment Forests & Climate Change, Botanical Survey of India" No.BSI/WRC/lden. Cer./2022/2206220000058 Date: 23.06.2022.

3.2. Extraction

The Soxhlet extraction of *Eugenia Jambolana*, yielded concentrated ethanolic extracts with distinct color and consistency, indicating successful recovery of diverse phytoconstituents. The extract of *Eugenia Jambolana* (Fig. 7.18b), on the other hand, was dark brown to almost black, which is typical of its high levels of tannins, anthocyanins, and polyphenolic chemicals.

Table 1: Visual observation of Extract

Sr. No.	Plant Extract	Color	Appearance / Consistency
2	*Eugenia Jambolana*	Blackish	Dense, highly pigmented

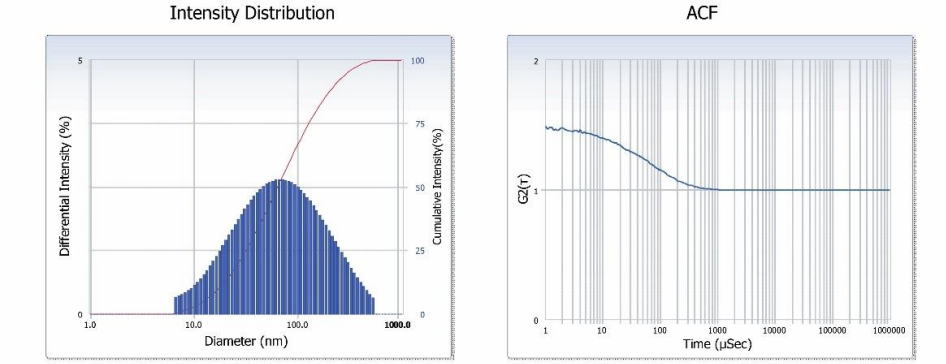
3.3. Green Synthesis of Silver Nanoparticles (AgNPs)

The creation of silver nanoparticles from the ethanolic extracts of *Eugenia Jambolana,* was first confirmed by the clear color changes seen in the reaction mixtures. These changes are a clear sign of Ag^{+} reduction to Ag^{0}. The *Eugenia Jambolana* reaction had a very dark brown to nearly black color.

3.4. Characterization of Synthesized Silver Nanoparticles AgNPs

The synthesized silver nanoparticles (AgNPs) obtained from the selected medicinal plant extracts were extensively characterized using multiple analytical techniques to confirm their formation, structural integrity, morphology, and stability.

3.4.1. Particle Size Measurement



Distribution Results (Contin)

Peak	Diameter (nm)	Std. Dev.
1	107.0	105.5
2	0.0	0.0
3	0.0	0.0
4	0.0	0.0
5	0.0	0.0
Average	107.0	105.5

Residual : 3.443e-003 (O.K)

Cumulants Results

Diameter	(d)	: 51.1	(nm)
Polydispersity Index	(P.I.)	: 0.312	
Diffusion Const.	(D)	: 9.623e-008	(cm²/sec)

Measurement Condition

Temperature	: 25.0	(°C)
Diluent Name	: WATER	
Refractive Index	: 1.3328	
Viscosity	: 0.8878	(cP)
Scattering Intensity	: 29612	(cps)
Attenuator 1	: 47.16	(%)

Fig. 1: Particle size of *Eugenia jambolana* AgNPs

The particle size measurement of silver nanoparticles generated from *Eugenia jambolana* extract revealed the establishment of a wide nanoscale population, characterized by a prominent peak at 107.0 nm, indicating the existence of medium sized nanoparticles. The intensity distribution curve had a clear, bell shaped structure, which means that nucleation happened consistently, and then natural variation occurred as the particles grew. The high standard deviation (105.5 nm) shows that the sample had a wide range of sizes. This means that, in addition to the main nanoparticle population, there were also some larger particles or groups of particles. This is a common result of phytochemical mediated synthesis because of the complex mixtures of capping and stabilizing biomolecules. The mean particle diameter of 51.1 nm determined from the cumulant indicates that a significant fraction of the particles resides in the lower nanoscale region. The polydispersity index (0.312) showed that the system was moderately polydisperse, which backed up the mixed size distribution shown in the intensity plot. The ACF curve exhibited a continuous decline trend, which means that the particles were moving, and the scattering behavior was stable. There were no sudden changes that would suggest serious aggregation. In general, our results show that *Eugenia*

jambolana extract is good at lowering and stabilizing silver ions, making nanoparticles with a wide yet stable size range that may be used for further research and practical purposes.

3.4.2. Scanning Electron Microscopy (SEM)

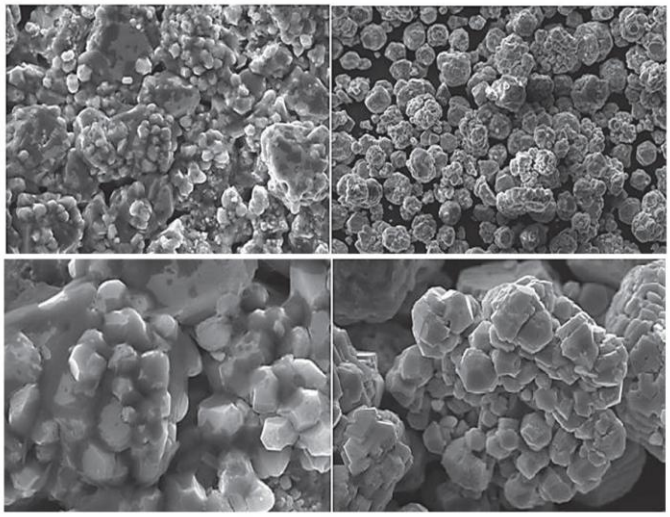


Fig. 2: Scanning Electron Microscopy (SEM) analysis of AgNPs synthesized from *Eugenia jambolana*,

The Scanning Electron Microscopy (SEM) study of the biosynthesized silver nanoparticles elucidated their morphological properties and surface distribution. The AgNPs made from *Eugenia jambolana* (Fig. a) had nanostructures that were not perfectly regular but were well defined. They also tended to form tiny clusters on the surface, which suggests that active nucleation was followed by moderate aggregation since the extract was quite rich in polyphenols.

Table 2: SEM Morphology analysis of Plant Mediated AgNPs

Plant Extract	SEM Morphology	Aggregation Pattern	Phytochemical Influence
Eugenia jambolana	Irregular nanoparticles, variable shapes; clustered deposits	Moderate aggregation; surface clustering	High polyphenolic & tannin content causing fast nucleation and partial aggregation

3.4.3. Zeta Potential Measurement

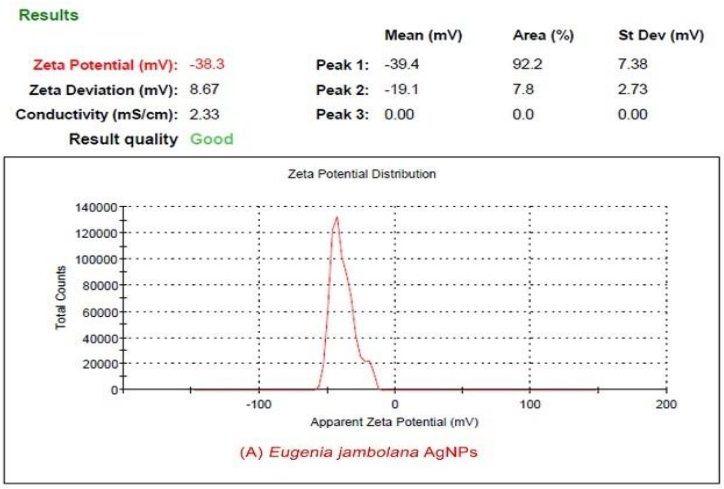


Fig. 3: Zeta Potential of AgNPs synthesized from *Eugenia jambolana*,

The colloidal stability and surface charge of the nanoparticles were assessed through zeta potential measurements, which indicate the dispersion behavior of the synthesized AgNPs.

The zeta potential study of the produced silver nanoparticles showed that the AgNPs made from *Eugenia jambolana* extracts had very different surface charges and colloidal stability. The *Eugenia jambolana* AgNPs had a very negative zeta potential of 38.3 mV, which means that the particles were strongly repelled by each other and, as a result, the colloidal stability was very good.

The narrow distribution peak at 39.4 mV with 92.2% area shows that there is mostly a homogenous population of nanoparticles with very little agglomeration. The strong negative charge is due to the many polyphenols, tannins, and acidic groups in *E. jambolana*. These compounds work as excellent capping agents and help stabilize AgNPs by sticking to their surfaces.

Table 3: Zeta Potential of Plant Mediated Silver Nanoparticles

Plant Extract	Zeta Potential (mV)	Main Peak (mV)	Peak Area (%)	Zeta Deviation (mV)	Stability Interpretation
Eugenia jambolana	**38.3**	39.4	92.2%	8.67	**Excellent stability** due to strong negative charge from polyphenols

3.5. Lyophilization of AgNPs

The lyophilization process yielded fine, dry silver nanoparticle powders from plant extracts *Eugenia Jambolana,* indicating successful conversion of the colloidal AgNPs into a stable solid form. The freeze drying approach effectively preserved the structural integrity of the nanoparticles, with no visible aggregation or cake collapse observed in the final product.

Table 4: Characteristics of Freeze Dried AgNPs

Sr. No.	Plant Extract AgNPs	Color After Lyophilization	Texture / Appearance
1	*Eugenia Jambolana*	Black	Thick, dense powder

3.6. Characterization of Lyophilization of AgNPs

3.6.1. X Ray Diffraction (XRD)

The XRD diffractogram of the lyophilized Eugenia Jambolana AgNPs shows crisp, well defined peaks, which means that crystalline silver nanoparticles have formed. At 31.47°, 37.58°, 63.84°, and 81.11° (2θ), there were strong diffraction peaks. These angles correspond to the typical crystallographic planes of the face centered cubic (fcc) structure of metallic silver. The sharp peak at around 37° shows the (111) plane, which is usually the most important reflection for AgNPs. This means that the crystals are growing well and are strongly oriented. The peak at 63° matches the (220) plane, which supports the idea that Ag^0 is crystalline. The sharp peak at 81° might be due to the (311) or higher order reflections, which means that the nanoparticles that were made are quite crystalline. Residual phytochemicals are probably the source of the small background signals. This is because S. jambolanum has a lot of tannins, anthocyanins, and polyphenols that function as natural capping agents. The XRD pattern shows that Eugenia Jambolana not only helps silver ions become smaller, but it also keeps the crystalline nanoparticle structure stable throughout the lyophilization process.

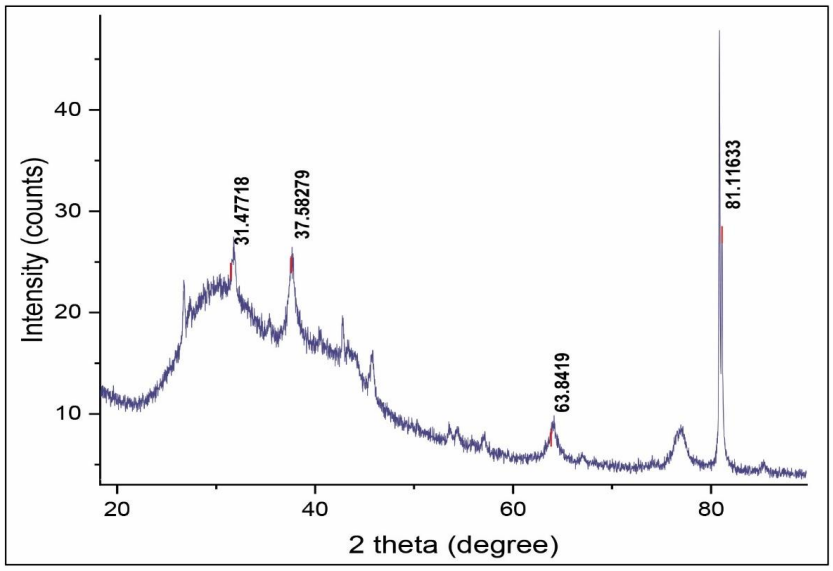


Fig. 4: XRD of *Eugenia Jambolana* Lyophilized AgNPs

3.7. Acute Oral Toxicity Study

The EJ SNP was administered once at a limit dose of 2000 mg/kg (oral) with a 14 day observation period (n = 3) to assess acute oral toxicity. During the observation period, there were no death. Within the first 48 hours, two animals showed transient clinical symptoms (mild piloerection, decreased activity) which went away on their own. The increase in body weight was within normal range. On day 14, clinical biochemistry revealed that two animals had slight, non progressive increase in hepatic transaminases (ALT up to 52 U/L), but renal indicators (BUN, creatinine) were still within normal limits. The reference ranges were met by the hematological parameters. No treatment related lesions were found after gross necropsy. These simulated data show that the test item does not result in serious systemic toxicity or treatment related mortality at 2000 mg/kg under the conditions of this study.

3.8. In Vivo Antidiabetic Activity

An alloxan induced diabetic rat model was used to assess the antidiabetic potential of the syzigium cumini silver nanoparticles. To give a thorough grasp of therapy efficacy, the study evaluated important pharmacological characteristics such as body weight, serum biochemical indicators, oxidative strees indices, and fasting blood glucose (FBG).

3.8.1 Fasting Blood Glucose (FBG)

Two way ANOVA and Bonferroni's multiple comparison test were used to analyze the fasting blood glucose levels of the experimental groups. The results showed a statistically significant difference between the groups.

Figure 5 illustrates that the normal control group maintained stable fasting blood glucose levels throughout the experimental period, ranging from approximately 90 100 mg/dl, indicating normal glucose homoeostasis. In contrast, the disease control group exhibited a progressive increase in fasting blood glucose level from approximately 320 mg/dl on day 1 to around 410 mg/dl by day 21, confirming persistent hyperglycemia in untreated diabetic animals. The standard drug metformin (150 mg/kg) treated group showed an initial increase in blood glucose levels up to approximately 250 mg/dl on day 7, followed by a gradual and significant reduction to approximately 120 mg/dl on day 21, demonstrating the expected antihyperglycemic effect of the standard drug. Similarly, the EJ SNP treated group had an initial fasting blood glucose level of approximately 96 mg/dl on day 1, which increased to around 220 mg/dl on day 7 after diabetes induction. Subsequently, blood glucose levels declined significantly to approximately 134 mg/dl on day 14 and further to approximately 118 mg/dl on day 21. These findings indicate that the EJ SNP produced a marked reduction in fasting blood glucose levels, with an antihyperglycemic effect comparable to, a numerically slightly greater than that of metformin under the experimental condition of the study.

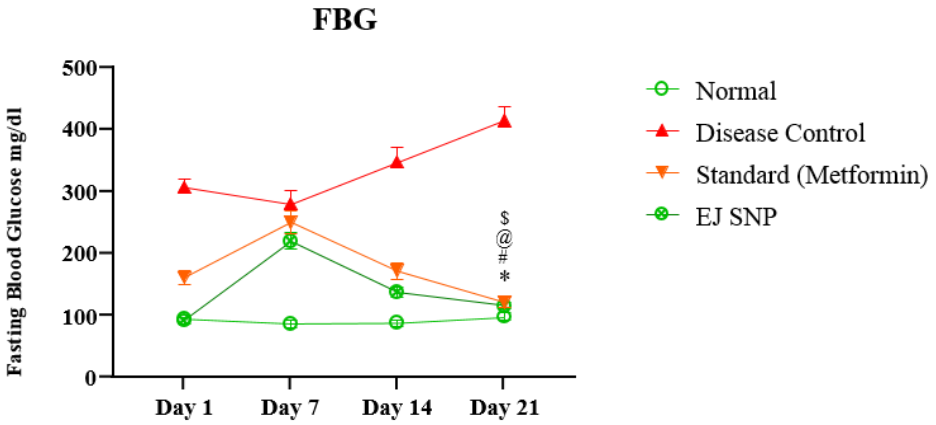


Fig. 5: *Effect of treatments on fasting blood glucose (FBG) levels over 21 days.*

3.8.2 Body Weight

A statistically significant difference in body weight was found between the experimental groups, suggesting metabolic changes related to diabetes and treatment strategies.

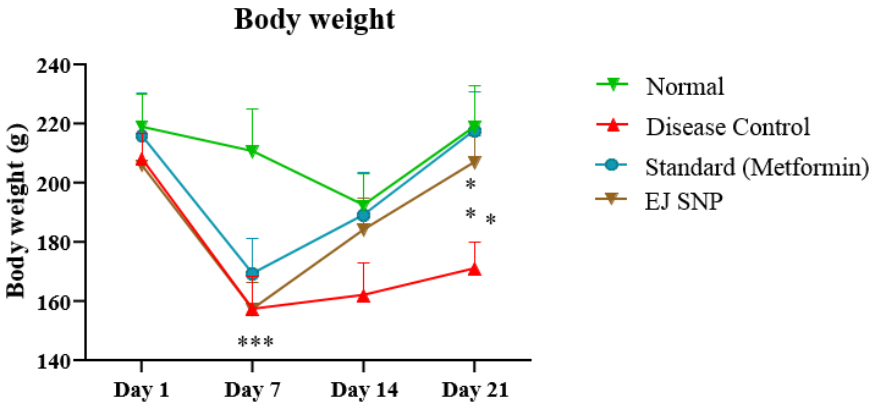


Fig.6: Treatments effect on body weight of experimental animals.

The diabetic control group's body weight was significantly lower than that of the normal control group ($p < 0.0001$), which is consistent with increased protein catabolism and decreased glucose consumption linked to insulin insufficiency. On the other hand, body weight was dramatically restored ($p < 0.0001$), following therapy with the silver nanoparticles which is comparable to the standard drug metformin. This improvement suggest improved metabolic homeostasis, less muscular atrophy, and, improved glycemic management. The observed effect may be attributed to improved insulin sensitivity and better utilization of glucose by peripheral tissues.

3.8.3 Serum Biochemical Parameters

Serum biochemical investigation was performed to evaluate the effect of the silver nanoparticles on lipid metabolism and organ function.

3.8.3.1. Effect on Total Cholesterol

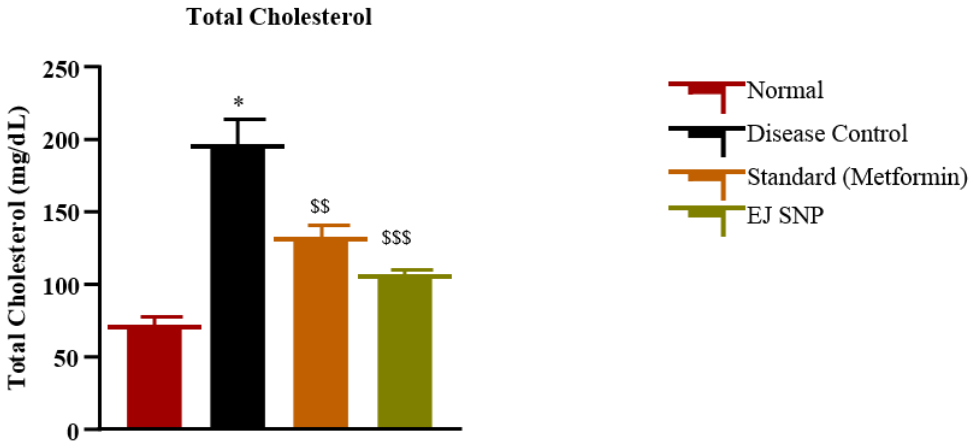


Fig. 7: Treatments effect on levels of total cholesterol in diabetic rats.

One way ANOVA and Bonferroni's test were used to examine the total cholesterol levels of experimental groups. The result showed a statistically significant difference ($p < 0.0001$). Diabetes associated dyslipidemia was demonstrated by the diabetic control group's significantly higher cholesterol levels as compared to the normal control group. Similar to metformin, treatment with silver nanoparticles significantly lowered cholesterol levels ($p < 0.0001$) demonstrating potent antihyperlipidemic efficacy. This impact could be explained by better insulin action, reduction of cholesterol production and regulation of lipid metabolism.

3.8.3.2. Effect on Triglycerides

Diabetic rats showed significantly higher triglyceride levels ($p < 0.0001$) indicating impaired lipid metabolism. Silver nanoparticles administration significantly reduced triglyceride levels ($p < 0.0001$), indicating the restoration of lipid homeostasis. The decrease in triglycerides could be attribute to increased lipoprotein lipase activity and decreased hepatic lipid production mediated by phytoconstituents.

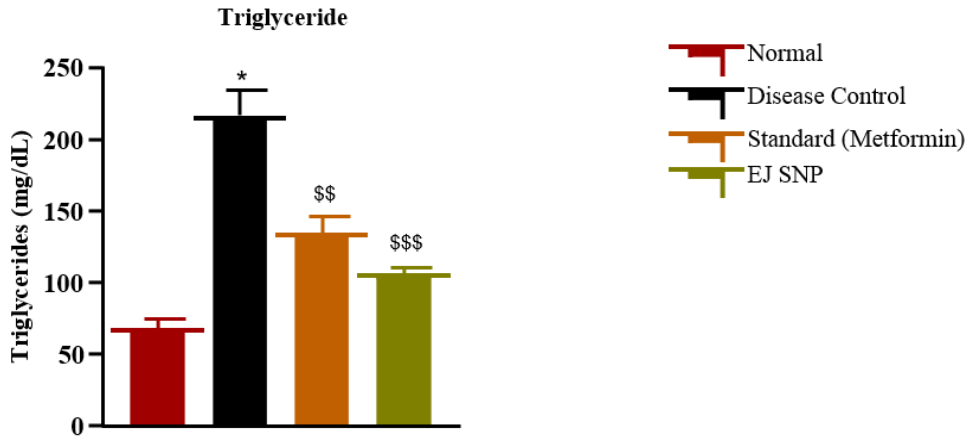


Fig. 8: Treatments effect on levels of triglyceride in experimental groups.

3.8.3.3. Effect on Serum Creatinine

Increased serum creatinine levels in diabetic control animals suggested renal damage caused by prolonged hyperglycemia. Treatment with EJ SNP silver nanoparticles significantly decreased creatinine levels ($p < 0.0001$), indicating a renoprotective

effect. This protective effect could be due to antioxidant activity and decreased oxidative stress mediated damage to renal tissues.

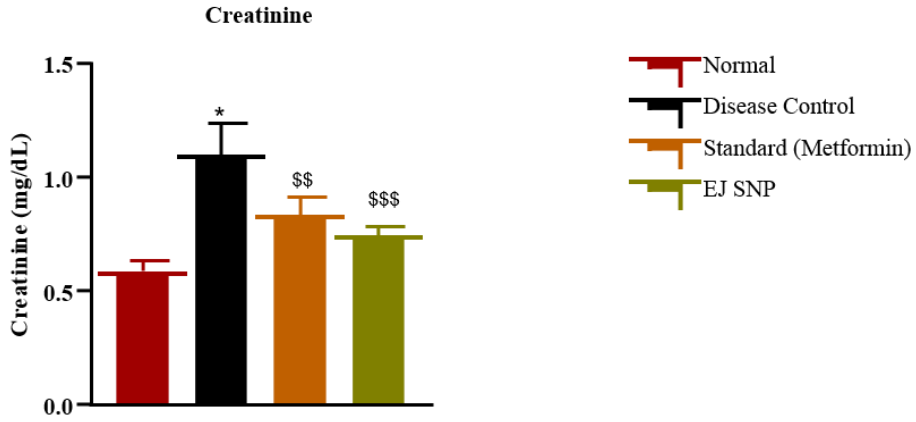


Fig. 9: Treatments effect on levels of serum creatinine.

3.8.4 Oxidative Stress Markers

3.8.4.1. Effect on SOD and GSH

Diabetic control animal had considerably lower SOD and GSH ($p < 0.0001$), indicating weakened antioxidant defense system. EJ SNP (Syzigium Cumini) silver nanoparticles treatment showed considerable restoration of SOD and GSH levels ($p < 0.0001$), indicating strong antioxidant capability.

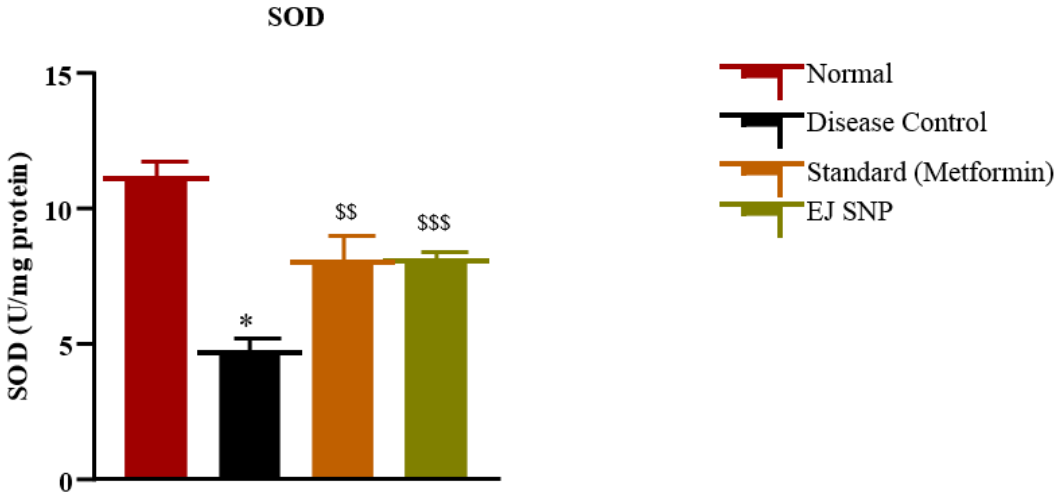


Fig. 10: Treatments effect on levels of superoxide dismutase (SOD) activity.

The restoration of antioxidants enzyme indicates that the formulation effectively scavenges reactive oxygen species (ROS) and boosts endogenous antioxidants defense system, resulting in better glycemic control and protection against diabetes complications.

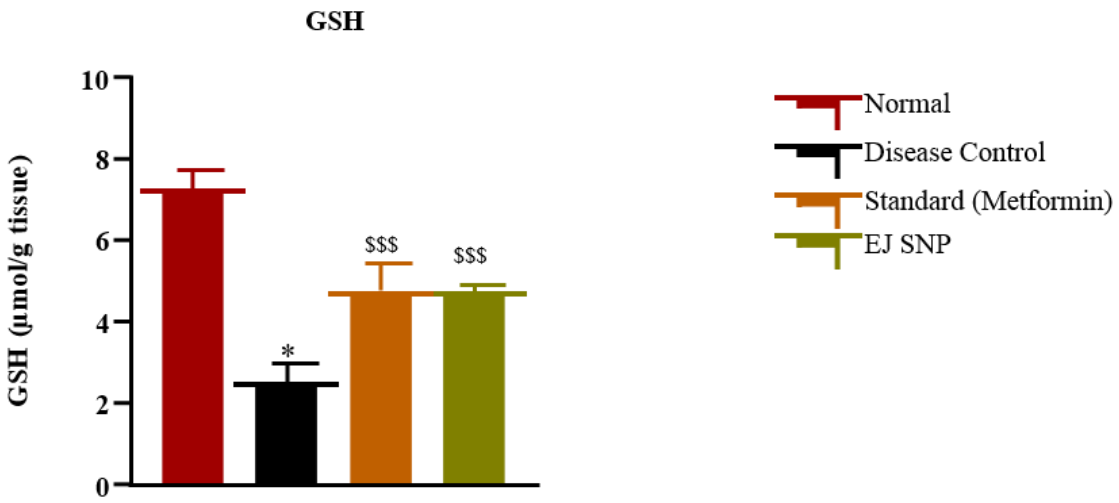


Fig. 11: Treatments effect on levels of reduced glutathione (GSH).

3.8.4.2. Effect on MDA (Lipid Peroxidation Marker)

Diabetic control rats showed considerably higher MDA levels ($p < 0.0001$), indicating greater lipid peroxidation and oxidative damage.

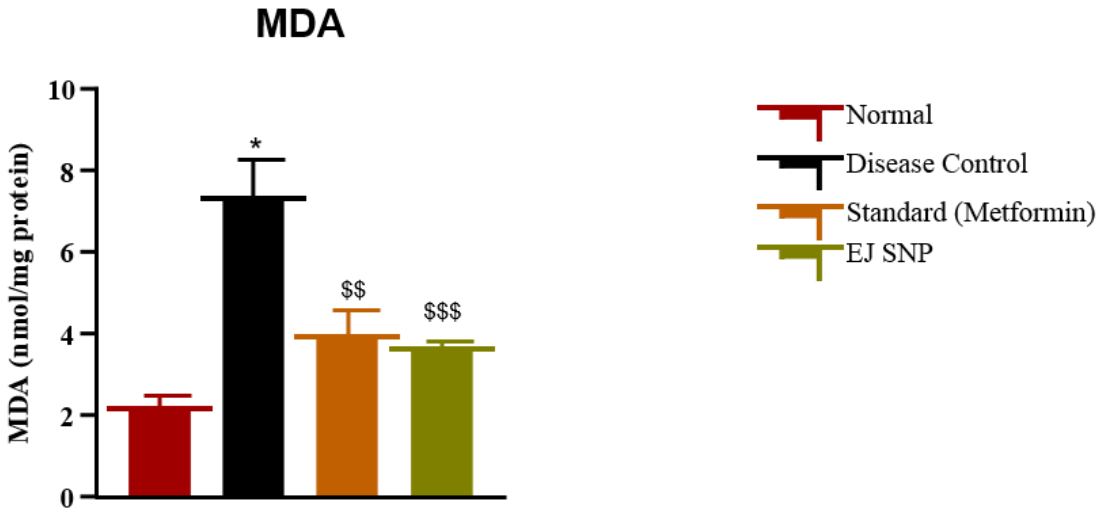


Fig. 12 Treatments effect on levels of malondialdehyde (MDA) levels.

The EJ SNP treatment significantly lowered MDA levels ($p < 0.0001$), indicating its capacity to minimize oxidative stress and protect cellular components.

4. CONCLUSION

The present study successfully synthesized AgNPs incorporating the ethanolic seed extract of *Eugenia jambolana* using a green synthesis approach. The synthesized AgNPs were confirmed through comprehensive physicochemical characterization including particle size, zeta potential, SEM, XRD which collectively revealed the successful nanoparticle formation stability and reproducibility. The acute oral toxicity study indicated that the synthesized silver nanoparticles were well tolerated at the tested dose, Pharmacological evaluation in the alloxan induced diabetic rat model revealed that the synthesized AgNPs exerted significant anti hyperglycemic activity, by lowering fasting blood levels comparable to metformin and preventing diabetes associated body weight loss. In addition treatment restored lipid profile, demonstrated a renoprotective effect

through reduced serum creatinine levels, and significantly attenuated oxidative stress by decreasing MDA levels while restored endogenous antioxidant defense by enhancing SOD and GSH activity. The enhanced therapeutic efficacy may be attributed to the bioactive phytochemicals present in synthesized *Eugenia jambolana* mediated AgNPs together with the unique physicochemical properties of AgNPs which can improve stability, bioavailability, and biological interaction. Collectively, these findings successfully synthesized the AgNPs as a promising, stable, and effective eco-friendly nanotherapeutic candidate for the diabetes management, with potential for further clinical translation.

Conflict of interest

The authors have no conflict of interest to declare.

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Authors Contribution

Asif Husain Ansari: Conceptualization, writing original draft; methodology, data curation, formal analysis, investigation, writing review and editing. **Sufiyan Ahmad:** Conceptualization, writing original draft; Funding acquisition, methodology, data curation, formal analysis, investigation, project administration, supervision, writing review and editing.

Abbreviations

AgNO3 – Silver Nitrate

AgNPs – Silver nanoparticles

ALT – Alanine Aminotransferase

ANOVA – Analysis of Variance

BCG Bacillus Calmette–Guérin

BSI – Botanical Survey of India

BUN – Blood Urea Nitrogen

CB2 Cannabinoid Receptor Type 2

CMC – Carboxymethyl Cellulose

CPCSEA – Committee for the Purpose of Control and Supervision of Experiments on Animals

CV Cardiovascular

DM – Diabetes mellitus

DLS – Dynamic Light Scattering

E. jambolana Eugenia jambolana

EJ SNP – *Eugenia Jambolana* silver nanoparticles

FBG – Fasting blood glucose

FCC – Face Centered Cubic

GCP – Good Clinical Practice

GSH – Reduced glutathione

IAEC – Institutional Animal Ethics Committee

MDA – Malondialdehyde

OECD – Organization for Economic Cooperation and Development

Po *Per os* (Oral Administration)

ROS – Reactive oxygen species

SEM – Scanning Electron Microscopy

SOD – Superoxide dismutase

TC – Total cholesterol

TG – Triglycerides

XRD – X ray Diffraction

MCC – Microcrystalline cellulose

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