

In vivo Therapeutic Potential of Biologically Synthesized Silver Nanoparticles

Kalakotla Shanker, G. Krishna Mohan

Abstract—Nowadays, nanoparticles are being used in pharmacological studies for their exclusive properties such as small size, more surface area, biocompatibility and enhanced solubility. In view of this, the present study aimed to evaluate the antihyperglycemic potential of biologically synthesized silver nanoparticles (BSSNPs) and *Gymnema sylvestre* (GS) extract. The SEM and EDAX analysis divulges that the BSSNPs were spherical in shape. EDAX spectrum exhibits peaks for the presence of silver, carbon, and oxygen atoms in the range of 1.0-3.1 keV. FT-IR reveals the binding properties of active bio-constituents responsible for capping and stabilizing BSSNPs. The results showed increased blood glucose, huge loss in body weight and downturn in plasma insulin. The GS extract (200 mg/kg, 400 mg/kg), BSSNPs (100 mg/kg, 200 mg/kg) and metformin 50 mg/kg were administered to the diabetic rats. BSSNPs at a dose level of 200 mg/kg (b.wt.p.o.) showed significant inhibition of ($p < 0.001$) blood glucose levels as compared with GS extract treated group. The results obtained from study indicate that the BSSNP shows potent anti-diabetic activity.

Keywords—BSSNP, *G. sylvestre*, wistar rats, antihyperglycemic activity.

I. INTRODUCTION

DIABETES is the most common chronic non-communicable disease, approximately 85-95% of all cases are type 2 diabetes, and the global explosion of this disease is an important health care freight. It is estimated that nearly 380 million people worldwide will have diabetes by 2025 [1]. In contemporary years, BSSNPs have enticed the scientific community in the meadow of nanotechnology and pharmacology due to their distinctive characters and biological applications. Green biosynthesis of nanomaterials is considered as non-toxic, environmental friendly methods analogize to chemical method [2]. The BSSNPs have been extensively used in many biological applications such as anti-diabetic activity [3], anti-cancer activity [4] and also used in industries. Nanotechnology-based methodologies hold significant potential for enhancing the compliance of patients with diabetes [5]. Thus, herbal remedies encircling anti diabetic potential may serve as a relevant and intact alternative or as an adjunct candidate in the management of diabetes.

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GS is an indigenous plant, and traditionally, it has got great importance since ancient times. GS is belonging to family *Asclepiadaceae*, and the therapeutic properties of GS have been well documented [6]. However, there are no reports available on *in-vivo* anti-diabetic activity of silver nanoparticles synthesized from GS plant. The current study was thus focused on intense to synthesize biological silver nanoparticles by simple, single, efficient method using gymnemic acid of GS. The BSSNPs were tested against STZ induced diabetic wistar rats model.

II. MATERIALS AND METHODS

A. Materials

AgNO₃ was purchased from SR Life Science Pvt. Ltd, Hyderabad, India. Gymnemic acid has been procured from ICT Hyderabad. All other chemicals used in the reaction were of analytical grade. Fresh leaves of GS were collected from Dawasaaz medicinal plants, Hyderabad, India. Streptozotocin purchased from SRL Private Limited.

B. Biosynthesis of Silver Nanoparticles

10 ml of filtered Gymnemic acid solution was added drop wise to 45 ml of 1 mM AgNO₃ solution and stirred vigorously with the help of a magnetic stirrer for 6, 12, and 24 hours. The conical flasks were incubated at room temperature for 12-24h (Figs. 1 (A)-(C)). A color change of the solution from pale yellow to dark brown was monitored. The fully reduced solution has been centrifuged at 5000 rpm for 20 minutes. The residue was retained after discarding the supernatant. The final residue has been washed with sterile distilled water and dried.

C. Characterization of BSSNPs

Synthesized BSSNPs were characterized by using High Resolution Transmission Electron Microscopy and X-ray Diffraction.

D. Experimental Animals

Male Wistar rats, weighing between 160-200 gm, were obtained from Mahaveer enterprises, Hyderabad, India. Protocol for conducting animal study was approved by Institutional Animal Ethical Committee (IAEC) through its reference number 1684/PO/a/13/CPCSEA. The selected animals were housed six per each of acrylic cages at 25 °C, 45-55% humidity and 12h/12h light/dark under controlled environment. Rats were fed with standard laboratory diet and water was given *ad libitum*.

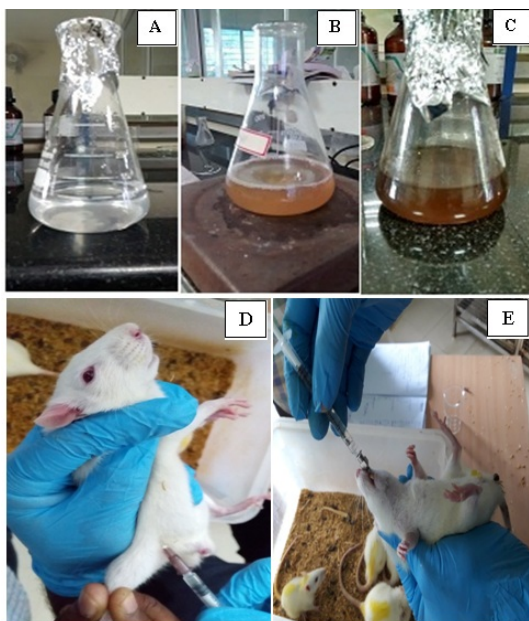


Fig. 1 (A) Formation of BSSNPs at different time intervals, i.e. 0 hour, (B) 12 hours, (C) 24 hours, (D) Injection pattern of STZ (E) Dosing pattern of BSSNPs and extract

E. Induction of Diabetes

Prior to the experiment, freshly prepared solution of streptozotocin (40 mg/kg in 0.1 M citrate buffer, pH 4.5) was injected intraperitoneally to overnight fasted Wistar rats (Figs. 1 (D) and (E)). The rats elicited hyperglycemia after 48 hours of STZ administration [7], [8]. The rats with blood glucose levels of 250 mg/dl or above were chosen for the study.

F. Experimental Design

The experiment has been carried out in seven groups of six rats each:

- Group I- Normal control rats received saline.
- Group II- Diabetic control received STZ (40 mg/kg)
- Group III- Diabetic rats treated with Metformin (50 mg/kg).
- Group IV- Diabetic rats treated with GS extract (200 mg/kg).
- Group V- Diabetic rats treated with GS extract (400 mg/kg)
- Group VI- Diabetic rats treated with BSSNP (100 mg/kg)
- Group VII- Diabetic rats treated with BSSNP (200 mg/kg)

F. Statistical Analysis

Results were expressed as mean \pm S.E.M. For statistical analysis of the data group, mean was compared by a one-way analysis of variance (ANOVA) followed by Dunnett's test. $p < 0.001$ was considered to be statistically significant.

III. RESULTS AND DISCUSSION

A. Characterization of BSSNPs

The crystalline nature of the BSSNPs was demonstrated using X-ray diffraction analysis. The SEM images confirm

that the obtained BSSNPs were at nano-scale and spherical in shape, most of them being mono-dispersed (Figs. 2 (A) and (B)). The BSSNPs showed strong absorption peak at 440 nm in UV spectroscopy (Fig. 3) [9]. The EDAX profile apparently delineates the optical absorption of silver. Silver is present at 2.5 keV, carbon and potassium present at 1.0 and 1.2 keV, respectively (Fig. 4). The functional groups present in BSSNPs were identified by FT-IR analysis. The phenolic group present in the GS has shown an affinity binding with the BSSNPs. Hence, it was substantiated that the phytochemical contents may be attached with the BSSNPs during the green synthesis process (Fig. 5).

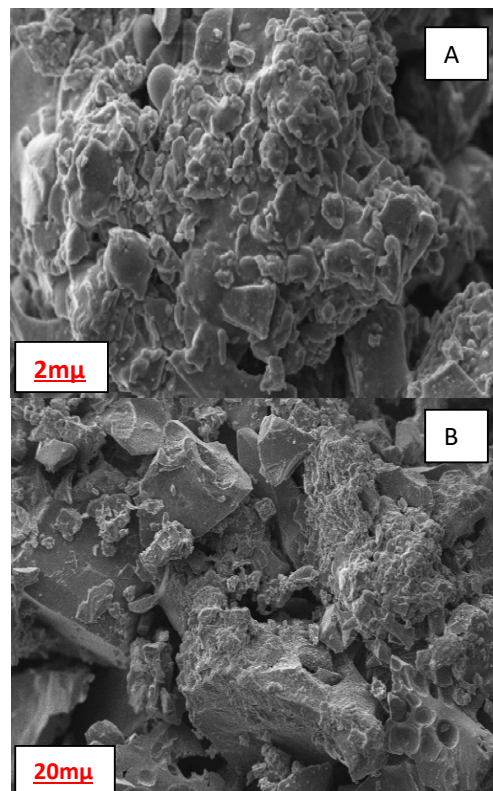


Fig. 2 (A) and (B) SEM micrographs of BSSNPs at different magnifications

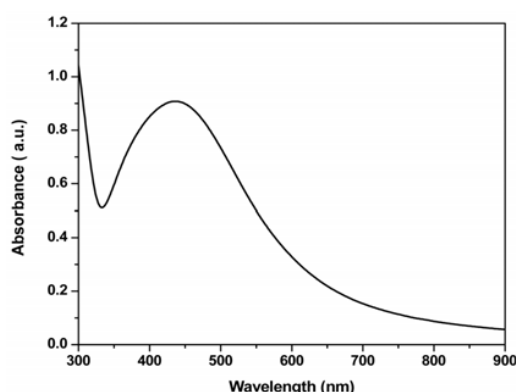


Fig. 3 UV spectrum of BSSNP

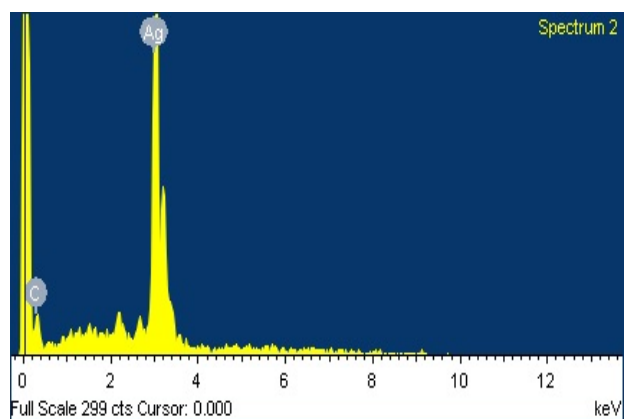


Fig. 4 EDAX spectrum of BSSNP

B. Bioactivity of BSSNPs

1. Effect of GS Extract and BSSNPs on Glucose Levels of STZ Induced Diabetic Rats

GS has profound effect in controlling diabetes by regulating blood glucose level as reported by previous researchers on rats [10]. It ought to be noticed that diabetic rats treated with BSSNPs altogether ($p < 0.001$) brought the parameters to normal level, demonstrating the beneficial outcome of BSSNPs (Table I). The enhanced activity of BSSNPs over

crude extract in controlling glucose levels may be due to their greater surface area, thus increasing the surface area phenomena and may increase the pharmacokinetic properties from pharmacological prospective. The impacts of oral hypoglycemic medications rely upon numerous kinetic parameters, for example absorption, metabolism and excretion, and the action of drug starts from inside the cell, thus it is trusted that BSSNPs are small in size and can easily carried across the cell membrane by transport mechanism and may evoke the huge biological effect [11].

2. Effect of GS Extract and BSSNPs on Serum Insulin Level in Diabetic Rats

Fasting insulin levels in BSSNPs treated diabetic rats restored the levels significantly ($p < 0.001$) to normalcy as compared to diabetic control rats (Table I). In the present study, STZ significantly induced hyperglycemia and oral administration of BSSNP for a period of 14 days produced a significant reduction in blood glucose levels. The promising mechanism by which BSSNPs mediated its anti-diabetic effect could be by potentiation of pancreatic secretion of insulin from existing β -cells of islets, as was apparent by the critical increment in the level of insulin in the BSSNPs treated animals.

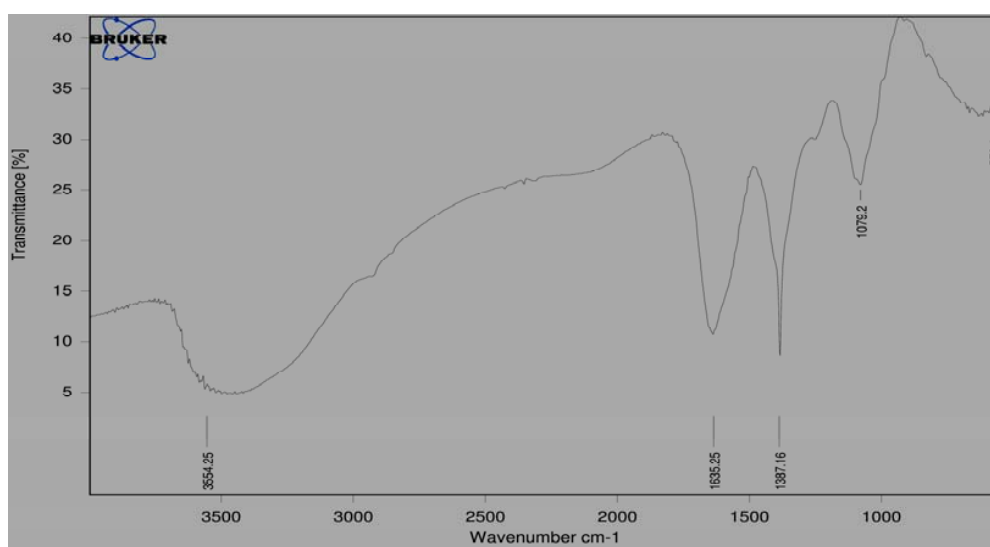


Fig. 5 FTIR spectrum of BSSNP

TABLE I
EFFECT OF GS EXTRACT AND BSSNPs ON BLOOD GLUCOSE AND INSULIN LEVELS IN STZ INDUCED DIABETIC RATS

Treatment and dose	Day 0	Day 4	Day 7	Day 11	Day 14	Insulin levels (μ U/ml) (day 14)
Normal Control	95.40 \pm 3.80	95.83 \pm 3.01	99.05 \pm 3.10	98.97 \pm 1.45	99.19 \pm 0.86	89.13 \pm 0.51
Diabetic control (40 mg/kg)	272.65 \pm 5.05 ^a	268.33 \pm 4.01 ^a	272.89 \pm 3.95 ^a	269.46 \pm 3.13 ^a	270.99 \pm 4.76 ^a	4.14 \pm 0.83 ^a
DC+ Metformin (250 mg/kg)	258.75 \pm 13.87 ^a	201.57 \pm 1.99	152.65 \pm 7.14	125.21 \pm 2.54	112.20 \pm 2.98	4.00 \pm 0.46
DC+ Extract (200 mg/kg)	271.08 \pm 5.08	253.08 \pm 2.01	241.08 \pm 3.99	240.05 \pm 3.27	230.55 \pm 3.70 ^{NS}	5.23 \pm 0.43
DC+ Extract (400 mg/kg)	269.76 \pm 3.59	244.43 \pm 2.81	230.40 \pm 4.21	211.56 \pm 3.90	174.78 \pm 4.12	4.80 \pm 0.47
DC+ BSSNPs (100 mg/kg)	263.13 \pm 2.21	243.56 \pm 3.10	238.27 \pm 1.92	223.55 \pm 2.90	169.32 \pm 3.99 ^a	6.12 \pm 0.82 ^a
DC+ BSSNPs (200 mg/kg)	259.98 \pm 10.99	203.55 \pm 5.75	159.22 \pm 2.4 ^a	136.44 \pm 6.14 ^a	117.07 \pm 5.21 ^a	8.59 \pm 0.83 ^a

The data are expressed as mean \pm S.E.M.; $n=6$ in each group. ^a $p < 0.001$, significant when compared to diabetic control.

IV. CONCLUSION

As per experiment findings, administration of the BSSNPs has intense antihyperglycemic property and could likewise improve different entanglements of diabetes. Current global excitement in the use of eco-friendly and cost effective resources drives direct the application of highly hailed medicinal plants to the green synthesis of nanoparticles that acquire diverse pharmacological properties. Accordingly, the BSSNPs were synthesized using gymnemic acid of *GS* plant and characterized using XRD and HRTEM. In addition, the hypothesis for the synthesis of BSSNP was to associate BSSNPs (enhanced biological properties) with a natural GS and to increase the surface area of drug to achieve greater and potent pharmacological activity over extract. Hence, it is recommended BSSNPs to be utilized as compelling nanomedicine for treatment of diabetes mellitus; however, further pharmacokinetic studies are empowered.

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