Green Biosynthesis, Characterization, In vitro Antidiabetic Activity, and Investigational Acute Toxicity Studies of Some Herbal-mediated Silver Nanoparticles on Animal Models

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ABSTRACT
Diabetes is a metabolic disorder characterized by hyperglycemia, altered carbohydrate, lipid and protein metabolism. In recent studies, Nanoscience and nanotechnology are blazing fields for researchers; for researchers; of late there has been a prodigious excitement in the field of nanopharmacology to study silver nanoparticle (SNP) synthesis using natural products. Biological methods have been used to synthesize SNPs using medicinally active plants having an antidiabetic role, and this made us to assess the biologically synthesized SNPs from the seed extract of Psoralea corylifolia using 1 mM silver nitrate solution. The synthesized herbal-mediated SNPs (HMSNPs) were subjected to various characterization techniques such as X-ray diffraction analysis (XRD), energy dispersive X-ray (EDX) analysis, transmission electron microscope (TEM), and differential light scattering (DLS), respectively. In the current study the HMSNPs were tested to observe the in vitro antidiabetic activity and possible toxic effects in healthy female albino mice by following OECD guidelines-425. Huge data from biochemical, cellular, mouse, and chemical inhibitor studies have recognized protein tyrosine phosphatase 1B (PTP1B) as a major negative regulator of insulin signaling. In addition, corroboration suggests that insulin action can be enhanced by the inhibition of PTP1B. Keeping in view of the above fact, the PTP1B assay was done to determine the PTP1B inhibitory effect of HMSNPs. It can be concluded that medicinal plants can be a good source for the synthesis of HMSNPs. This study can be used for the development of valuable nanomedicines to treat various ailments, and it also highlights the safety and biocompatibility of SNPs within a biological cell; in vivo parameters need to be considered for further discoveries.

Key words: Female albino mice, herbal-mediated silver nanoparticles (HMSNPs), 425 OECD guidelines, protein tyrosine phosphatase 1B (PTP1B) in vitro antidiabetic assay, Psoralea corylifolia, toxicity of silver nanoparticles.

SUMMARY
In present research, acute oral toxicity studies and in vitro anti diabetic activity of Herbal mediated silver nanoparticles (HMSNPs) has been investigated. Characterization techniques employed to determine the crystallinity, size, shape and elemental composition of HMSNPs. The results obtained from acute oral toxicity studies and histopathological studies showed that the synthesized HMSNPs were non-toxic and safe. and also had good in vitro anti diabetic activity. The results would provide certain references to screen out more pharmacological activities of silver nanoparticles using green biosynthesis methods.

INTRODUCTION
Diabetes mellitus is an unending metabolic issue in which commonness has been expanding relentlessly everywhere throughout the world. As a consequence of this pattern, it is quick turning into a pandemic in a few nations of the world with the quantity of individuals influenced anticipated that would twofold in the following decade because of expansion in maturing populace, in this way adding to the effectively existing weight for human services suppliers, particularly in ineffectively created nations. Two forms of diabetes (types 1 and 2) differ in their pathogenesis, but both have hyperglycemia as a common character.
In type 2 diabetes, hyperglycemia is caused due to impairment in insulin secretion combined with or without impairment of insulin action.\(^\text{[10]}\) WHO reported that the global population is in the midst of a diabetes epidemic. The people in South Asia and Western Pacific have been under greater risk, and the majority of patients have type 2 diabetes. The development of biological methods for the synthesis of silver nanoparticles (SNPs) is evolving into an important branch of Nanoscience and technology. Several methods of synthesizing SNPs such as chemical reduction of silver ions in aqueous solutions,\(^\text{[4]}\) photo reduction in reverse micelles, and radiation chemical reduction\(^\text{[8]}\) have been reported in previous nanoscience and technology area of research. Most of these methods involve the use of hazardous, toxic chemicals that may possess biological risks. Biological methods can be adopted for the synthesis of SNPs using microorganisms\(^\text{[6]}\) and plant extracts,\(^\text{[7,8]}\) which have been suggested as potential environmental-friendly alternatives compared with physical and chemical methods.

Silver has long been recognized as a useful metal having several commercial benefits as well as pharmacological benefits, such as antibacterial, antidiabetic,\(^\text{[9]}\) etc. Physical and chemical properties of herbal-mediated SNPs (HMSNPs) have raised a concern that nanoparticles synthesized from herbs may interact in new unknown ways with the biological system. *Psoralea corylifolia* is a widely used medicinal plant shown in Figure. 1, plant is commonly known as Baguchi in Sanskrit and Bavachi in Hindi. This plant has been used since many years for its antidiabetic, antioxidant, antiacne, and antidermatitis effects.\(^\text{[10,11]}\)

Although there have been numerous toxicological studies performed and articles published on nanoparticles, it is still difficult to write definite conclusions about their toxicity, as the number of experiments have been performed without thorough characterization and description of the nanoparticles and solvents used under different experimental conditions. Yet it is not clear to which degree the obtained silver ions show toxicity.\(^\text{[12,13]}\) Though the toxicity of silver ions (chemically synthesized) is known, determination of the dose at which SNPs produce toxic effect in a biological cell is a principal criterion. Keeping in view of the above criteria, SNPs must be synthesized by biological methods using different plant extracts for reducing toxicity. Therefore, we investigated the toxicity of HMSNPs by following OECD guidelines 425 (OECD Guideline (2001)).\(^\text{[14]}\)

### MATERIALS AND METHODS

Silver nitrate (AgNO\(_3\)) was procured from SR Life Science and used as a precursor for the synthesis of HMSNPs. Millipore water was used throughout the reactions. All glass wares were washed with dilute HNO\(_3\) and distilled water.

### Plant material collection and soxhlet extraction

The seeds of *P. corylifolia* were obtained from an authorized medicinal plant supplier in Hyderabad, Telangana; 250 g of powdered seeds of *P. corylifolia* was placed in the body tube of the soxhlet extractor and successive solvent extraction was done for 18 h in an increasing order of polarity using hexane, chloroform, and water. Methanol and water extracts were concentrated in the rotary evaporator (Heidelberg; Schwabach, Nuremberg, Germany) and crude extract was kept in the desiccator. Further preliminary phytochemical analysis was done using standard test procedures to confirm the availability of active phytochemicals in the plant extract has been reflected in Table 1.

### Synthesis of HMSNPs

The crude extract was filtered using Whatman filter paper no 1 and 10\(^-2\) mM of AgNO\(_3\) solution was prepared and stored in brown bottles. Conical flasks were incubated at room temperature. The color change of the seed extracts from pale yellowish green to dark brown was checked periodically. The formation of dark brown color suggested that the HMSNPs synthesized from herbs the formed nanoparticles centrifuged at 5000 rpm for 15 minutes.

### Characterization of the synthesized HMSNPs

1. X-ray diffraction (XRD) analysis: Crystalline nature of the SNPs was confirmed by XRD analysis.
2. Differential light scattering (DLS) analysis: The size distribution and average size of the HMSNPs determined by DLS. Malvern DLS instrument used for the current study.
3. Transmission electron microscope (TEM) analysis: Morphology of the HMSNPs was determined by using TEM.
4. Energy dispersive X-ray (EDX) analysis: Elemental composition was determined by EDX analysis. This analysis provided information regarding the presence of silver in the test sample.

### Acute oral toxicity studies

Sensitive female albino mice, weighing 20–30 g, were procured from National Institute of Nutrition, Hyderabad. The animals were housed in clean cages and had free access to standard pallet diet and water. During the experiment, the mice were kept under

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**Table 1 : Phytochemical profile of *P. corylifolia***

| S. No | Name of the test     | HE | CE | WE |
|-------|----------------------|----|----|----|
| 1     | Test for Alkaloids   | –  | –  | –  |
| 2     | Test for carbohydrates| –  | –  | –  |
| 3     | Test for Saponin Glycoside | +  | +  | +  |
| 4     | Test for Proteins    | +  | +  | +  |
| 5     | Test for Volatile oils| –  | –  | –  |
| 6     | Test for fats and fixed oils| –  | –  | –  |
| 7     | Test for Steroids    | –  | –  | –  |
| 8     | Test for Flavonoids  | +  | +  | +  |
| 9     | Test for Tannins     | –  | –  | –  |

CE = chloroform extract, HE = hexane extract, WE = water extract
a controlled environment of 12-h light/dark cycle. All the animals were accommodated to laboratory conditions for 1 week prior to commencement of the research work. Present study proceed from the guidelines outlined in the OECD 425, after getting an approval from the Institutional Animal and Ethics Committee (IAEC) at Department of Pharmacology and Toxicology, Pullareddy Institute of Pharmaceutical Sciences, Hyderabad, Telangana.

Albino mice breed weighing 20–30 g were used in Central Animal House facility of Pullareddy institute of Pharmacy, Hyderabad (Reg. No. 1684/PO/a/13/CPCSEA). All the animals were maintained under standard laboratory conditions, such as temperature 20°C ± 2°C, humidity 45–55%, and a 12 : 12 h light/dark cycle. The animals had free access to standard pellet diet (Amrut feeds, Bangalore), with water provided ad libum under strict hygienic conditions.

Selected animals were divided into 3 groups of 3 animals in each. The normal control and control group received normal distilled water (2 mL/kg) and SNPs, respectively. The other groups received graded dose (100, 200, 300, 600, 800, 1000, and 2000 mg/kg) of the HMSNPs, respectively. Immediately after dosing of drug, the animals were investigated regularly for the first 4 h for any behavioral changes and death, if any, intermittently for the next 6 h, then again at 24 h after dosing. They were then kept under observation for up to 14 days after drug administration to identify mortality, if any. The observations were made twice daily, one at 8 a.m. and another at 8 p.m. (T Ghosh, 2007 et al., and OECD 2001 - Guideline on Acute oral toxicity (AOT).[15]

Histopathological study

On the 15th day, the selected group of mice was killed with 30 mg/kg Phenobarbital administered intra peritoneally, and organ tissues such as the heart, liver, and kidney were surgically detached for performing histopathological studies. The isolated sections were examined carefully under the microscope. The histopathological changeover deviants from the normal were carefully recorded.

In vitro antidiabetic activity

Phosphatase 1B (PTP 1B) inhibitory assay: PTP inhibitory activity was carried out with respect to the modified method of Goldstein et al.[15] The liver homogenate of rat was used as a source of PTP 1B. The whole liver was quickly removed from rat, and 100 mg of wet hepatic tissue was placed in the ice-cold 0.25 m solution of sucrose. The mixture was homogenized at 4°C for 1 min and diluted to 2 mL/100 mg wet liver with the solution of sucrose. The homogenate was centrifuged at 4°C 12000 g for 30 min. The supernatant fluid was collected and frozen for the assay. The test compounds (5–50 μM, 5 μL) were preincubated with liver homogenate (3 μL) in HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid) buffer (total volume 50 μL) for 30 min. Drug assay was performed in a final volume of 200 μL in a test mixture containing 10 mM of p-nitro phenyl phosphate in 50 mM HEPES buffer with pH 7.0. Postliminary 10 min of incubation at 37°C, the reaction was stopped by the addition of 50 μL of 0.1 N sodium hydroxide and the absorbance was detected at 410 nm. Sodium orthovanadate was used as the standard for this enzyme assay.

RESULTS AND DISCUSSION

Transmission electron microscope analysis

Figure 2 shows the TEM image of HMSNPs synthesized using P. corylifolia seed extract, which predominates in spherical triangle, cuboidal, and tetrahedral shapes ranging from 15 to 25 nm with an average size of 18.0 nm. Most of the HMSNPs were roughly circular in shape with smooth edges. The phytochemical constituents such as saponin glycoside, alkaloids, proteins, and flavonoids in P. corylifolia seeds may be entrapped and act as reducing agents during the synthesis of HMSNPs.

X-Ray diffraction analysis

Figure 3 represents presence of peaks at 2θ values 28.1°, 33.09°, 47.36°, and 56.29° corresponding to (111), (200), (202), and (311), planes of silver, respectively. Thus, the XRD spectrum confirms the crystalline nature of HMSNPs. No peaks of other impurity crystalline phases were detected.
Differential light scattering
The particle size was analyzed under the category of intensity of laser light on the sample particles. Laser diffraction revealed that the particles obtained are aggregated mixtures with size ranging from 18 to 20 nm, as shown in Figure 4. The average particle diameter was found to be 20 nm.

Energy dispersive X-ray analysis
The energy dispersive spectrum [Figure 5] revealed a clear identification of the elemental composition of the synthesized HMSNPs. From EDX it is clear that the strong signal of silver in the spectra confirms the formation of SNPs.

ACUTE ORAL TOXICITY RESULTS

Histopathology
The result obtained from histopathological sectioning was in agreement as there was no apparent damage to the heart, liver, and pancreas.

Table 2: Dose progression and acute oral toxicity results of Herbal-Mediated silver nanoparticles

| S No | Identification parameters | Group 1 Control | Group 2 SNP | Group 3 HMSNPs |
|------|--------------------------|-----------------|-------------|----------------|
| 1    | Alertness                | Normal          | Normal      | Normal         |
| 2    | Restlessness             | No              | No          | No             |
| 3    | Passive/Active           | Active          | Active      | No             |
| 4    | Aggressiveness           | No              | No          | No             |
| 5    | Tremors                  | No              | No          | No             |
| 6    | Touch response           | Normal          | Normal      | Normal         |
| 7    | Pain response            | Normal          | Normal      | Normal         |
| 8    | Convulsion               | No              | No          | No             |
| 9    | Gripping strength        | Observed        | Observed    | Observed       |
| 10   | Writhing                 | No              | No          | No             |
| 11   | Pupils                   | Normal          | Normal      | Normal         |
| 12   | Urination                | Normal          | Normal      | Normal         |
| 13   | Salivation               | No              | No          | No             |
| 14   | Skin color               | Normal          | Normal      | Normal         |
| 15   | Respiration              | Normal          | Normal      | Normal         |
| 16   | Lacrimation              | No              | No          | No             |
activity than that of HMSNPs. Hence, the HMSNPs showed almost equal percentage inhibition of PTP 1B when compared with the standard compound. This is the first report demonstrating the toxicity parameters of HMSNPs synthesized using *P. corylifolia* and using sensitive Female albino mice. Since the HMSNPs are effective in inhibiting the PTP 1B enzyme, according to the molecular mechanism of insulin, they could be useful in treating type 2 diabetes. Further *in vivo* pharmacological investigations will clearly elucidate the mechanism of action and help in projecting the efficacy of currently synthesized HMSNPs as a therapeutic target in treating type 2 diabetes.

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**Conflicts of interest**

None

**REFERENCES**

1. Devanjee S, Das AK, Sahu R, Gangopadhyay M. Antidiabetic activity of Diospyros *peregrina* fruit: effect on hyperglycemia, hyperlipidemia and augmented oxidative stress in experimental type 2 diabetes. Food Chem Toxicol 2009;47:2679-85.
2. Davis S, Brunton L, Lazo J, Parker K. Insulin, oral hypoglycemic agents and the pharmacology of the endocrine pancreas. In: The Pharmacological Basis of Therapeutics 2006: New York: McGraw Hill; 2006. p 1613.
3. Lin Y, Sun Z. Current views on type 2 diabetes. J Endocrinol 2010;204:1-11.
4. Raveendran P, Fu J, Wallen SL. A simple and “green” method for the synthesis of Au, Ag, and Au-Ag alloy nanoparticles. Green Chem 2006;8:34-8.
5. Sun YP, Atorngitjawat P, Meziani MJ. Preparation of silver nanoparticles via rapid expansion of water in carbon dioxide microemulsion into reductant solution. Langmuir 2001;17:5707-10.
6. ShivaKrishna P, Ram Prasad M, Krishna G, Singara Chaya MA. Synthesis of silver nanoparticles from marine bacteria *Pseudomonas aerogenosa*. Octa J Biosci 2013;1:108-14.
7. Dubey M, Bhaduria S, Kushwah BS. Green synthesis of nano silver particles from extract of *Eucalyptus hybridus* (Safedal leaf). J Nanomater Biobstruct 2009;4:537-43.
8. Jae YS, Beom SK. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. Bioprocess Biosyst Eng 2009;32:79-84; Langmuir 2009;17:2329-33.
9. Swamalatha L, Rachel C, Ranjan S, Baradwaj P. Evaluation of *in vitro* antidiabetic activity of *Sphaeranthus Amaranthoids* mediated silver nanoparticles. Int J Nanomater Biobstruct 2012;2:25-9.
10. Kamboj J, Sharma S, Kumar S. In vivo anti-diabetic and anti-oxidant potential of *Psoralea corylifolia* seeds in Streptozocin induced type-2 diabetic rats. J Health Sci 2011;57:108-14.
11. Kiran B, Raveesha KA. Evaluation of antioxidant potentiality of seeds of *Psoralea corylifolia* L. World Appl Sci J 2010;8:985-90.
12. Landsiedel R, MA-Hock L, Kroll A, Hahn D, Schnekenburger J, Wiench K. Testing metal-oxide nanomaterials for human safety. Adv Mater 2010;22:6017-27.
13. Maynard AD, Warheit DB, Philbert MA. The new toxicology of sophisticated materials: nanotoxicology and beyond. Toxicol Sci 2011;120:Suppl.1 S109-29.
14. OECD Guideline. (2001) on acute oral toxicity (AOT) Environmental health and safety monograph series on testing and adjustment number 425. https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl425-508.pdf
15. Goldstein BJ, Bittner-Kowalczyk A, White MF, Harbeck M. Tyrosine dephosphorylation and deactivation of insulin receptor substrate-1 by protein-tyrosine phosphatase 1B. J Biol Chem 2000;275:4283-9.