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ORIGINAL ARTICLE

# 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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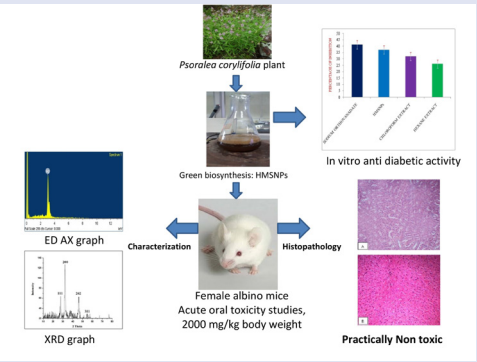
## ABSTRACT

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 PTP1 B inhibitory effect of HMSNPs. It can be concluded that medicinal plants can be a good source for the synthe sis 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 Crystallanity, 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.




**Abbreviations used:** HMSNPs: Herbal mediated silver nanoparticles, XRD: X-ray diffraction, EDX: Energy dispersive X-ray analysis, TEM: Transmission electron microscope, PTP1B: Protein tyrosine phosphotase 1B, OECD: Organization for economic cooperation and development

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## 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[1,2] Two forms of diabetes (types 1 and 2) differ in their pathogenesis, but both have hyperglycemia as a common character.



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**Figure 1:** *Psoralea corylifolia* plant.

In type 2 diabetes, hyperglycemia is caused due to impairment in insulin secretion combined with or without impairment of insulin action.[3] 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,[4] photo reduction in reverse micelles, and radiation chemical reduction[5] 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[6] and plant extracts,[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,[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.[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.[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).[14]

**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

## 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 (Heidolph; 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 Whatmann filter paper no 1 and $10^{-3}$ 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

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 labium 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.

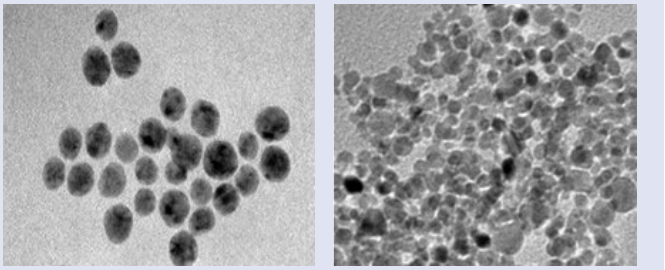

**Figure 2:** TEM image of HMSNP.

# 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.

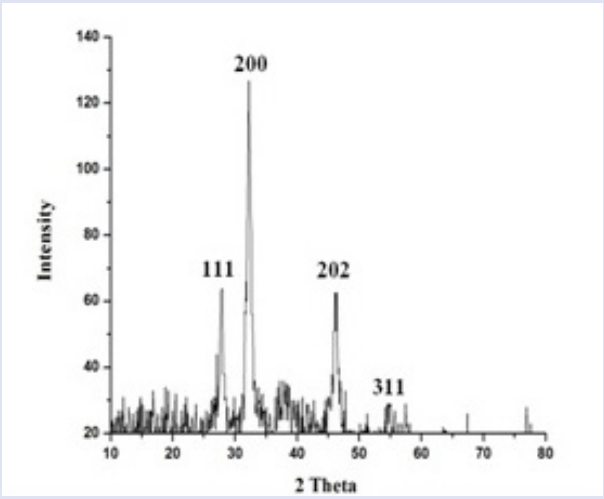


**Figure 3:** XRD graph of HMSNPs.

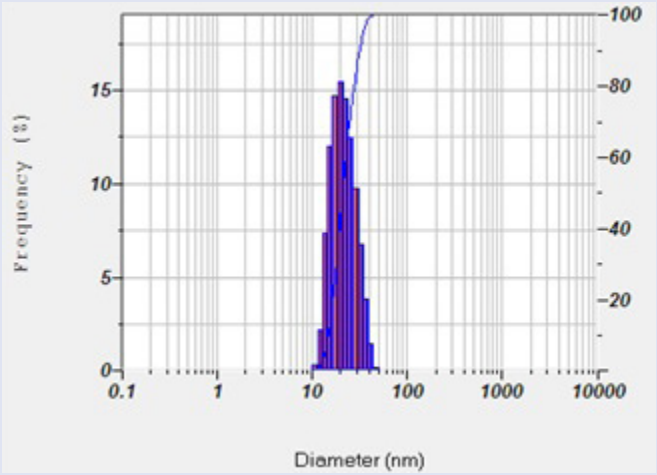


**Figure 4:** DLS graph of HMSNPs.

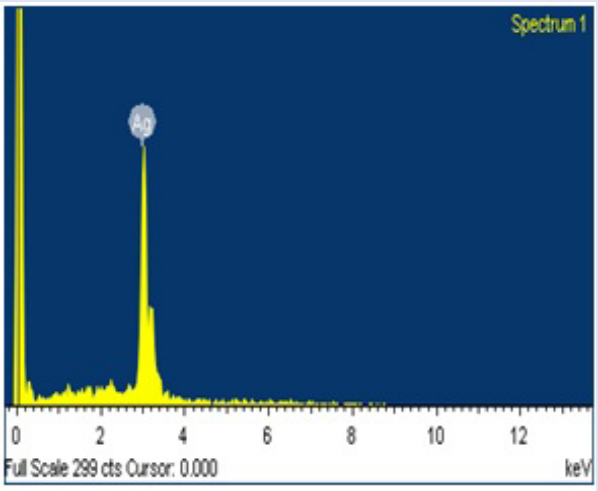


**Figure 5:** EDX graph of HMSNPs.

## 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

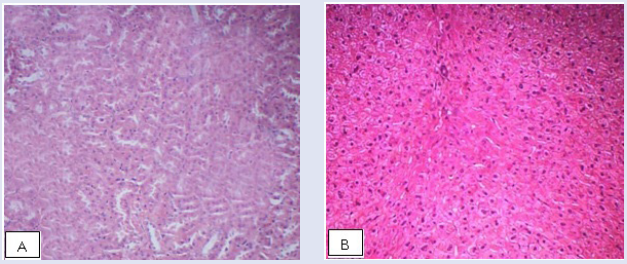


**Figure 6:** (a) Photograph of section of the heart (control). (b) HMSNPs treated.

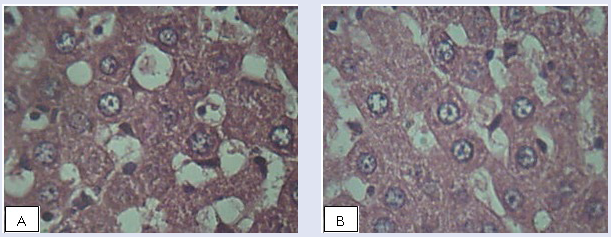


**Figure 7:** (a) Photograph of section of the liver (control). (b) HMSNPs treated.

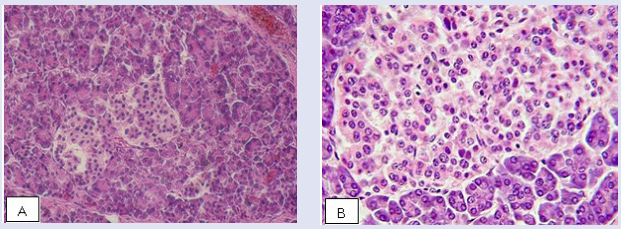


**Figure 8:** (a) Photograph of section of the pancreas (control). (b) HMSNPs treated.

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

| S No | Identification parameters | Group 1 | Group 2 | Group 3 |
|---|---|---|---|---|
| | | Control | SNP | HMSNPs |
| 1 | Alertness | Normal | Normal | Normal |
| 2 | Restlessness | No | No | No |
| 3 | Passive/Active | Active | Active | Active |
| 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 |

**Table 3:** *In-vitro* antidiabetic activity of HMNPs

| Sl.No | Drug | Percentage of Inhibition |
|---|---|---|
| 1 | Sodium Orthovanadate (standard) | 41.21% |
| 2 | HMSNPs | 37.16% |
| 3 | Chloroform extract | 32.09% |
| 4 | Hexane extract | 26.08% |

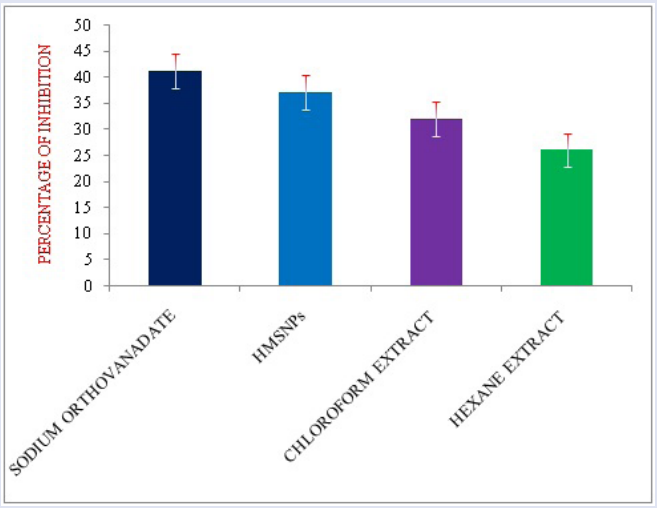


**Figure 9:** Graphical representation of *in-vitro* antidiabetic activity of HMNPs.

[Figures 6–8] observed in the treated groups when compared with the control group; this study therefore confirmed that the HMSNPs were nontoxic to the heart, liver, and pancreas within the treatment durations. Test compounds did not cause any mortality at the dose level tested (i.e., 2000 mg/kg body weight) until the end of 14 days of observation.

## *In vitro* antidiabetic activity results

HMSNPs possess potent percentage inhibition of PTP enzyme. Sodium orthovanadate was used as the standard compound (concentration = 10 μM). As the standard compound showed 41.21% of inhibition, comparatively HMSNPs showed 37.16% inhibition, chloroform extract showed 32.09% inhibition, and hexane extract showed partial inhibition of 26.08% of PTP enzyme. Chloroform extracts possess potent percentage of inhibition of PTP 1B enzyme [Figure 9, Tables 2 and 3].

# CONCLUSIONS

The HMSNPs were successfully obtained from bioreduction of $AgNO_3$ using *P. corylifolia* plant extract. The SNPs were appropriately characterized and confirmed using different methods viz., UV-vis spectroscopy, XRD, DLS, scanning electron microscope (SEM), and EDX analysis. $LD_{50}$ studies were conducted on albino mice; the HMSNPs did not cause any mortality at the dose level tested (i.e., 2000 mg/kg body weight) till the end of 14 days of observation and were considered safe. Goldstein method was followed to determine *in vitro* antidiabetic activity of HMSNPs; sodium orthovanadate (standard) showed 41.21% of inhibition of PTP 1B. HMSNPs showed 37.16% of inhibition, and plant extracts without SNPs showed less 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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## Financial support and sponsorship

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## Conflicts of interest

None

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