Development of Plant-Mediated Silver Nanoparticles & Their Pharmacological Evaluation

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Research Article

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Abstract

Diabetes is among the most common debilitating and non-transferable diseases on the planet. The idea of using nanoparticles as a drug to treat diabetes mellitus seems intriguing. The Ag nanoparticles (Ag NPs) were effectively produced utilizing *Moringa olifera* (family: *Moringaceae*) plant extract employing a simple, cheaper, faster, and environmentally friendly green synthesis process. The antidiabetic effect of the produced Ag NPs was also tested in vivo. In the presence of plant extract, silver nitrate was converted to silver ions (Ag). XRD, FTIR, UV, XPS, and HRTEM studies characterize the formed Ag NPs. Ag NPs that have been biosynthesized, crystal nature was confirmed through XRD analysis and confirmed by UV-visible spectroscopy. FT-IR spectra were used to verify the presence of various functional groups in the biomolecules, forming and stabilizing the nanoparticles. The size of the NPS was in the range of 20-40 nm determined by HRTEM. The induction of diabetes using STZ showed increased blood glucose, cholesterol, triglycerides, LDL, VLDL, massive loss in body weight. These changes were reversed following the treatment of diabetic rats for 28 days and showed significant inhibition ($p < 0.001$) at a dose range of 0.2 mg/kg leaf extract and 0.2 mg/kg Ag NPs compared with the extract-treated group. These obtained results suggested that plant-mediated Ag NPs have shown promising antidiabetic and anti-hyperlipidemic activity compared to the crude extract.

Highlights

- Plant mediated silver nanoparticles synthesized and characterized by XRD, FTIR, UV, XPS, and HRTEM.
- The size of the nanoparticles was in the range of 20-40 nm determined by HRTEM
- Induction of diabetes using STZ showed increased blood glucose, cholesterol, triglycerides, LDL, VLDL, massive loss in body weight
- The *M.olifera* mediated silver nanoparticles shown excellent anti-diabetic and anti-hyperlipidemic activity among other compounds.
- The *M.olifera* mediated green silver nanoparticles may be considered as an alternative herbal nanomedicine for treatment of hyperlipidemia and diabetes.

Introduction

Transformation in ways of life diminished physical action, expanded weight, and quick changes in natural aggravations add to the increasing quantity of outcomes of diabetes [1]. Universally, an expected 422 million grown-ups were living with diabetes in 2014, contrasted with 108 million out of 1980, ascending from 4.7 to 8.5% in the grown-up populace. As per the WHO, diabetes will be the seventh leading cause of death by 2030. Moreover, this figure was already reached by 2011 as per the International Diabetes Federation [2, 3]. Diabetes is a chronic metabolic disorder characterized by hyperglycemia. Among other metabolic scatters, our general public wishes to have an urgent solution for

diabetes mellitus. Although various investigations have been completed to disclose prescriptions from plant sources, it still needs extensive enhancement.

For a long time, silver has been regarded as a valuable metal with numerous commercial and pharmacological benefits, including antidiabetic and anti-bacterial properties [4, 5]. Chemical and physical properties of plant-mediated silver nanoparticles have raised a worry that nanoparticles orchestrated from herbs may interface in new obscure routes with the biological framework. In recent years, plant-mediated silver nanoparticles (PMSNPs) may increase uncommon consideration from researchers in the region of nanotechnology and pharmacology because of their unique characters and biological applications. Green synthesis of silver nanoparticles is considered a non-dangerous, ecological neighborly technique analogized to synthetic strategy [6]. PMSNPs have been widely used in various bio-applications, such as the antidiabetic movement [7, 8], the anti-cancer movement [9], and pharmaceutical businesses. Nanotechnology and its based procedures hold significant potential for upgrading the consistency of patients with diabetes [10]. Thus, plant-mediated nanoparticles encircling antidiabetic potential may serve as a relevant and intact alternative candidate in the treatment of diabetes.

Moringa olifera (MO) is a widely used medicinal plant shown in Figure 1, is generally disseminated in Asia, America, and Africa. Different kinds of medicinally active plants have been utilized for a few centuries worldwide as dietary enhancements and traditional treatment regimens for some ailments. So far, many proven plant prescriptions have been tried for diabetes, and some of them have shown promising therapeutic potential. Among these plants, a rapidly developing enduring tree, *Moringa oleifera*, belonging to the *Moringacea*e family, has good medicinal potential. The MO plant was used by the Romans, Greeks, and Egyptians. Romans, Greeks, and Egyptians have utilized MO plants. In the Indian natural therapeutic arrangement of medication, MO is accounted for to have a hypoglycemic effect. Since it is a critical wellspring of vitamin C, proteins, beta-carotene, iron, potassium, and different supplements [11]. Nowadays, a few experiments have been accounted for the antidiabetic capability of MO leaves and seeds. Regardless, the vast majority of them were centered around the leaf [12–16].

Materials And Methods

Silver nitrate ($AgNO_3$-99.99%) was purchased from Sigma Aldrich chemicals. The *Moringa oleifera* leaves (southern Indian common name of plant drumstick plant) from *Moringaceae* family and deionized water. Albino Wistar rats weighing 150 ± 20 g were used in the in-vivo diabetes model. Animals have maintained a 12h light/12 h dark cycle throughout the experiment at a temperature of 22° C. They were supplied with a regular chow pellet diet. And the water was kept ad libitum. The study was started after getting the approval from institutional animal Ethical committee with resolution number 1684/PO/a/13/CPCSEA.

Collection of plant material and preparation of extract:

Fresh leaves of the plant *(Moringa oleifera)* were collected from the JSS College of Pharmacy, Ooty campus. The collected fresh leaves were washed with deionized water and chopped into pieces by preparing a hot water extract of the leaves, 10g of leaves in 100 mL of distilled water and boiled at 80^{o} C for 15 minutes. The extract solution was obtained by filtration using Whatman filter paper, and the extract was stored at 4^{o}C for further use. The extract solution acts as a reducing and stabilizing agent for the synthesized silver nanoparticles.

Synthesis of Ag nanoparticles:

100 mL (1mM) aqueous solution of silver nitrate was prepared in a conical flask. Then 10 ml of leaf extract was added separately to 50mL aqueous silver nitrate solution kept in separate beakers at room temperature and stirring for 2hrs. The answer was to change the color slowly yellow to dark yellow. Finally, the answer turns yellow to dark brown, indicating the formation of silver nanoparticles [17].

Diabetes induction:

Diabetes was induced in male albino Wistar rats by treating with Streptozotocin 40 mg/kg. Streptozotocin is dissolved in citrate buffer (40 mg/kg in 0.1 M citrate buffer, pH 4.5) and administered to all the animals. After 48 h, blood glucose levels were measured by glucose check (Abbott) for all the induced animals. Animals with more than 250 mg/dL were considered for the diabetic activity, and they were grouped and marked. [18]

Experimental design:

Animals were divided into five groups with six animals each randomly, and they were treated for 28 days.

Group I: Normal (treated with saline)

Group II: Diabetes (Streptozotocin 40 mg/kg i.p)

Group III: Diabetes + Glibenclamide (0.5 mg/kg p.o)

Group IV: Diabetes + AgNP (0.2 mg/kg p.o)

Group V: PMAgNP (0.2 mg/kg p.o)

Acute oral toxicity studies:

Acute oral toxicity studies were carried out by following OECD guidelines 425. From the studies, it was confirmed that 0.2 mg/kg PMAgNPs did not produce any toxicity.

Biochemical parameters: Cholesterol, Triglycerides, SGOT, SGPT

Animals were fasted for 3 hours before administering the test drugs. On the 28th day, animals were sacrificed by the decapitation method. The serum was separated immediately after the blood was

collected. Serum was used for the estimation of various biochemical parameters. Few of the major organs were managed and weighed. These organs were stored in 10% buffered formalin for histopathological studies. The serum was used to estimate various biochemical parameters like cholesterol, triglycerides, SGOT, SGPT, Alkaline phosphatase were measured using respective kits from Erba, Germany.

Body weight measurement:

The animal's body weight was monitored on 1, 7, 14, 21 and 28 days during the study in STZ induced diabetic model.

Statistical Analysis:

Whole data were expressed as mean ± SD (n=6). Statistical significance was assessed by one-way ANOVA followed by Bonferroni's multiple comparison test using Graph Pad Prism 5.01.

Histopathology:

After 28 days, animals were sacrificed, and various body organs were collected. The organs were weighed and washed in phosphate buffer. The samples were stored in buffered 10% formalin and further processed for histopathological techniques. All the tissues were embedded in paraffin wax, and sections with 4.5- to 5-µm were processed. The teams were additionally stained with hematoxylin and eosin.

XRD ANALYSIS OF SILVER NANO PARTILCES:

The X-ray diffraction patterns of silver nanoparticles (Ag NPs) are shown in Fig 2. Careful analysis of X-ray diffraction of Ag Nanoparticles from fig. 2 (A) suggests that it has crystalline nature. The characteristics peaks appear at 2θ = 38.22°, 44.41°, 64.57° and 77.46° corresponding to the planes (111), (200), (220) and (311) respectively. The obtained lattice planes are in good agreement with the JCPDS card No. 04-0783 and can be indexed as a face-centred cubic phase with lattice parameters a = b = c =0.40862 nm. Debye determined the crystalline size–Scherrer's formula and Williamson Hall Plot method (fig.2(B)) D = 0.9*λ/(βCosθ and $\beta\cos\theta$ = + 4$\epsilon\sin\theta$, Where β is full width at half maximum in radians, and λ is the wavelength of X-rays, and θ is the Bragg's angle. The average crystalline size Ag NPS was calculated to be 13.49 nm and 12.61nm. The Ag NPs through green synthesized can reduce the dislocations.

Fig. 2 (C) shows the FT-IR spectra of *Moringa olifera* plant silver nanoparticles. The broadband between peak at 3500–3800 cm—1 was assigned to OH stretching vibrations of free alcohol, at 3400-3500 cm—1 was an indication of the phenols and hydrogen-bonded alcohols, a weak peak at 2924 cm—1 was an indication of the C–H stretching vibrations of poly- saccharide. The band at 1000–1200 cm—1 was attributed to the C–O antisymmetric trying in the C–O–H and C–O–C groups of polysaccharides. It can be observed that the characteristic absorption peak of OH for the *Moringa olifera* plant silver nanoparticles was at 3450 cm—1, and 1500-500 cm-1 represents the nitro compounds. Hydroxyl and

carboxyl present in *Moringa olifera* silver nanoparticles act as reducing agents and conclude it worked good in diabetic activity. UV -VIS absorption spectra of *Moringa olifera* synthesized silver nanoparticles as in fig. 2 (D) showed that the absorbance and peak bordering at around 412 nm indicated that silver nanoparticles were monodispersed and showed (SPR) surface Plasmon resonance with UV visible light. The size of the particles has a strong influence on this absorption.

The present study XPs technique was done to detect the composition of *Moringa olifera* plant silver nanoparticles (Ag NPS). The binding energy of C1s was referenced to the original is at 284.85 eV, as shown in fig.3(C). The XPS survey wide scan identifies the elementals found at a depth of 10nm from the surface materials. It will give you different binding energies for different elements. The high-resolution XPS gives information about the chemical state of the elements. Fig.3(D) shows the wide XPS survey scan spectra of silver nanoparticles that conformed to detecting C, O, N and Ag nanoparticles. No other absorbed peaks were found that showed the high purity of the Silver nanoparticles. The binding energies of C 1s and O 1s were resolved in two peaks at arose at 284.25 eV (fig3.(B)) and 525.9eV(fig.3(C)), respectively. The N peak at 401.04eV scan suggested the presence of charged nitrogen atoms, which showed the electrostatic interaction with the silver surface. High resolution can be performed on Ag 3d core levels, which gave information about the nature of silver element and spectra, as shown in figure 3. The binding energies for Ag 3d5/2 and Ag 3d3/2 were found at 366.6eV and 37.45eV (fig .3(A)) respectively. Moreover, the obtained narrow peaks showed that only a single -element was present in the system. Fig.3 (A-D) represents the formation of Ag NPs with negligible impurities, which showed good diabetic activity.

Moreover, Ag NPs absorbed 412nm (fig. 2(B)), which corresponds to the surface Plasmon resonance, and this is confirmed that the Ag NPS synthesized with the ignorable amount of impurity. It showed the weight percentage of Ag. Furthermore, the particle size and shape as indicated by HRTEM, which conformed spherical in fig 4(A), confirmed the size of silver nanoparticles in the range of diameter 5-10 nm in fig 4(B). The d spacing of Ag NPs d=1.229, hkl (311) obtained from *Moringa Olifera* in fig.5(C) is matched with JCPDF card no of silver 04-0783. From Fig 4 (D), the SEAD pattern shows the brighter spots in the ring pattern indicating Ag of (111), (200), (220) and (310) surfaces.

Results

Diabetes was induced using STZ (40 mg/kg), there were increased glucose levels, and other serum parameters in the diabetic animals and these changes were reversed in the treatment group. There were significant ($P < 0.05$) differences in the diabetic group and the remaining groups. The animals treated with only silver nanoparticles, the results were compared with the normal there were no significant differences between the two groups. The body weight of the diabetic group animals was decreased significantly. In contrast, in the standard and AgNP groups, the body weight was reduced in the initial days. Eventually, at the last week of study, the importance of animals improved compared to the diabetic groups. LDL, HDL, TG, cholesterol, SGOT, SGPT and ALP (Fig. 5A-5C) were significantly higher in the diabetic control among other groups, whereas regular group and AgNP groups, the result was

comparable, and There was no noticeable difference between the groups. Liver function markers were standard in the remaining groups (leaf extract, AgNPs, and glibenclamide treated groups). Histopathological examination microscopic observation of liver sections from the experimental rats revealed that both the aqueous leaf extract and AgNPs minimized cellular damage induced by STZ (Fig. 6A4-D4). Histological sections from the standard control group exhibited typical cell architecture (Fig. 6A1-D1). Extracts from the diabetic control group showed classic architecture but congested central veins and minimal to moderate peripheral inflammation. In the leaf extract-treated group, standard architecture was observed, and the morphology of the hepatocytes appeared normal. Similarly, in the AgNPs-treated group, typical architecture was observed, and the hepatocytes seemed to be expected (Fig. 6A2-D2). Sections from the glibenclamide-treated group exhibited standard architecture with congestion of the portal vessels (Fig A6-D6). In the kidneys, histological sections from the common control group showed typical cell architecture. Although changes to the glomeruli were observed in the diabetic control group, namely mild thickening, the renal tubules exhibited normal cell morphology (Fig. 6A3-D3). Sections from both the leaf extract and AgNPs-treated groups showed standard architecture with glomeruli and renal tubules that appeared normal.

Discussion

An attempt was made to elucidate the antidiabetic activity of the silver nanoparticles synthesized from *Moringa olifera* and determined the acute toxicity studies. Hyperlipidemia leads to obesity, which is one of the critical causes of diabetes. Carbohydrate and lipid metabolism will be impaired in diabetes, and it further leads to severe complications. The current study's findings indicate that body weight increased in the diabetic treatment groups due to improved glycemic control. Food consumption and increased body weights were comparable with the extract, and AgNPs treated groups. One of the most important organs is the liver which is Responsible for storage, metabolism and detoxification. SGOT, SGPT and ALP are the key liver biomarkers for liver function. In the diabetic group of animals, the liver was necrotized, and increased markers were observed. This increase in markers might be due to the leakage of the enzymes from the liver cytosol. This was reversed in the treatment groups significantly in *Moringa olifera* and standard groups. These results agreed with the histopathology results for the group treated with the reference drug glibenclamide to evaluate the enhanced effect of the preparation.

Diabetes is one of the oxidative stress disorders. The β-cell of the pancreas will be destroyed in the STZ induced diabetes; It further leads to damage of DNA. *Moringa olifera* was already proven to have many antioxidants compounds, and these antioxidants reverse oxidative stress. The present study demonstrates the effect of AgNP prepared from *Moringa olifera* on STZ induced diabetic models. After 28 days, the blood glucose levels were controlled compared to the diabetic group, which might be due to the restoration of the β-cell of the pancreas by its antioxidant activity mediated by their active constituents. Biologically prepared silver nanoparticles were shown to improve liver functions and kidney functions. It also restored the lipid profile, body weights and food intake significantly compared to the diabetic animals. The study findings suggest that AgNPs are potent antidiabetic agents.

The induction of diabetes using STZ increased blood glucose levels and reduced body weight [18]. These changes were reversed following the treatment of diabetic rats for 28 days with 0.2 mg/kg leaf extract and 0.2 mg/kg AgNPs, with significant reductions recorded in blood glucose levels. In addition, treatment with 0.2 mg /kg leaf extract and 0.2 mg /kg AgNPs increased rats' bodyweight compared with both the standard control and untreated diabetic groups. Furthermore, insulin levels were lower in untreated diabetic rats (Group II) than in the joint management, and treatment with both leaf extract and AgNPs significantly increased insulin levels ($P < 0.01$) similar to those in the standard control group. Aqueous leaves extract of *M. olifera* consists of antioxidant compounds like Vitamins C, E and β-carotene in concentrated. Antioxidants might be responsible for antidiabetic activity. The aqueous extract already proven for the presence of many polyphenols may be responsible for the antioxidant activity mediated antidiabetic activity [19, 20]. Many plant-based silver nanoparticles compounds have been proven scientifically for antidiabetic activity. The present study explored the AgNPs biologically synthesized from *M. olifera* leaf extract to treat diabetes.

Conclusion

In the current study, we acute toxic studies of *M. olifera* mediated AgNPs. Upon repeated administration, the AgNPs showed a very negligible amount of toxicity or mortality as evaluated by the behavior of albino mice and the microscopic histopathological examination of the isolated organs. According to the present study, the administered plant-mediated AgNPs showed potent anti-hyperglycemic, anti-hyperlipidemic properties compared to the *M. olifera* extract and likewise improved different predicaments of diabetes. Current global excitement is cost-effective and eco-friendly. Resources drive the application of highly hailed medicinally active plants to direct the green synthesis of nanomaterials that acquire distinct pharmacological effects. Subsequently, the Ag NPs were synthesized using *M.olifera* and characterized using FT-IR, UV, XRD and XPS. In addition, the rationale of the synthesis of nanoparticles was to accomplice green synthesis with a natural plant (enhanced biological activity, non-toxic and chemically inert) and to improve the surface area of the molecule to achieve the potential therapeutic activity. Hence, it is recommended that the *M. olifera* mediated AgNPs are more potent than the plain extract and may be utilized as fascinating advanced nanomedicine to treat diabetes mellitus. Further scientific molecular level studies are required to figure out the exact mechanism of action of plant-mediated silver nanoparticles.

Declarations

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Figures

Figure 1

Moringa olifera plant

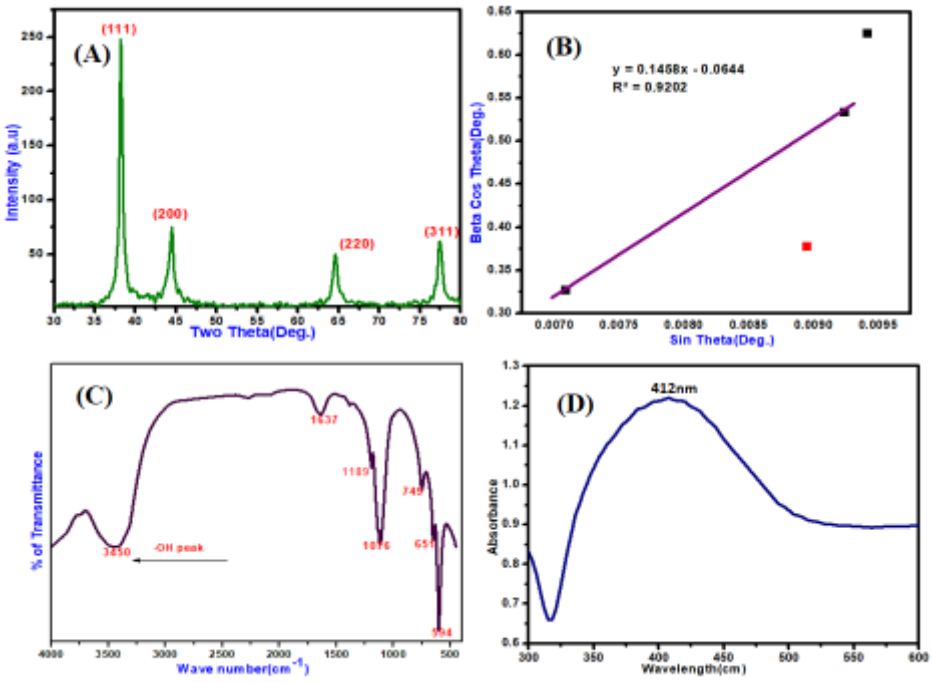

Figure 2

(A) XRD (B) Williamson Hall Plot (C) FTIR Spectra of *Moringa olifera* silver nanoparticles (D) UV- Visible spectra of *Moringa olifera* AgNPs

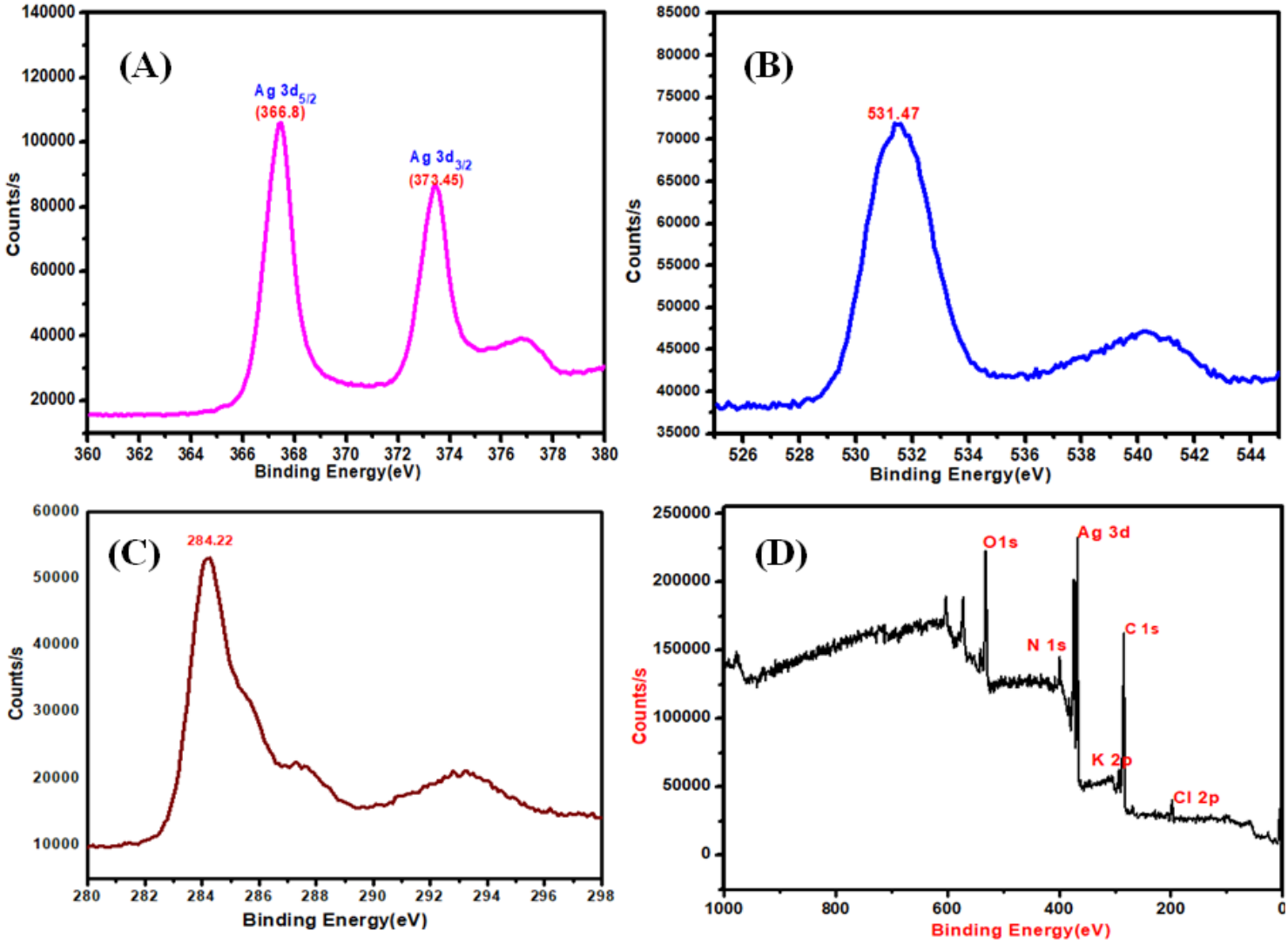

Figure 3

(A) 3D spectra of *Moringa Olifera* Ag NPs (B)O 1s scan of Ag NPs(C) C 1s scan of Ag NPs (D) Wide survey scan X-ray photoelectron spectra.

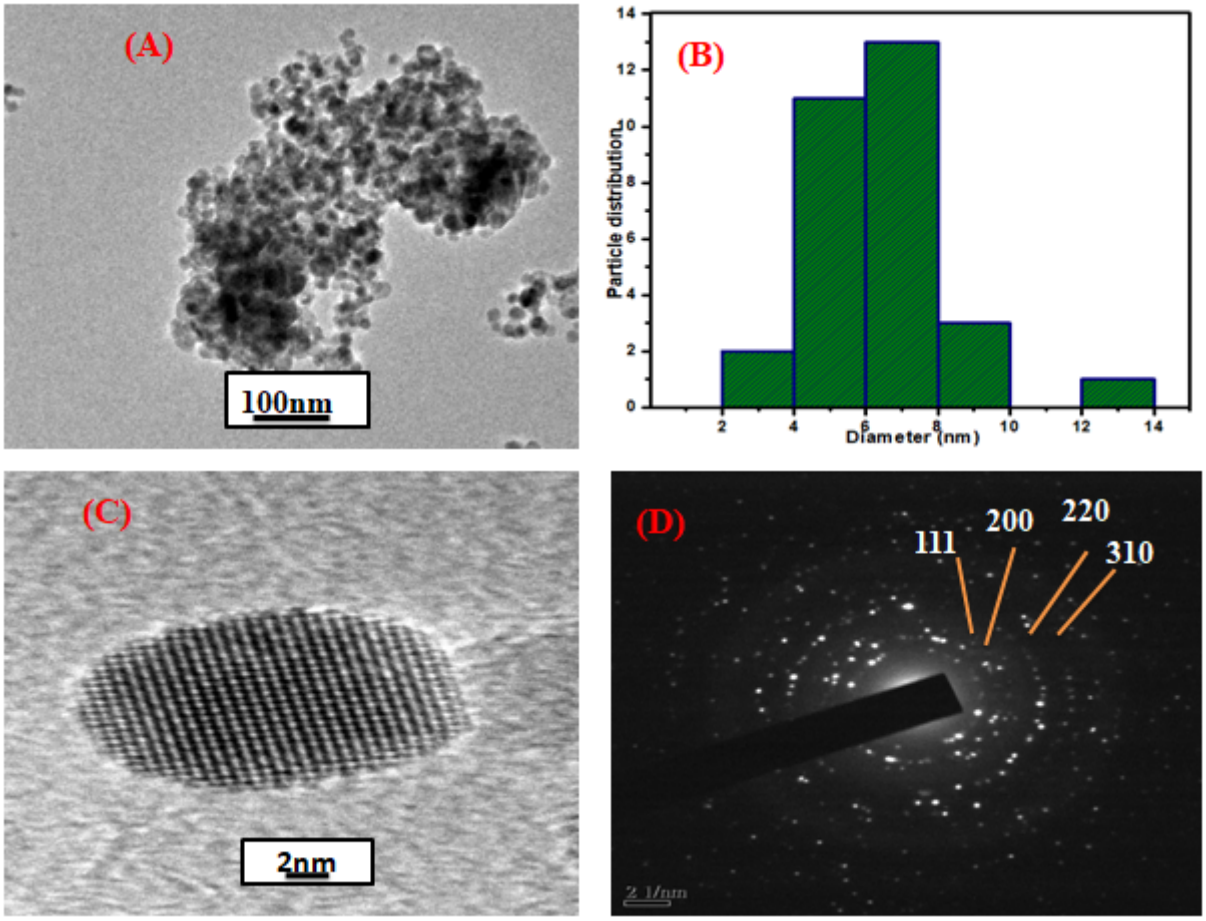

Figure 4

HRTEM results 4 (A) AgNPs at 100nm resolution 4 (B) size of the AgNPs, 4 (C) d-spacing, 4 (D) SEAD pattern of Ag NPS

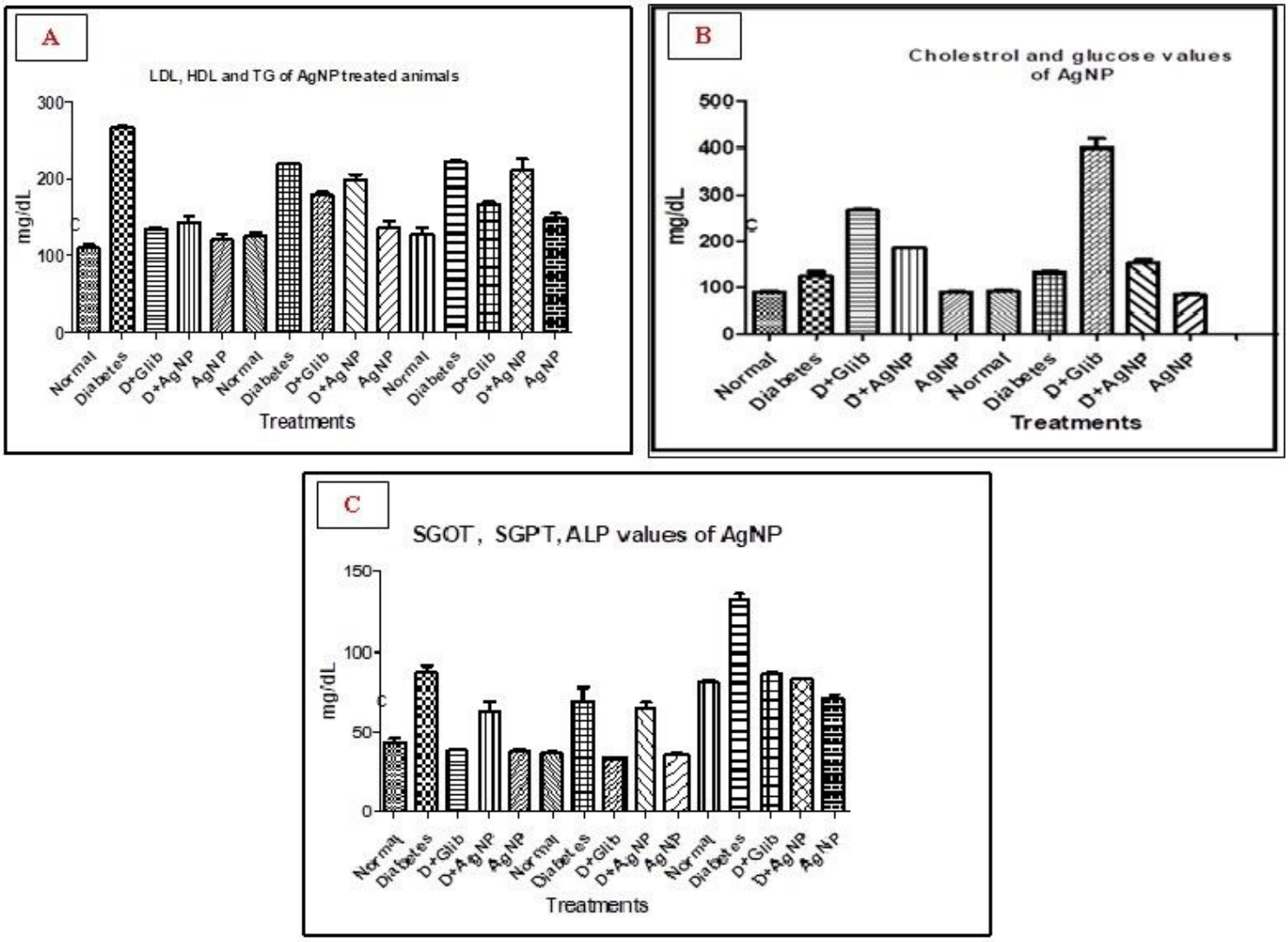

Figure 5

(A) LDL, HDL AND TG of AgNPs, 5 (B) Cholesterol and glucose levels of the AgNPs treated animals, 5 (C) SGOT, SGPT and ALP values of Ag NPs

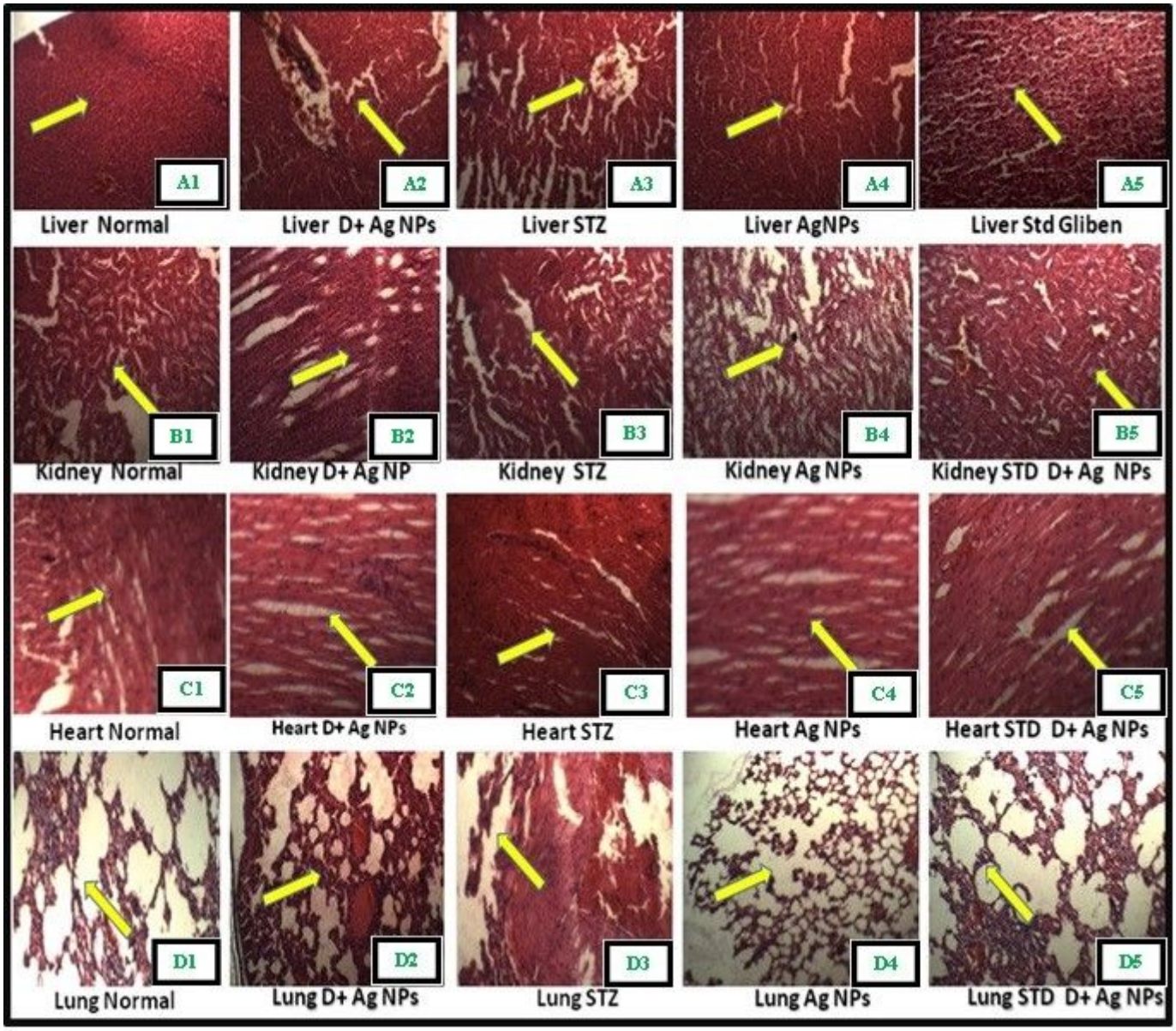

Figure 6

Histopathology of rat's liver, kidney, heart and lungs for normal control group (A1, B1, C1, D1); histopathology of liver, kidney, heart and lungs for D + Ag NPs treated group (A2, B2, C2, D2); histopathology of liver, kidney, heart and lungs for STZ treated group (A3, B3, C3, D3); histopathology of liver, kidney, heart and lungs for Ag NPs treated group (A4, B4, C4, D4, and E4); histopathology of liver, kidney, heart and lungs for Standard drug treated group (A4, B4, C4, D4).

Supplementary Files

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