 doi:10.20944/preprints202106.0045.v1

Minocycline Derived Silver Nanoparticles for Assessment of Their Antidiabetic Potential against Alloxan induced Diabetic Mice

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Abstract

Diabetes is a life-threatening disease and chronic diabetes affects the parts of the body including the liver, kidney and pancreas. The root cause of diabetes is mainly associated with oxidative stress

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produced by reactive oxygen species. The minocycline is a polyphenolic drug with excellent antioxidant activities. The objective of the present study was to investigate the antidiabetic potential of minocycline modified silver nanoparticles (Mino/AgNPs) against alloxan-induced diabetic mice. The Mino/AgNPs were synthesized using minocycline as reducing and stabilizing agents. UV-vis, FTIR, X-ray diffraction (XRD) and transmission electron microscopy (TEM) were applied for the characterization of Mino/AgNPs. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was conducted to determine the antioxidant potential of newly synthesized Mino/AgNPs. The results revealed that the Mino/AgNPs showed higher radical scavenging activity (IC50 = 19.7 µg/mL) as compared to the minocycline (IC50 = 26.0 µg/mL) and ascorbic acid (IC50 = 25.2 µg/mL). Further, the Mino/AgNPs were successfully employed to examine their antidiabetic potential against Alloxan-induced diabetic mice. Hematological results showed that the mice treated with Mino/AgNPs demonstrated a significant decrease in fasting blood glucose level and lipid profile as compared to the diabetic group. The histopathological examination confirmed that the diabetic mice treated with Mino/AgNPs showed significant recovery and revival of histo-morphology of kidney, central vein of liver and islet cells of the pancreas compared to the diabetic mice. Hence Mino/AgNPs have good antidiabetic potential and could be an appropriate nanomedicine to prevent the development of diabetes.

Keywords: Minocycline, Silver Nanoparticles, Tetracycline, Antidiabetic, *In Vivo*, Nanomedicine

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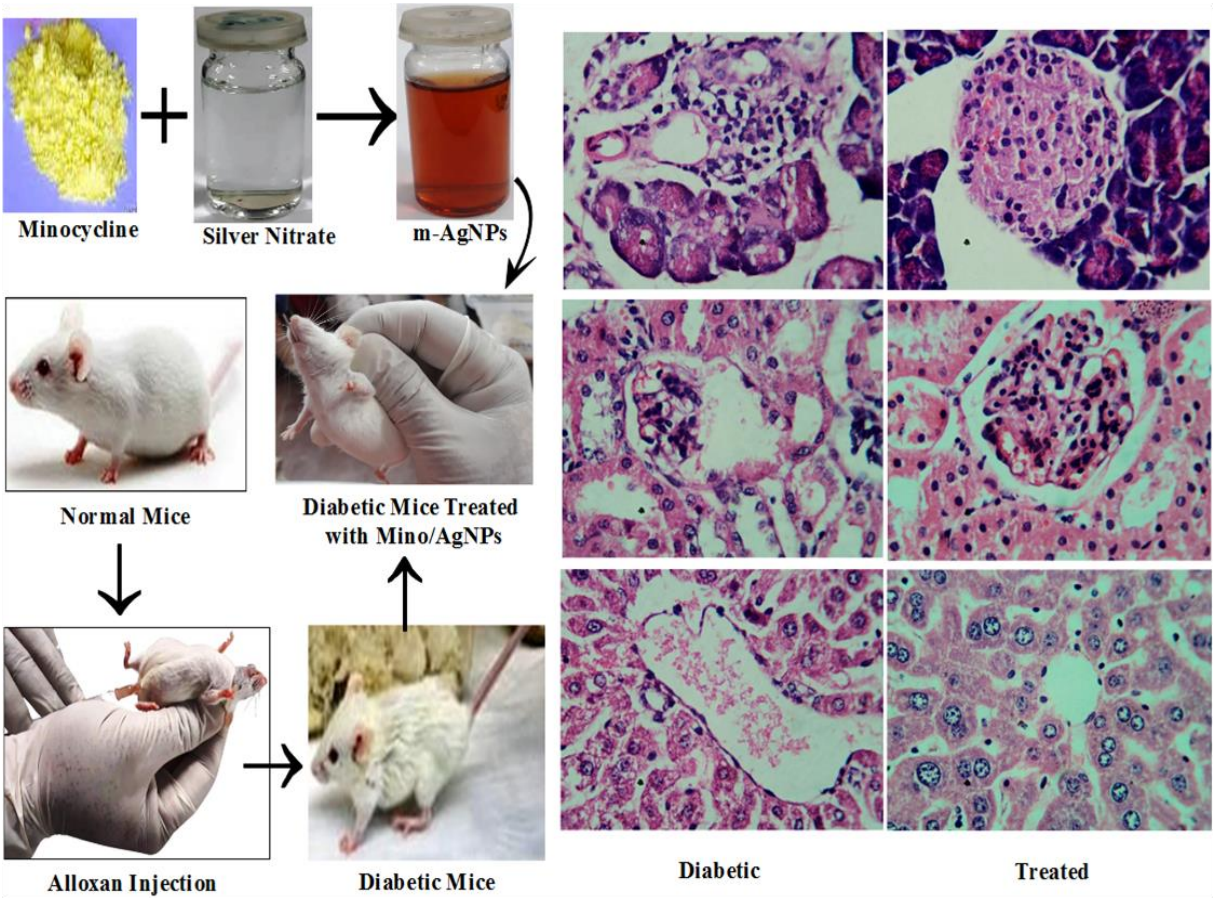

Graphical Abstract. Schematic presenting the Synthesis and *in vivo* Antidiabetic Potential of Mino/AgNPs

1. INTRODUCTION

Diabetes mellitus along with its secondary complications continued to be a major threat to human health all over the world (Z. Zhang et al., 2016). It is one of the five main causes of death globally (Mohammadi Arvanag et al., 2019). A group of metabolic disorders occurred as a consequence of hyperglycemia and glucose intolerance, known as diabetes mellitus (DM). Two types of diabetes mellitus are conventionally known. Insufficient secretion of the hormone insulin from β-cells of the pancreas is classified as type-1 DM and the development of insulin resistance in the body is classified as type-2 DM (Hussein et al., 2019). Globally more than 90 % of diabetes

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patients suffer from type-2 DM (Dhas et al., 2016). Alarmingly, the numbers are increasing at a dreadful rate. According to Veiseh *et al.,* more than 280 million adults are suffering from diabetes mellitus and the high prevalence of DM may cause the 400 million adults to be affected till 2030 (Veiseh et al., 2014).

The high prevalence of DM is mainly associated with modernization of lifestyle, lack of physical activity, obesity, ethnicity, older age and genetic polymorphism (Malapermal et al., 2017; Samadder, 2014). People with type-2 DM develop insulin resistance in the body; consequently cells unable to take glucose from the blood which finally resulted in the rise of blood glucose level known as hyperglycemia. When left untreated, the prolonged hyperglycemia resulted in the metabolic disorder of many organs like the kidney, liver, heart and pancreas. These metabolic disorders become fatal for life, often resulted in diabetes-associated secondary complications such as kidney disease, heart disease, liver disease, blindness and erectile dysfunction (Thorve et al., 2011; Veiseh et al., 2014).

The root cause of diabetes is mainly associated with oxidative stress produced by reactive oxygen species (ROS) that induced the β-cells dysfunction, insulin resistance and impaired glucose tolerance. Excess food and lack of physical activity contribute to the overload of glucose and fatty acid that leads to the formation of ROS (Wright et al., 2006). According to Rohdes, the pancreatic β-cells are highly sensitive to physiological and pathological stressors, resulting in loss of insulin, triggered by apoptotic cell death (Rhodes, 2005). The studies of Volpe et al. also reported the effect of oxidative stress on pancreatic β-cell death and associated diabetic complications. According to them, diabetes-associated complications that are induced by hyperglycemia are mainly because of an imbalance between ROS, which leads to higher oxidative stress and cellular death (Volpe et al.,

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2018). Thus, these diabetic complications can effectively be controlled by down-regulating the generation of ROS.

The change of lifestyle, diet and oral administration of antidiabetic agents are the key factors to down-regulate the generation of ROS regarding the treatment of diabetes (Veiseh et al., 2014; Wright et al., 2006). The primary objective for both type-1 DM and type-2 DM is maintaining the persistent control of glucose level within the normal glycaemic range (70-140 mg/dL) (Veiseh et al., 2014). The selection of a suitable drug is a common problem in the treatment of diabetes. Various antidiabetic drugs and hypoglycemic agents have been introduced for the treatment of diabetes such as sulfonylureas and biguanides but these drugs do not provide persistent control over the blood glucose level. In addition, the prolonged use of these drugs induces toxicity and undesirable adverse effects such as gastrointestinal discomfort, hypoglycemia, pancreatic degeneration and liver impairment in the body which renders them less common (Campbell & Taylor, 2010). Therefore to find new drugs and hypoglycemic agents with minimal side effects and higher efficacy is interesting and the focus of prior research elsewhere.

Nanobiotechnology is among the demanding areas of research that make use of the biological substances at the nanoscale and find their applications in different fields such as biosensor (Kazmi et al., 2020), diagnostics (Hu et al., 2013), bio-imaging (Zhou et al., 2018), catalysis (Raza et al., 2017), drug delivery system (Kazmi et al., 2019) and nanomedicine (Wicki et al., 2015). In comparison to the conventional drug formulations, nanomedicine has retrieved more attention for the last few years due to its benefits such as more precise diagnosis, a higher percentage of recovery, and more effective therapies (Kouame et al., 2019). Especially the silver nanoparticles have become more desirable in the field of nanomedicine because of their fascinating properties such as ease of synthesis, colloidal stability, biocompatibility, bioavailability, low

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toxicity, and ability of surface modification (Burdușel et al., 2018; Park et al., 2011; Siddiqi & Husen, 2017). The previous studies have reported the administration route and bioavailability of AgNPs in an animal model. The small size AgNPs could be easily absorbed into the gastrointestinal tract and released into the bloodstream followed by excretion from the body via feces and urine (Jiménez-Lamana et al., 2014). Park et al. have reported the bioavailability and excretion of citrate coated AgNPs with an average size of 7.9 nm. According to them, the bioavailability of rats administrated orally with 1 mg/kg AgNPs was 1.2% and 4.2% in the rats exposed to the 10 mg/kg AgNPs (Park et al., 2011).

The AgNPs can reduce the oxidative stress caused by the imbalance between reactive oxygen species (ROS). The DPPH free radical scavenging activity of AgNPs has been reported by many workers (Ahn et al., 2019; Elemike et al., 2017; Khorrami et al., 2018; Küp et al., 2020; Vijayan et al., 2018). The H_2O_2 is an important metabolic signal for glucose-stimulated secretion of insulin from β-cells (Pi et al., 2007) whereas excessive generation of H_2O_2 can be harmful for the integrity and function of β-cells (Kaneto et al., 2007). The studies of Campoy et al. demonstrated the use of EP/AgNPs for the protection of INS-I cells from H_2O_2 induced oxidative injury. They used the *Eysenhardtia polystachya* (EP) extract to synthesize AgNPs. They reported that the cells that were exposed to H_2O_2 showed marked inhibition in the insulin secretion whereas the cells that were treated with EP/AgNPs before exposure to H_2O_2 showed a significant increase in insulin secretion. They anticipated that the polyphenolic compounds present in *Eysenhardtia polystachya* may protect the insulin-secreting cells from oxidative stress (Campoy et al., 2018). Keshari et al. have also reported the strong H_2O_2 scavenging potential of AgNPs as compared with standard vitamin C. The study demonstrated that the antioxidant properties of AgNPs arsis because of functional groups present on the surface of AgNPs (Keshari et al., 2020). Khorrami et al.

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proposed that the enhanced antioxidant activities of AgNPs are because of the simultaneous action of polyphenols as antioxidants and nanoparticles as catalysts (Khorrami et al., 2018). Several studies have been published reporting the possible mechanisms for antioxidant properties of AgNPs. However, it is necessary to note that the antioxidant potential of AgNPs largely depends on the chemical composition of the compound with which it is modified. The nanoparticles prepared using extracts rich in phenolic compounds and flavonoids showed high scavenging activities (Ahn et al., 2019; Bedlovičová et al., 2020).

It is therefore minocycline was selected to synthesize AgNPs. In addition to antimicrobial properties, minocycline has shown strong antioxidant potential and free radicals scavenging activities. The minocycline is a semi-synthetic antibiotic from the tetracycline group. It has been used for more than 30 years as a drug of choice for the treatment of diseases related to bacterial infections. Nowadays, non-antibiotic characteristics of minocycline such as anti-tumor, anti-inflammatory, and antioxidant (Pourgholami et al., 2012; Soory, 2008) have dragged the attraction of scientists towards this second-generation antibiotic. The minocycline has a polyphenol structure with multiple ionizable functional groups. At C4 carbon, Minocycline has a dimethylamino group which is mainly responsible for the enhanced antioxidant potential of minocycline (Murakami et al., 2020). Lee et al. have reported the antioxidant activities of minocycline against the oxidative stressor (H_2O_2). According to them, the flies treated with minocycline showed more resistance to hydrogen peroxide (H_2O_2) and died less as compared to the flies which did not receive minocycline treatment (G. J. Lee et al., 2017). The MURAKAMI et al. has also reported the free radicals scavenging activity of minocycline. The study demonstrated that the antioxidant activity of minocycline is 200 to 300 times more potent than that of tetracycline. According to them, minocycline is a chain-breaking antioxidant with antioxidant activities comparable to that of

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Trolox and α-tocopherol (Murakami et al., 2020). Several previous reports have been published demonstrating that the minocycline is an effective antioxidant with free radical scavenging potency similar to vitamin C and E (Kraus et al., 2005).

Considering the antioxidant potential of minocycline and AgNPs, the present study was aimed to check the antidiabetic potential of minocycline modified silver nanoparticles (Mino/AgNPs) against the alloxan-induced diabetic mice. The Mino/AgNPs were synthesized and extensively characterized using UV-vis, X-ray diffraction (XRD), FT-IR and transmission electron microscopy (TEM). Then, the synthesized Mino/AgNPs were successfully applied to examine their *in vivo* antidiabetic potential against alloxan-induced diabetic mice.

2. Experimental

2.1. Ethical statement

All animal trial techniques were directed as per local and worldwide controls. The nearby direction is the Wet op de dierproeven (Article 9) of Dutch Law (International) as detailed in our previous studies (Ali et al., 2020; Ali et al., 2020; Hussain et al. 2020; Ara et al. 2020; Ali et al. 2020; Khan et al. 2019; Ali et al. 2019; Mumtaz et al. 2019; Mughal et al. 2019; Dar et al. 2019) and The Institutional Bioethics Committee at Government College University Lahore, Pakistan (No. GCU/IIB/21 dated: 08-01-2019).

2.2. Materials

Silver nitrate, Minocycline, Alloxan monohydrate and sodium chloride, were purchased from Sigma Aldrich. Sodium hydroxide was purchased from Fluka chemicals. The other chemicals were of analytical grade and used without further purification.

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2.3. Synthesis of Minocycline Derived Silver Nanoparticles (Mino/AgNPs)

The Mino/AgNPs were synthesized using minocycline as a reducing and stabilizing agent. 2 mL silver nitrate (0.8 mM) and 2 mL (0.8 mM) minocycline solution were taken in a conical flask. To the mixture, sodium hydroxide was added to accelerate the synthesis process. The resulting mixture was continuously stirred for 4 minutes. UV-vis spectrophotometer (Shimadzu UV-1700) was used to monitor the synthesis of Mino/AgNPs in the wavelength range 300-800 nm.

2.4. Characterization of the Mino/AgNPs

The size and morphology of prepared Mino/AgNPs were determined using a transmission electron microscope (TEM) using an FEI Tecnai t12 running at 80 kV with final emission of about 10 µA. A 2 k AMT camera was used to take micrographs. The sample was produced on a copper grid with carbon coating and formvar film, dropped 10 µL of Mino/AgNPs solution and was then dry overnight and analyzed using TEM.

The role of minocycline in the synthesis of Mino/AgNPs was examined through FT-IR analysis. To prepare the sample for FT-IR analysis, a colloidal Mino/AgNPs solution was centrifuged three times at 10000 rpm for 30 min and washed each time with deionized water. The pellet obtained after centrifugation was left overnight to dry under the fume hood. FTIR analysis was carried out with Bruker Alpha.

The crystalline nature of prepared Mino/AgNPs was studied using XRD. To prepare the sample for XRD studies, a colloidal solution of Mino/AgNPs was centrifuged three times at 10,000 rpm for 30 min and washed with deionized water each time. Then, the pellet obtained after centrifugation was left overnight to dry under the fume hood. XRD was carried out in the 2θ region,

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from 0 to 80˚. The scanning rate was 0.02˚ per minute. The Cu $K_{\alpha 1}$ radiation having wavelength (λ) 1.5406 A˚ was utilized along with 40 mA tube current and 40 kV tube voltages.

2.5. Antioxidant study – DPPH assay

The antioxidant potential of Mino, Mino/AgNPs and ascorbic acid were evaluated through DPPH free radical scavenging assay. Briefly, 100 µL of each Mino, Mino/AgNPs and Ascorbic acid at various concentrations (10, 25, 50 and 100 µg/mL) were added to the 2.9 ml of 0.1 mM DPPH solution in methanol. The resulting mixtures were kept in dark for 30 minutes. The DPPH solution (2.9 mL DPPH and 100 µL methanol) was used as a control solution. The absorbance of control and reaction mixtures was measured at 517 nm using a UV-vis spectrophotometer (Shimadzu UV-1700). The DPPH scavenging activity was expressed as a percentage and was calculated by the following formula:

$$DPPH\ Scavenging\ Effect\ (\%) = \frac{Absorbance\ of\ Control - Absorbance\ of\ Sample}{Absorbance\ of\ Control} \times 100$$

2.6. Experimental Animals

Thirty two albino mice were received from the University of Veterinary and Animal Sciences. Before any kind of experimentation, the mice were left in the animal house for two weeks at 25℃ with frequent access to water and food. This was done to acclimatize the mice to a new environment. The weight of the body of all mice was measured before and after the treatment. All the experiments performed during the in vivo studies were approved by the Bioethical Committee of Government College University Lahore, Pakistan.

2.7. Induction of Diabetes

Alloxan monohydrate is a toxic glucose analog that affects the β-cells of the Pancreas and is frequently used in an animal model to induce diabetes. The intraperitoneal injection of Alloxan

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monohydrate (100 mg/Kg body weight) resulted in the induction of diabetes to the overnight fasted mice. Subsequently, to protect the mice from hypoglycaemic effects, they were fed with glucose solution (10%) for 24 hours along with the normal food. To confirm the induction of diabetes, the fasting blood sugar level of mice was measured regularly with 3 days intervals, up to 14 days. Those mice were considered diabetic and were selected for further experimentation that carried the fasting blood sugar of more than 250 mg/dL.

2.8. Experimental Design

To conduct the antidiabetic studies, four groups were made with eight mice in each group. Group-I; Normal control, Group-II; Diabetic left untreated, Group-III; Diabetic treated with drug Glibenclamide (5 mg/Kg body weight), Group-IV; Diabetic treated with Mino/AgNPs (5 mg/Kg body weight). The mice were treated regularly for 28 days with Glibenclamide and Mino/AgNPs via oral administration.

2.9. Collection of sample

After the successful completion of twenty-eight days of treatment, the mice were fasted for 12 hours and subsequently, they were given anesthesia with chloroform. The mice were then dissected sacrificially and blood samples were obtained by heart puncture in three distinct tubes. The kidney, pancreas and liver were dissected followed by washing with phosphate buffer saline (to clear debris) and placed in a 10% formalin solution for further processing.

2.10. Biochemical Assay

Blood sugar level and hemoglobin were measured using commercially available kits. Serum lipid profiles such as triglycerides and total cholesterol were estimated using respective kits from BD, Bio-sciences, USA. Serum glutamic oxaloacetic transaminase (SGOT) and serum

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glutamate pyruvate transaminase (SGPT) were determined using a standard International federation of clinical chemistry (IFCC) kinetic method (BD, Biosciences, USA).

2.11. Histopathological Studies

For overnight fixation, the Kidney, Liver and Pancreas were added in the 10% formalin solution. Afterward, the dehydration of slices (3-4 mm) of liver, kidney and pancreas tissues were performed using ascending grades of alcohol, then cleared (alcohol was extracted) with xylene and embedded in paraffin wax (58– 60 ℃). Blocks were made and sectioned of 5 mm thickness with a microtome. The staining of tissue sections was done with hematoxylin and eosin staining (Fischer et al., 2008). The light microscope was used for the examination of prepared slides.

2.12. Statistical Analysis

The statistical analysis of the data was performed through ANOVA using Statistix 10 software and the data were presented as means ± standard deviation. The least significant difference (LSD) test was applied for multiple comparisons among the mean values. The differences were considered statistically significant at $p \leq 0.05$. The figures were plotted using Origin Pro. 8 software.

3. Results and Discussion

3.1 Strategy of Assay

Diabetes mellitus with its chronic metabolic disorders consistently remains a major threat to life. Down-regulating the generation of reactive oxygen species could be an alternative to reduce diabetes-associated complications. Minocycline is a semi-synthetic drug with excellent antioxidant properties similar to Vitamin C. Furthermore, the nanoparticles could work as a catalyst to increase the effectiveness of such phenolic antioxidants (Khorrami et al., 2018). Thus, in the present work,

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the Minocycline modified sliver nanoparticles (Mino/AgNPs) were prepared using minocycline as a reducing and capping agent. The prepared Mino/AgNPs were subjected to extensive characterization and successfully applied to examine their *in vivo* antidiabetic potential against alloxan-induced diabetic mice (**Graphical Abstract**).

3.2. Synthesis and Stability of Mino/AgNPs

UV-vis spectrophotometer is an important instrument to monitor the synthesis and stability of metal nanoparticles (X. F. Zhang et al., 2016). The mixture of silver nitrate, minocycline and sodium hydroxide was continuously stirred for 4 min at room temperature. The solution changed its color from colorless to yellowish-brown within 4 minutes which was the first indication of the synthesis of Mino/AgNPs (Hemmati et al., 2019). Furthermore, the synthesis of Mino/AgNPs was monitored through UV-vis. spectrophotometer (Shimadzu UV-1700) in the wavelength range 300-800 nm. A sharp LSPR band was observed at 395 nm **(Fig. 1)** which is a characteristic LSPR for silver nanoparticles (Lee & Jun, 2019). The stability of Mino/AgNPs was monitored for two weeks from plasmon wavelength (λmax) as aggregation causes the redshift of their spectra (X. F. Zhang et al., 2016). No significant shift in wavelength (λmax) was observed for LSPR of Mino/AgNPs colloidal solution until two weeks **(Fig. 2)** which describes the good stability of as-synthesized Mino/AgNPs.

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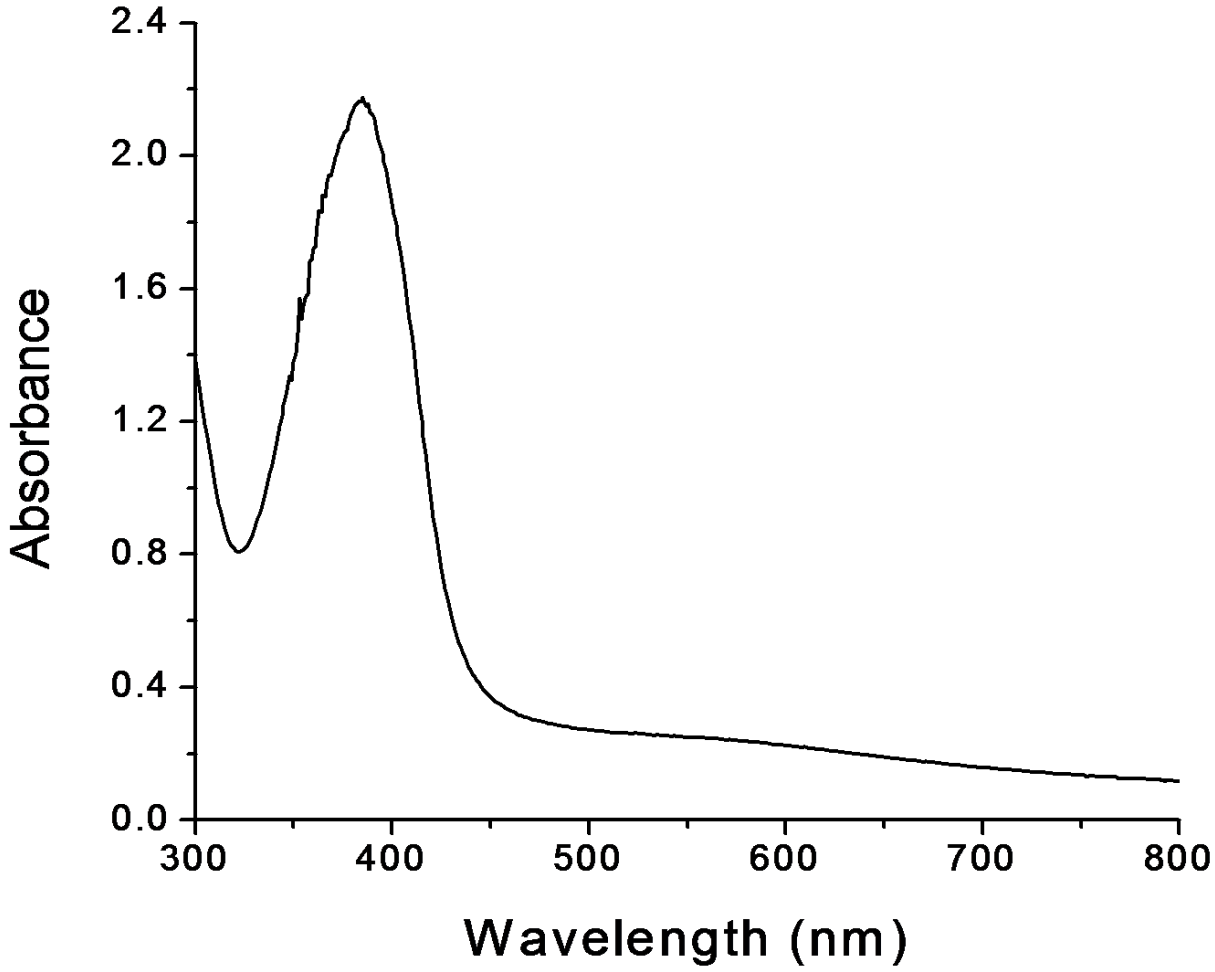

Figure 1. UV-vis spectrum of Mino/AgNPs

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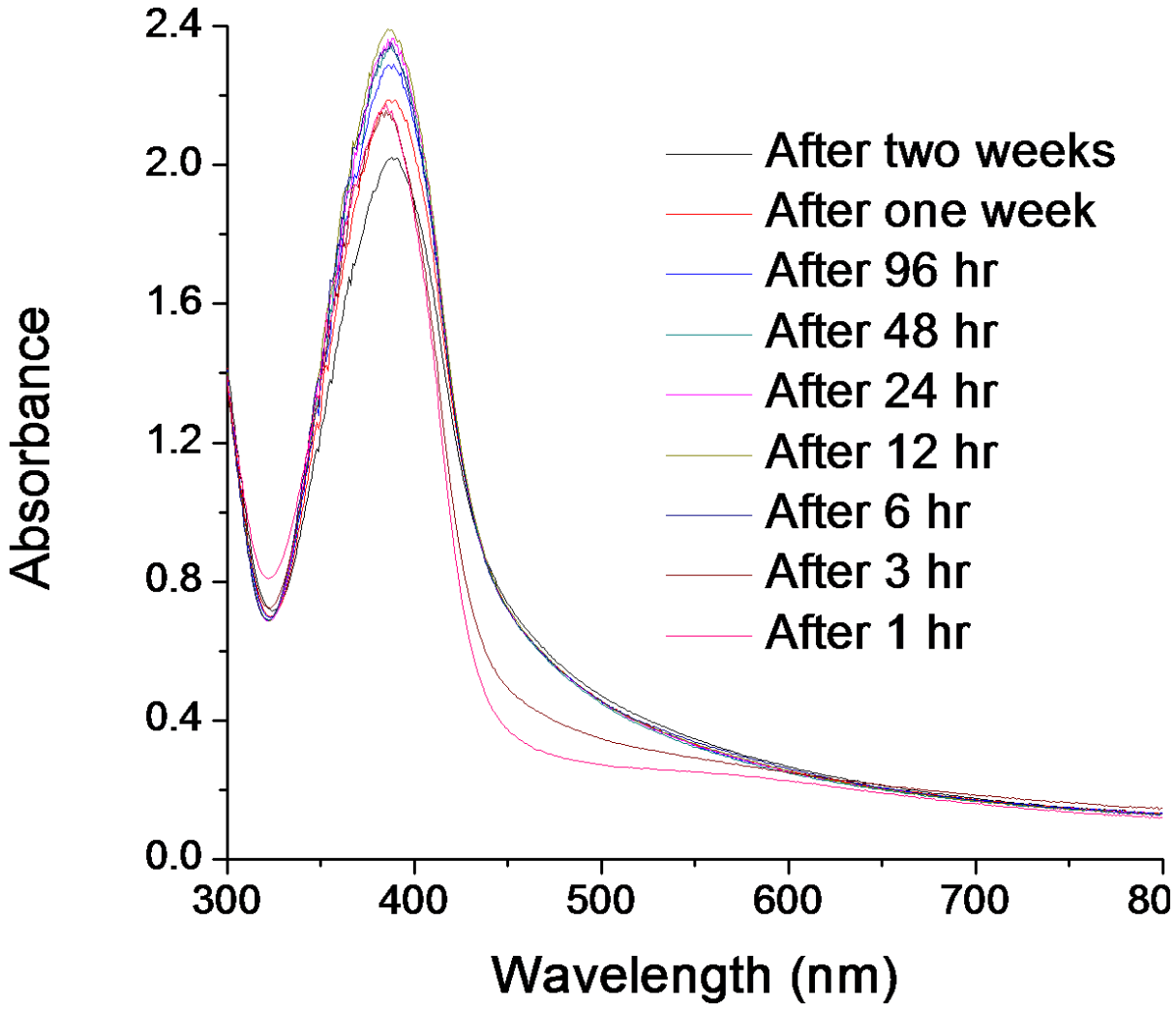

Figure 2. UV-vis spectra indicating the stability of Mino/AgNPs

3.3. Characterization of Mino/AgNPs

The size and morphology of prepared Mino/AgNPs were examined through TEM. Homogenously distributed spherical silver nanoparticles were obtained with this method **(Fig. 3)**. The average particle size of Mino/AgNPs calculated was 5.5 nm. The crystalline nature of Mino/AgNPs was examined through XRD analysis. XRD pattern of Mino/AgNPs showed strong diffraction peaks at 38.3, 44.5, 64.6 and 77.5° correspondings to (111), (200), (220), (311) which

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reflects the crystalline nature of Mino/AgNPs **(Fig. 4)**. Our XRD results of Mino/AgNPs are following the results reported elsewhere (Hemmati et al., 2019).

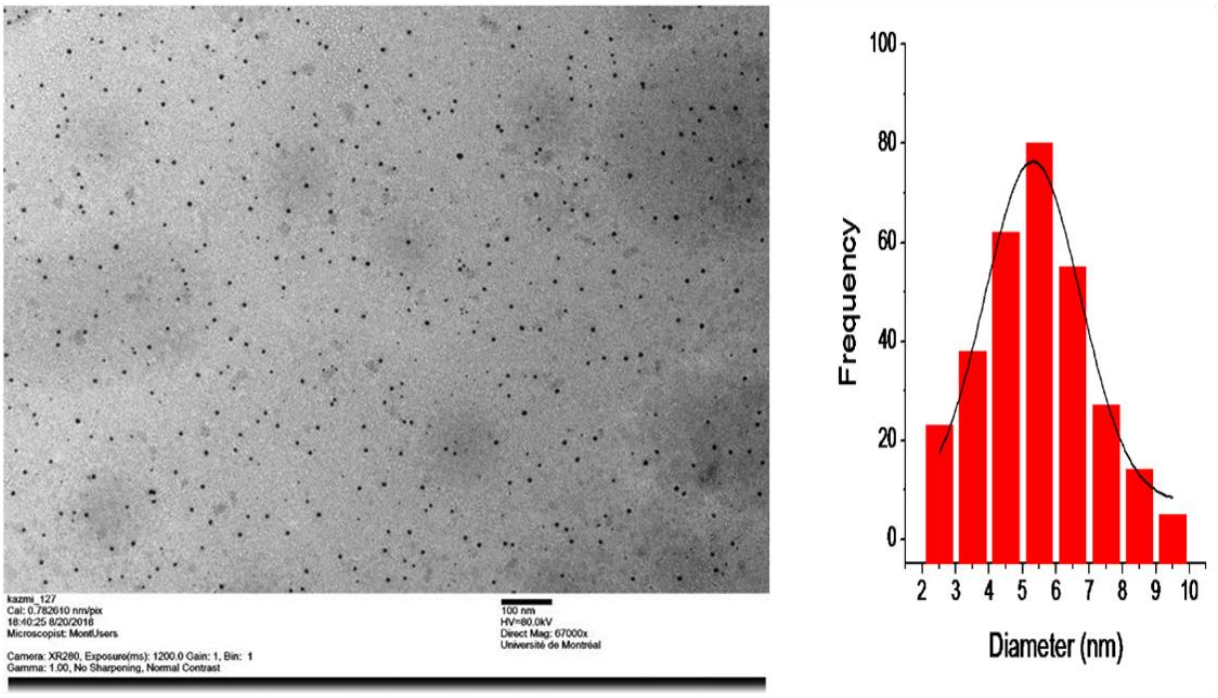

Figure 3. TEM and Histogram of Mino/AgNPs

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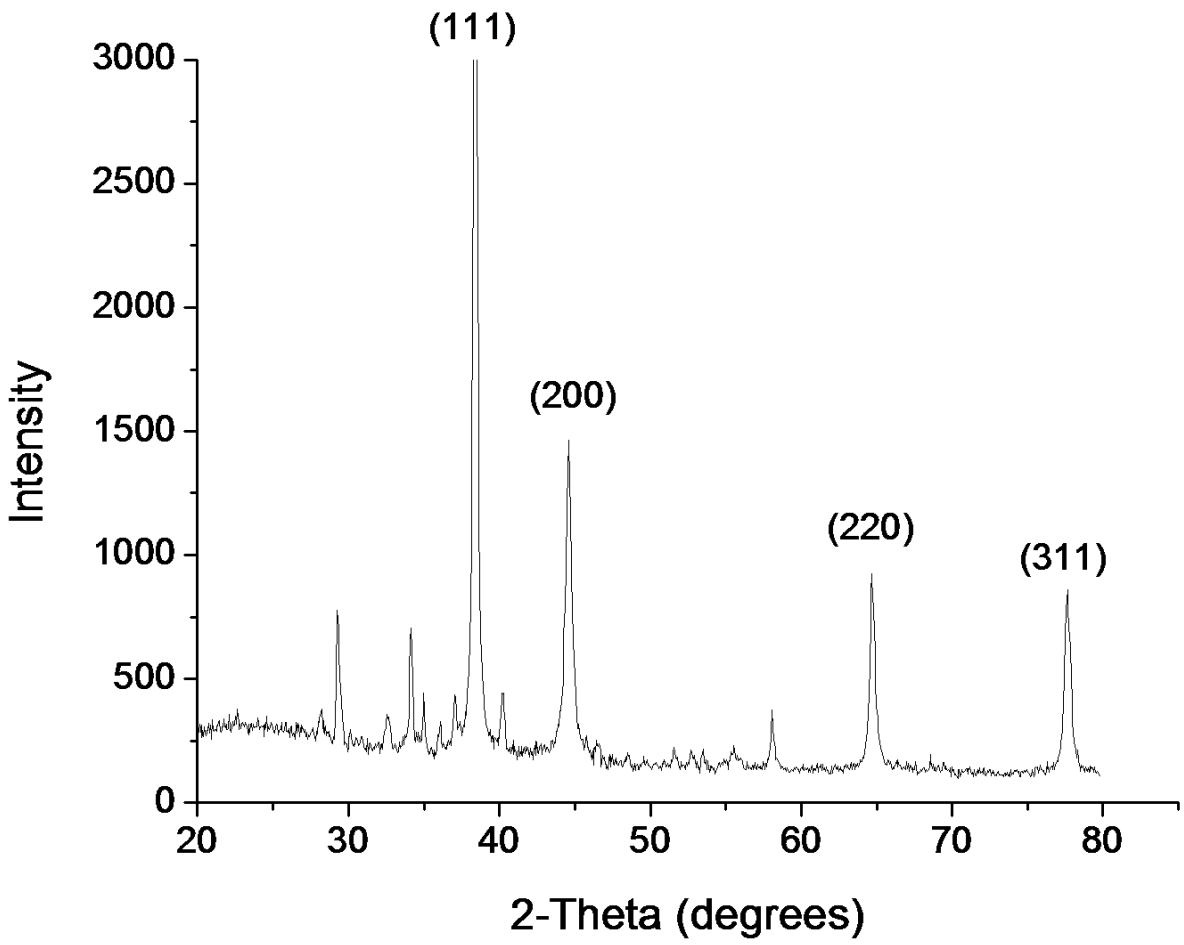

Figure 4. X-ray Diffraction of Mino/AgNPs

To examine the role of minocycline in the synthesis of AgNPs, FT-IR spectra of minocycline (red) and Mino/AgNPs (black) were compared advantageously **(Fig. 5)**. The spectrum of minocycline demonstrated two nominated signals at 3478.25 cm^{-1} for O-H bond stretching and 3340.11 cm^{-1} for N-H bond stretching. In addition to these two, some other signals were also present in the vicinity of these signals in the region of 3500-3100 cm^{-1} due to alkylic and vinylic alcohols. All of these signals became a single broad signal after the formation of nanoparticles which showed the involvement of these groups in the reduction of silver ions. The broadness of the signal also showed the presence of stretched N-H bonds involved in the stabilization of

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nanoparticles. The signal at 1580.41 cm^{-1} was attributed to the carbonyl of the amide group. This low value from 1680-1630 cm^{-1} for amidic carbonyl may be because of extended conjugation from C=C and NH_2. The shifting of this signal to 1633.65 cm^{-1} showed the involvement of the β-hydroxy group in the reduction of silver ions. The involvement of this hydroxyl group has resulted in the disappearance of C=C conjugation and so the signal for amidic carbonyl appeared in a normal range. The signal for carbonyl of ketone can also be observed in the vicinity of 1580.41 cm^{-1}. This signal is also shifted to a higher value like that of carbonyl of amide due to similar reasons.

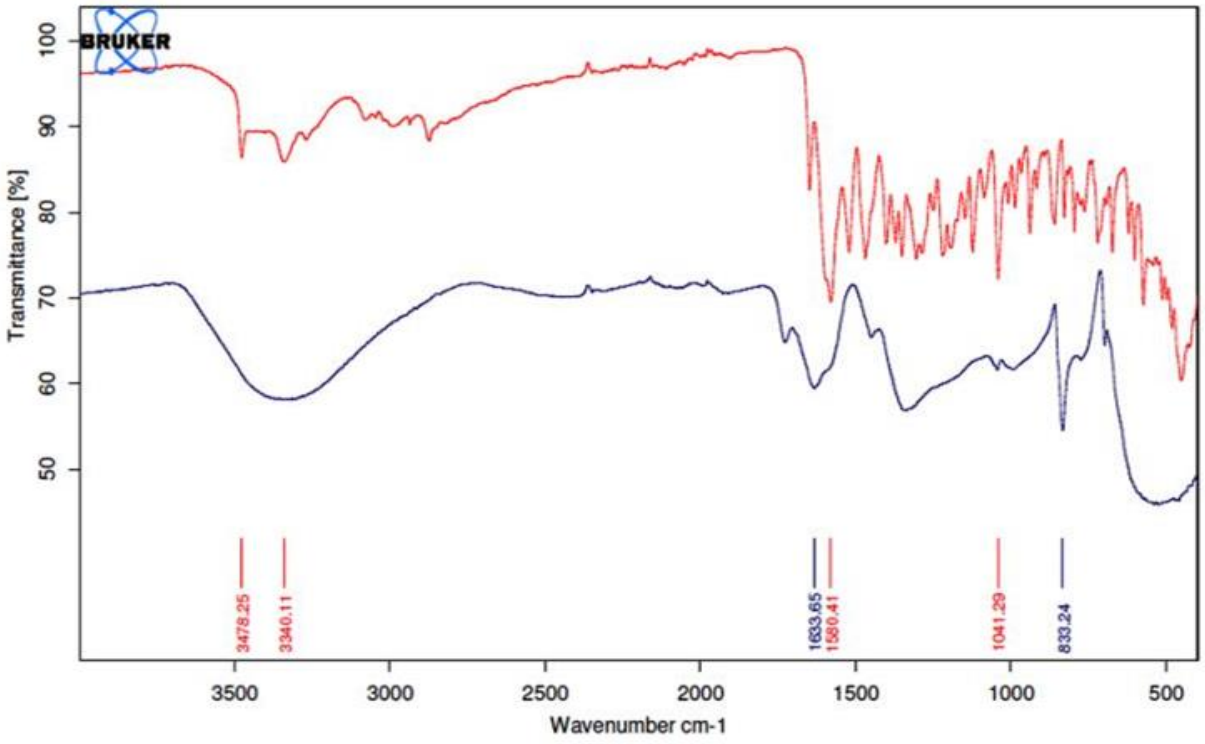

Figure 5. FT-IR Spectra of Minocycline (Red) and Minocycline modified Silver Nanoparticles (Blue)

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3.4 DPPH Radical Scavenging Assay

The free radical scavenging activities of minocycline, Mino/AgNPs and ascorbic acid were evaluated through DPPH assay. The results demonstrated that the percentage of inhibition was concentration-dependent and in general increased with the increase in concentrations of each analyte (**Fig. 6**). It was observed that the minocycline showed radical scavenging potency similar to that of ascorbic acid. Furthermore, the Mino/AgNPs showed higher radical scavenging activity (IC_{50} = 19.7 µg/mL) as compared to the minocycline (IC_{50} = 26.0 µg/mL) and ascorbic acid (IC_{50} = 25.2 µg/mL). We anticipated that the increased radical scavenging activity of Mino/AgNPs may be due to the additional effect of AgNPs as nanocatalysts. Elemike et al. also reported the catalytic effect of AgNPs to enhance the radical scavenging activity of *Costus afer* extract. The study reported that the Costus afer modified AgNPs (CA-AgNPs) showed higher DPPH radical scavenging activities as compared to the *Costus afer* leaf extract. They suggested that the increase in the antioxidant potential of CA-AgNPs can be due to the presence of phytochemicals such as flavonoids (with many hydroxyl groups) on the surface of AgNPs that contributed to the proceeding antioxidant activities through hydrogen atom transfer (HAT) and single electron transfer (SET) mechanism simultaneously (Elemike et al., 2017).

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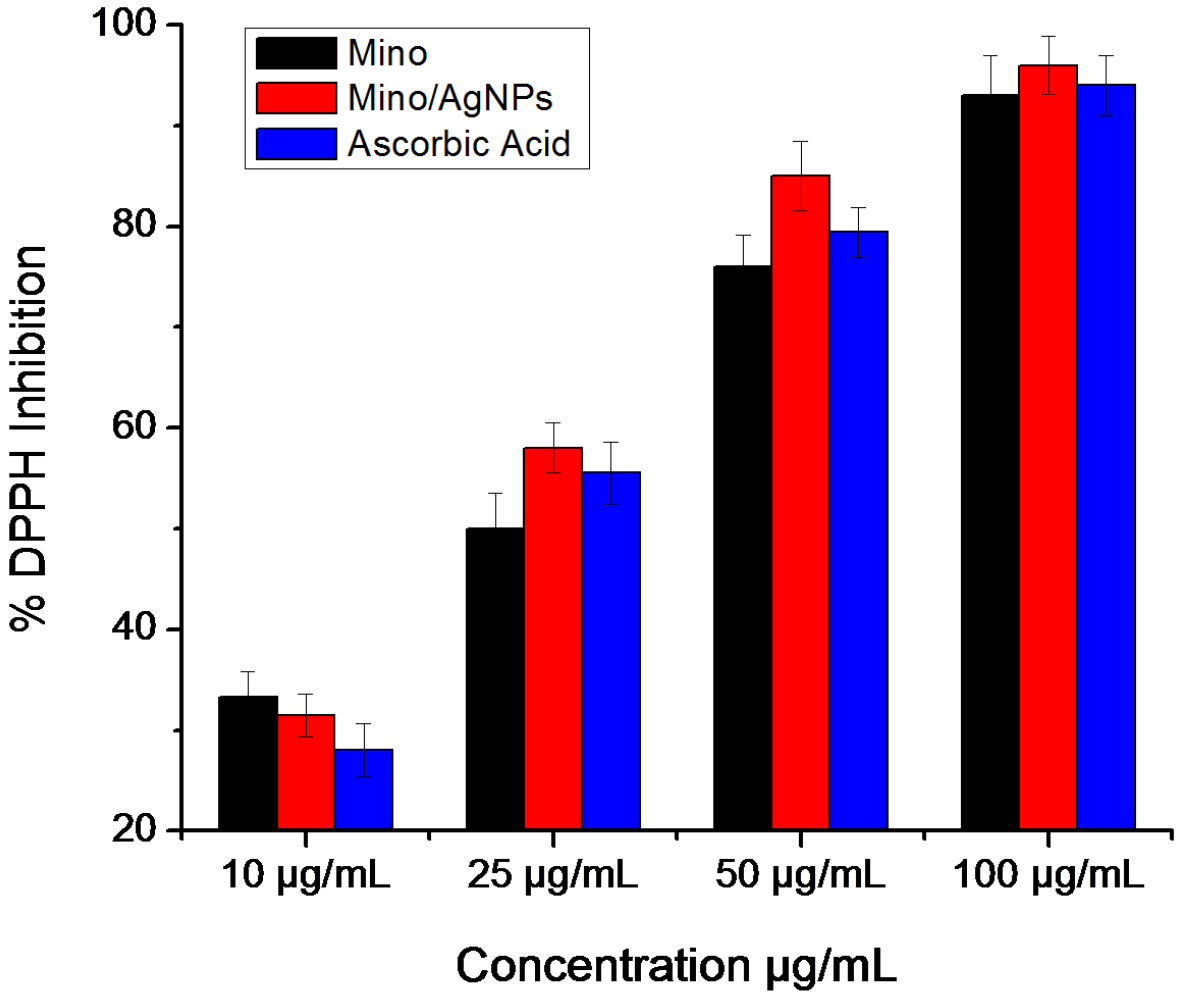

Figure 6. DPPH free radical scavenging assay

3.5. Antihyperglycemic activity of Mino/AgNPs in alloxan-induced diabetic mice

The diabetic mice carry the symptoms of diabetes mellitus such as hyperglycemia, weight loss, polyuria and decreased insulin level. The administration of standard drug glibenclamide and Mino/AgNPs to the diabetic mice resulted in a change of blood sugar level (BSL), cholesterol level, triglyceride level and hemoglobin level as compared to the diabetic mice. However, the Mino/AgNPs showed higher antidiabetic potential as compared to the drug glibenclamide. In

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diabetic mice, the BSL was notably high as compared to the normal mice. However, the oral administration of Mino/AgNPs to the diabetic mice resulted in a significant ($p \leq 0.05$) lowering of BSL relative to the diabetic mice left untreated **(Fig. 7)**. The hypoglycemic action of Mino/AgNPs can be attributed to its free radical scavenging activity that resulted in a decrease of ROS in the bloodstream. As a consequence of reduced oxidative stress, the insulin sensitivity was improved, thereby increasing cellular uptake of glucose from the bloodstream and thus down-regulate the blood sugar level in treated mice. Hurrle and Hsu also reported a similar effect of antioxidants on oxidative stress and insulin resistance. The study has reported the effect of ROS on different pathways in insulin receptor signal transduction that ultimately disrupt the expression of glucose transporter 4 (GULT4), a major glucose transporter in the cell. This affects the uptake of glucose from the blood into the cell that causes insulin resistance. However, the use of antioxidants reduces the oxidative stress that ultimately leads to the down-regulation of BSL in the bloodstream by improving insulin sensitivity (Hurrle & Hsu, 2017).

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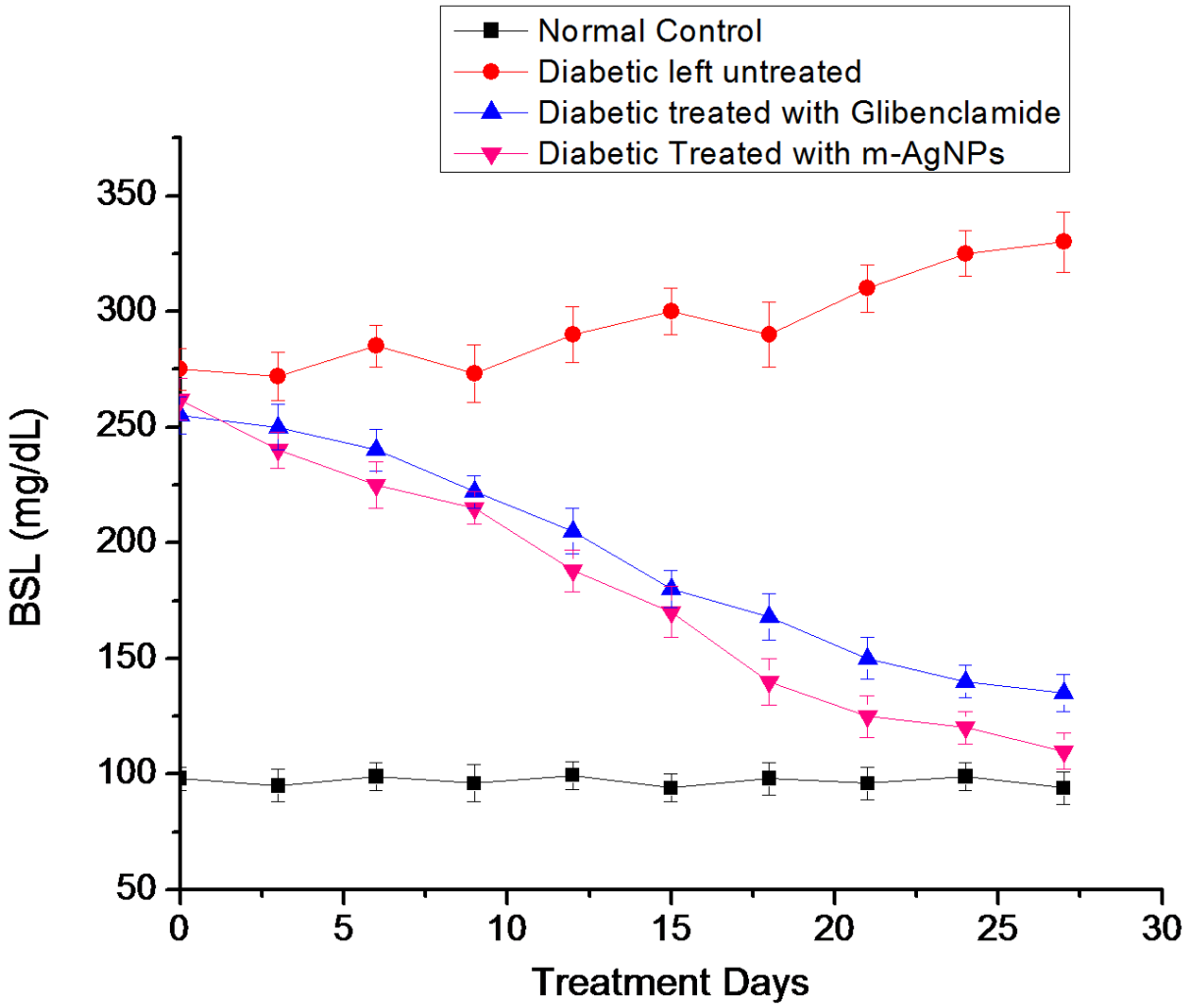

Figure 7. Blood Sugar Level (mg/dl) for various study groups

Furthermore, the weight loss and decrease in total hemoglobin level were also observed in diabetic mice as compared to the normal mice. In diabetic conditions, the reaction between excess glucose and hemoglobin converts hemoglobin to glycosylated hemoglobin as a result of which the level of hemoglobin decreases in diabetic mice (Dhas et al., 2016). However, the treatment of diabetic mice with Mino/AgNPs resulted in a Significant ($p \leq 0.05$) increase in body weight and hemoglobin level as compared to the untreated diabetic mice **(Fig. 8)**.

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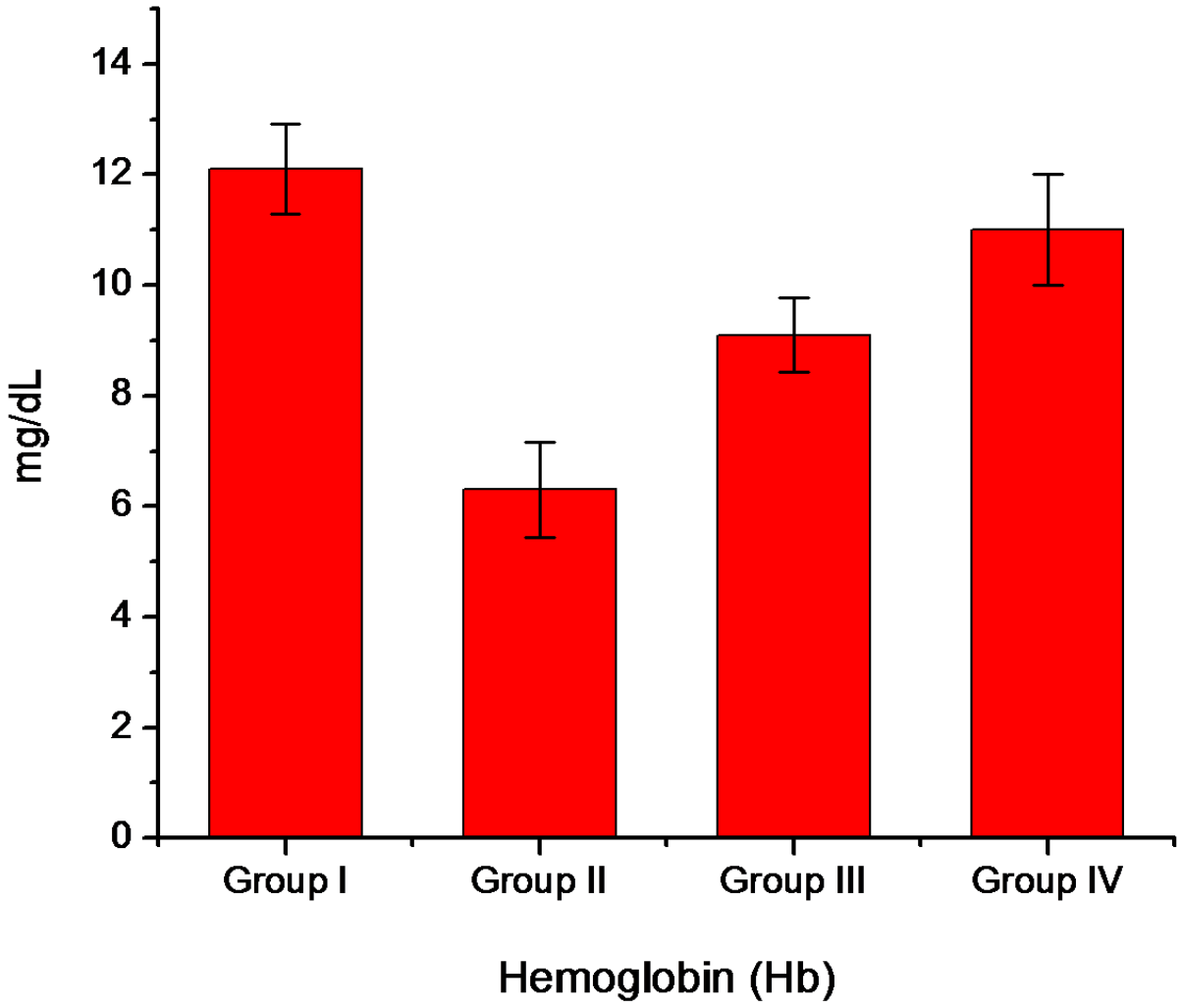

Figure 8. Hemoglobin Level (mg/dl) for the various study groups

Lipids have a very crucial part in the progression of DM. In a diabetic condition, the serum's lipids level is generally increased which indicates the risk of coronary heart disease (Al-Shamaony et al., 1994). Hypercholesterolemia and Hypertriglyceridaemia are the main risk factors that can cause atherosclerosis as well as coronary heart disease, the secondary complications associated with DM (Ananthan et al., 2003). The use of dietary or drug treatment seems to be effective in decreasing the lipids level in serum and consequently minimizing the risk of cardiovascular disease. In the present investigation, the cholesterol and triglycerides (TG) levels

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were notably high in diabetic mice. However, the oral administration of Mino/AgNPs to the diabetic mice resulted in a significant ($p \leq 0.05$) decrease in triglycerides and cholesterol levels as compared to the untreated diabetic mice **(Fig. 9)**. We anticipated that the increased level of ROS interferes with cell function, alter the cholesterol and triglyceride metabolism and thus resulted in higher TC and TG level. However the Mino/AgNPs treatment resulted in decreased oxidative stress by scavenging free radicals and ROS, normalization of cell function and consequently down-regulation of TC and TG levels (Murakami et al., 2020; Seo et al., 2019; Yang et al., 2008).

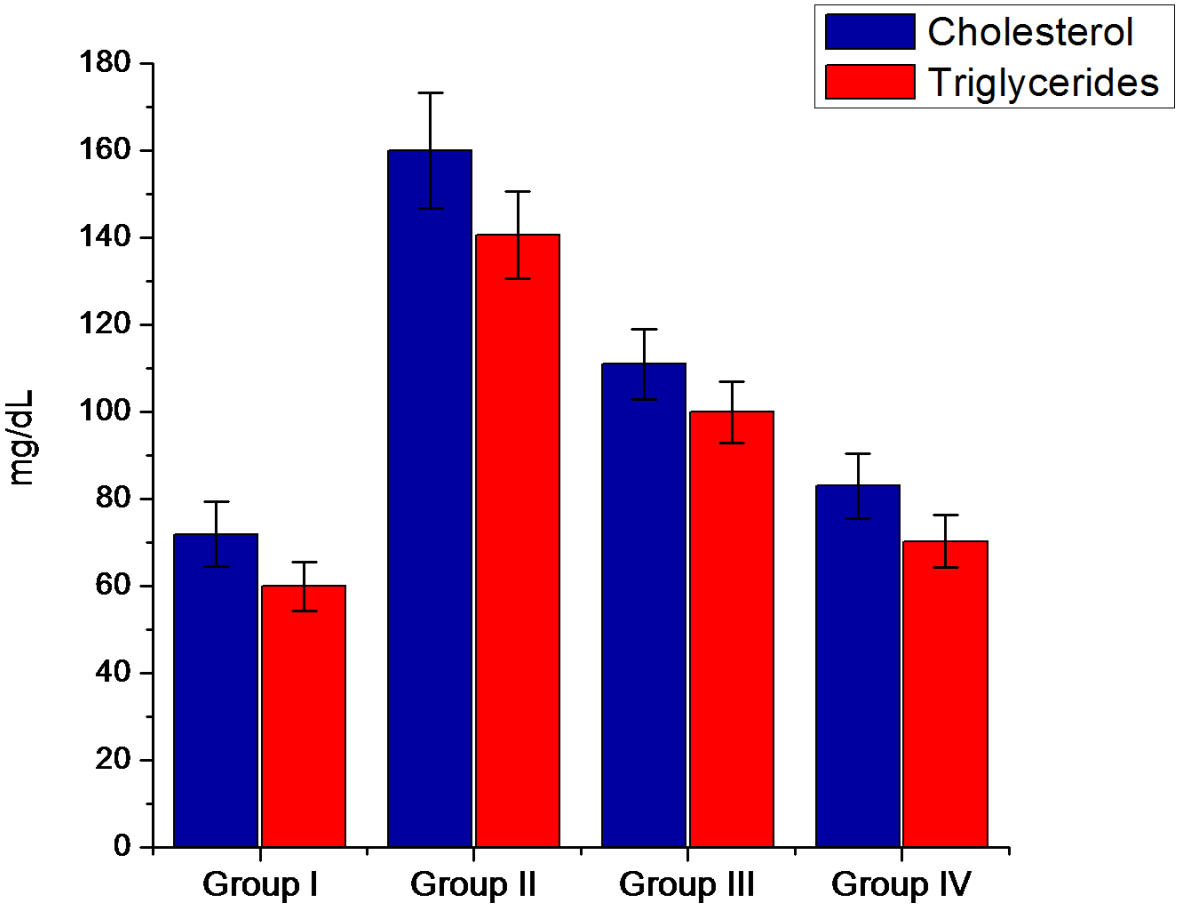

Figure 9. Lipid profile (mg/dl) for the various study group

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Furthermore, the administration of Mino/AgNPs to the diabetic mice also affected the activity of hepatic marker enzymes in the serum. In diabetic mice, the levels of SGOT and SGPT were raised. In diabetic conditions, the liver cells are damaged which causes the microsomal cells of the liver to excrete various enzymes such as SGOT, SGPT and ALP (Daisy et al., 2008). However, the oral administration of Mino/AgNPs to the diabetic mice maintained a significantly ($p \leq 0.05$) lower SGOT and SGPT as compared to the diabetic mice left untreated **(Fig. 10)**.

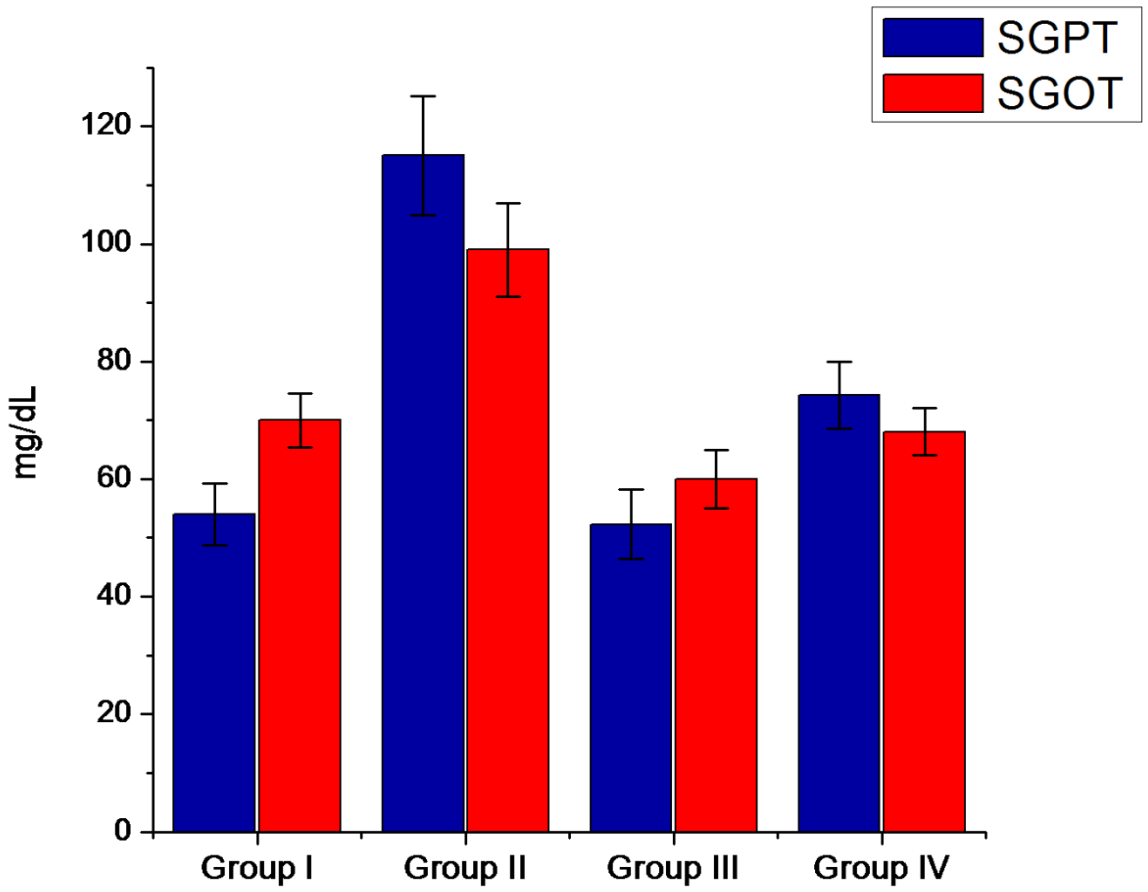

Figure 10. SGPT and SGOT Profile (mg/dl) for the various study groups

3.6. Histology Studies

In Histopathological studies, pancreas, kidney and liver sections of treated, untreated and normal control mice were examined. The pancreatic islet tissue of diabetic mice displayed irregular

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islet boundaries as well as mass distribution of cytoplasm relative to the normal mice (**Fig. 11)**. The treatment of diabetic mice with both glibenclamide and Mino/AgNPs displayed good regeneration and recovery of islet tissue of the pancreas. Nevertheless, the Mino/AgNPs showed more effectiveness in the regeneration and recovery of islet tissue than the glibenclamide. The β-cells mass was significantly higher in mice treated with Mino/AgNPs as compared to the diabetic mice left untreated **(Fig. 11)**. We anticipated that the Mino/AgNPs protected the β-cells of the pancreas from ROS and suppressed apoptosis in β-cells. The studies of Kaneto et al. also reported that the apoptosis induced by ROS in β-cells of the Pancreas was suppressed by the use of antioxidants (Kaneto et al., 1999).

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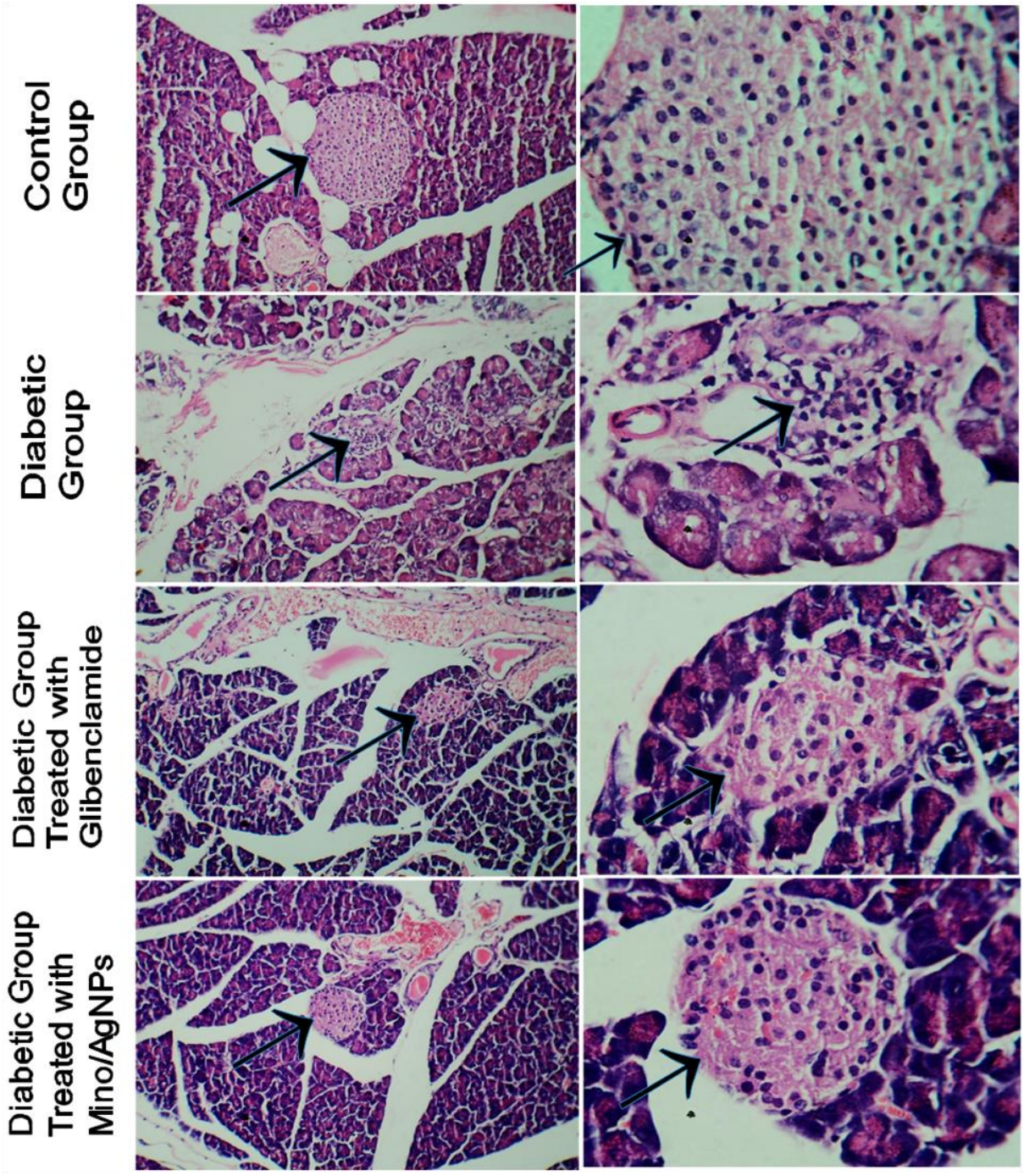

Figure 11. Histology of Islet cells of pancreatic sections of various study groups (Arrowhead pointing towards the islet tissue of pancreas)

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The tissue of the normal kidney section presented the normal architecture. The kidney section of diabetic mice showed distorted glomerular and dilated urinary space with Necrosis, vacuolation in the renal epithelial and some tubules with apoptotic cells **(Fig. 12)**. The treatment of diabetic mice with glibenclamide displayed limited improvement in the morphology of glomerular with some dilated urinary space whereas the treatment of diabetic mice with Mino/AgNPs displayed higher recovery and regeneration relative to the histo-morphology of kidney sections of normal mice. The kidney section of diabetic mice treated with Mino/AgNPs showed improved glomerular with improved urinary space very close to the architecture of normal mice **(Fig. 12)**.

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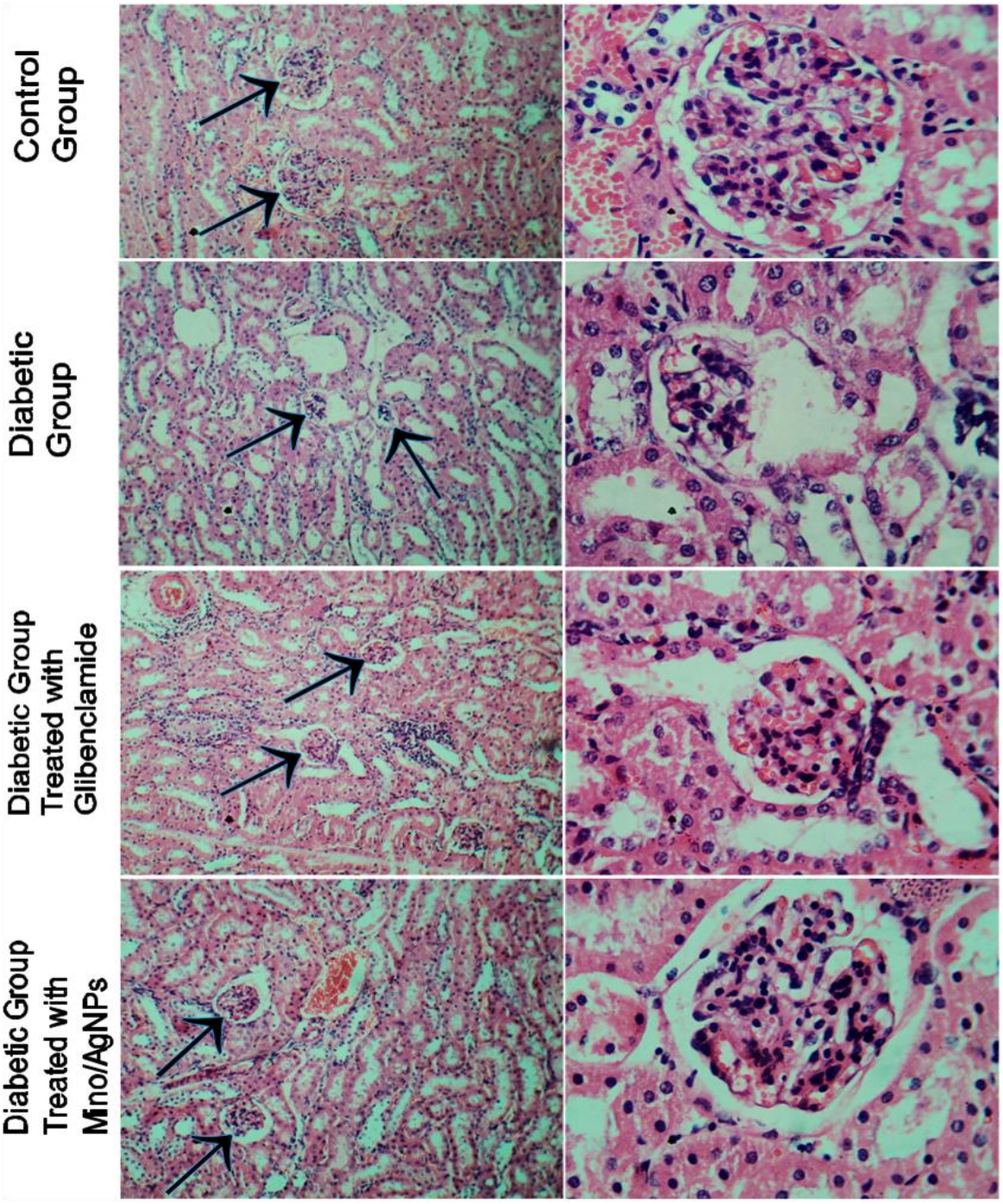

Figure 12. Histology of kidney sections of various study groups (Arrowhead pointing towards the glomerulus and urinary space of kidney)

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The hepatic sections of the normal liver showed normal architecture with intact central hepatic vein and slit-like sinusoids and prominent nuclei **(Fig. 13)**. The liver sections of diabetic mice displayed distorted central veins along with apoptotic nuclei. The oral administration of both drug and Mino/AgNPs to the diabetic mice showed significant recovery of the central hepatic vein. However, the treatment with Mino/AgNPs showed better recovery and revival effect as compared to the drug glibenclamide **(Fig. 13)**.

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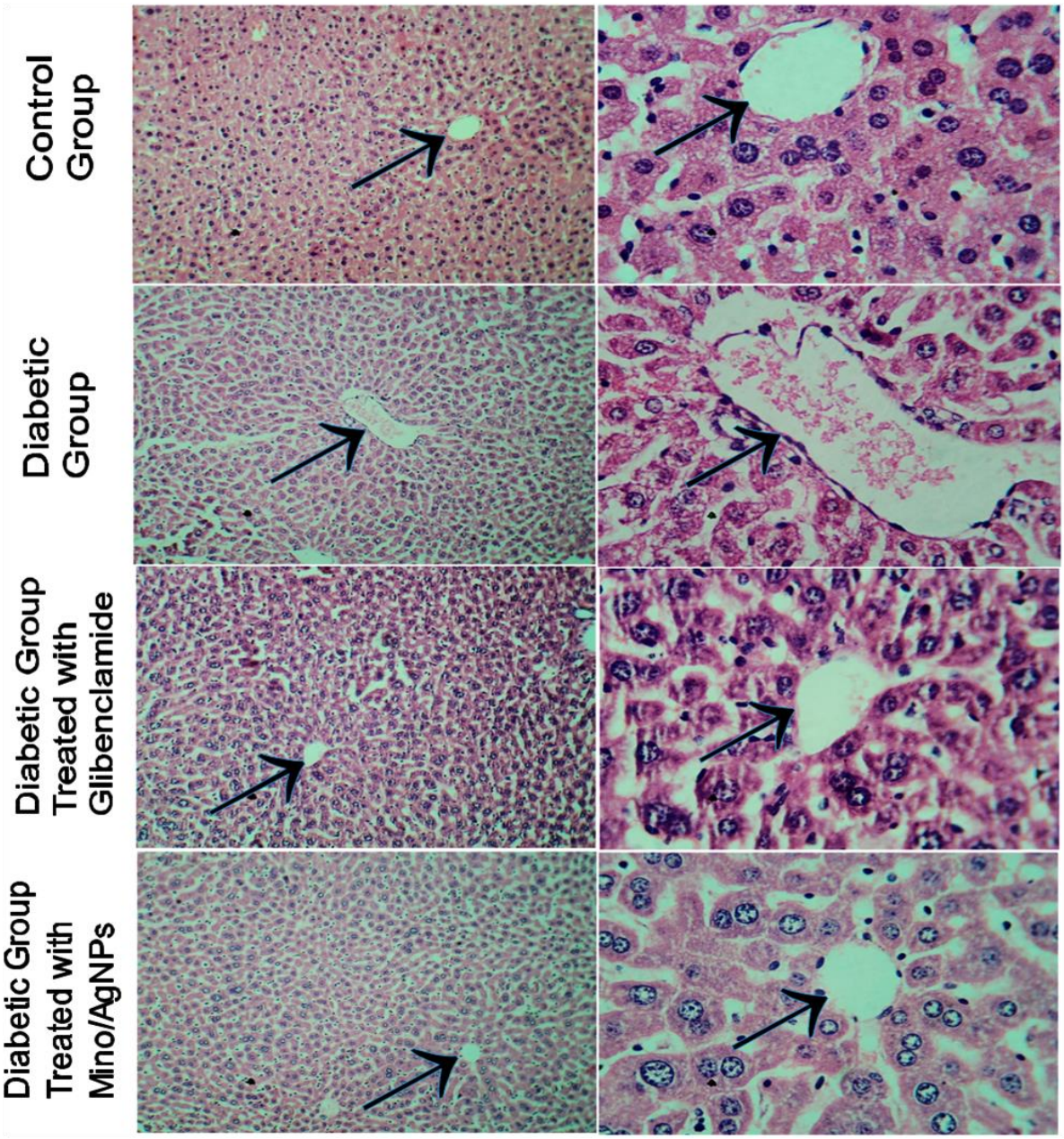

Figure 13. Histology of Liver sections of various study groups (Arrowhead pointing towards the central hepatic vein of the liver)

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4. Conclusion

Diabetes mellitus is a life-threatening disease all over the world and it demands significant efforts to be treated effectively. The antioxidants have shown to be very effective in many bioprocesses including disorders of diabetes mellitus. The present work was carried out to examine the antidiabetic potential of newly synthesized Mino/AgNPs against the alloxan-induced diabetic mice. The DPPH inhibitory assay was conducted to compare the antioxidant potential of Mino/AgNPs with that of minocycline and ascorbic acid. The Mino/AgNPs showed higher radical scavenging activity (IC_{50} = 19.7 µg/mL) as compared to the minocycline (IC_{50} = 26.0 µg/mL) and ascorbic acid (IC_{50} = 25.2 µg/mL). Further, hematological and histopathological analysis revealed that the Mino/AgNPs showed greater potential as an antidiabetic agent than the standard drug glibenclamide. The Mino/AgNPs showed more effectiveness in reducing Blood sugar, cholesterol and triglycerides levels. Furthermore, the treatment of diabetic mice with Mino/AgNPs also showed significant regeneration and revival of histo-morphology of kidney, central vein of liver and islet cells of the pancreas as compared to the normal control mice. Our results indicated that the as-synthesized Mino/AgNPs have good potential to reduce the disorders of diabetes mellitus and can be effectively used to treat diabetic conditions.

Acknowledgments

The authors are thankful to the Government College University Lahore and the HEC Pakistan.

Author's declaration

Conflict of Interest

The authors declare no conflict of interest.

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