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Silver/chitosan/ascorbic acid nanocomposites ameliorate diabetic nephropathy in the model of type 1 diabetes

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Abstract

Aims: The present study aimed to evaluate anti-diabetic properties of AgNPs/chitosan/ascorbic acid nanocomposites (Ag-NCs) in streptozotocin-induced diabetic rats.

Main methods: Eighteen male Wistar albino rats were divided into three main groups (6 rats/group); control, diabetic and Ag-NCs groups. Control group: after a single dose of citrate buffer (0.1 mol/l, i.p), the rats orally received 1 ml distilled water daily for four weeks. The diabetic model was induced by a single dose of streptozotocin (60 mg/kg, i.p) for type 1diabetes. Diabetic groups were treated orally with and Ag-NCs (0.25mg/Kg body weight) daily for four weeks.

Key findings: AgNPs/chitosan/ascorbic acid nanocomposite group showed a reduction in the concentrations of glucose, NO, MDA, creatinine, urea and uric acid. At the same time, it appeared a general increase in insulin, CAT, and SOD activities and GSH concentration. The histopathological investigation illustrated a clear improvement in renal architecture.

Significance: The suggested mechanism of action for Ag-NCs in decreasing diabetic nephropathy includes two pathways; the hypoglycemic activity and the antioxidant role of Ag-NCs

Keywords: Type 1 Diabetes; AgNPs/chitosan/ascorbic acid nanocomposites; Rats; Kidney functions; Oxidative stress

1. Introduction

Diabetes Mellitus (DM) is epidemic disorder of endocrine system which characterized by glucose intolerance due to high blood glucose levels and disturbed metabolism of carbohydrates, proteins, and lipids. DM is the fourth-largest cause of morbidity and mortality in the developed world [1]. The global diabetes prevalence in 2019 is projected to be 9.3% (463 million people), increasing to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045. Approximately the number of people living worldwide with diabetes is estimated to increase by 25% in 2030 and will be doubled to 51% in 2045 [2]. Diabetes mellitus is classified into type 1 diabetes (insulin dependent) which characterized by insufficient production of insulin hormone due to an autoimmune damage of the pancreatic β cells through T-cell mediated insulitis in addition to the response of a humoral (B cell) [3] and type 2 diabetes (non-insulin dependent) includes insulin resistance or impaired insulin secretion. Recently, a new term of diabetes would be described as type 3 diabetes which used for insulin resistance in brain [4]. There are long-term complications of diabetes involving macrovascular complications as coronary heart disease, stroke and peripheral vascular disease, and microvascular complications such as nephropathy, retinopathy and neuropathy [5]. One of the most destructive complications of type 1 diabetes mellitus (T1DM) is diabetic nephropathy (DN) that often results in end-stage renal disease (ESRD).

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The interface of nanotechnology in biomedical science has presented novel strategies for monitoring, imaging, repairing, targeted therapy and control of human biological systems. The application of nano-sized particles may be defined as a multidisciplinary area for glucose measurement and insulin delivery [6]. Additionally, the most distinctive feature of nano-based drug in diabetes management is early diagnosis, monitoring of disease progression, and introducing anti-diabetic agents. In previous researches, different types of biomedical nanoparticles are available and studied for insulin delivery in the treatment of diabetes mellitus including: polymeric biodegradable nanoparticles (nanospheres and nanocapsules), polymeric micelles, ceramic nanoparticles, dendrimers, and liposomes [7] Biocompatible nanoparticles with optimized physical, chemical, and biological properties can overcome the limitations of conventional anti-diabetic drugs [8]. Metal Nanoparticles are highly designed as medicinal agents for the of number of diseases such as cancer, diabetes, Parkinson's, Alzheimer, HIV/AIDS, arthritis, hepatitis, cirrhosis, spinal cord injury tuberculosis and cardio vascular diseases due to their unique physical, chemical and biological properties [9].

Silver nanoparticles (AgNPs) play an important role in the development of novel biomedical techniques because of their physiochemical properties and biofunctional features such as anti-inflammatory, anti-angiogenesis, antiplatelet, antiviral, antifungal, and antibacterial activities [10]. So that, they have been emerged in novel drug-delivery systems, catheter modification, wound healing and dental applications [11]. Indeed, genotoxicity and cytotoxicity of AgNPs depend on their dispersion rate, concentration, surface charge, size, morphology, and surface functionalization [12]. The green chemistry synthesis method was effective, clean, nontoxic, environment friendly, inexpensive and reliable to develop nanomedicines [9]. In a novel diabetic study reported that green synthesized AgNPs could reduce blood glucose level through inhibiting the activities of carbohydrate metabolism mediated enzymes such as α-amylase and α-glucosidase enzymes [13]. Other study claimed that plant extract mediated AgNPs significantly decreased pro-inflammatory cytokines IL-6 and TNF-α which led to accelerate wound healings in streptozotocin-induced diabetic rats [14]. Furthermore, green synthesized AgNPs were used for alleviation of diabetic neuropathy which is accounted as microvascular complication; this is because of their anti-inflammatory, anti-oxidant, anti-diabetic properties [15].

Chitosan is a promising biopolymer that evolved in the biomedical applications due to its chemical and pharmacokinetic properties. In addition to the benefits of chitosan as anti-diabetic agent for protecting and proliferating pancreatic beta cells, decreasing hyperglycemia, enhancing glucose tolerance, and preventing impaired lipid metabolism associated diabetes mellitus [16]. In previous animal investigations, chitosan showed remarkable ameliorating lipid accumulation and hyperglycemia that are considered the upstream risk factors of diabetic nephropathy; also it has the ability to prevent kidney injury with minimal side effects [17].

On the other hand, ascorbic acid is a powerful water-soluble antioxidant which has a scavenging effect on excessive free radicals that developed by diabetes disease and plays a protective role in tissue loss caused by oxidative stress [18]. In spite of advances of pharmaceutical drugs for hyperglycemic control, there is a great focus on innovative strategies for the utilization of anti-oxidants beyond anti-diabetic drugs to manage diabetic nephropathy [19]. Streptozotocin-induced hyperglycemia, pancreatic and renal injuries is commonly used for creating rodent models of type 1 diabetes which mimic the human diabetic nephropathy [20].

The ultimate goal of this work is the investigation of anti-diabetic activity of AgNPs/chitosan/ascorbic acid nanocomposites (Ag-NCs) on streptozotocin induced diabetes in rats in order to provide dual therapeutic action for type 1 diabetes management and its complications' control. The present era has witnessed the progress of next generation of nanotechnology as a prospective field in diabetes medication.

2. Material and methods

2.1. Chemicals

Medium molecular weight chitosan (1278 ± 10 KDa), acetic acid, silver nitrate, ascorbic acid, sodium hydroxide, streptozotocin, and sodium citrate were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of Ag-NCs through a green chemistry route

One gram of chitosan was added to 100 ml of 1% acetic acid then heated up to 95℃ using oil bath. And then 7 mM of $AgNO_3$ mixed with 1 mM of ascorbic acid solutions were wisely dropped in the following volume ratio: chitosan: $AgNO_3$: ascorbic acid, 100: 20: 20, respectively. This mixture was kept under heating and stirring for 12 hrs. Then dried in an electric oven (Pol-Eko) at 60°C till the complete evaporation of solvent. Lastly, the mixture was neutralized with 1 % NaOH solution and washed with deionized water then dried and kept in the dark until further use for the experiment [21].

2.3. Physical characterization of Ag-NCs

2.3.1. UV–Visible spectral analysis

The formation of Ag-NCs was confirmed by UV–Vis. absorption spectra. The reduction of the Ag^+ ions in solution was observed by periodic sampling of reaction mixture, and the maximum absorption was scanned by UV-Vis. spectrophotometer (Shimadzu UV-1601). The colour intensity of synthesized Ag-NCs was measured between the wavelengths 200 to 700 nm, and operated at an interval of 10 nm [22].

2.3.2. X-ray diffraction (XRD) analysis

A Bruker D2 diffractometer analyzed the XRD patterns at 40 kV and 50 mA. The secondary graphite monochromated Co K alpha radiation (l=1.7902A°) was used, and the measurements were recorded at a high angle 2 theta (2θ) in a range of 5°-90° with a scan speed of 0.01° [23].

2.4. Surface morphology analysis

2.4.1. Transmission electron microscopy (TEM) analysis

TEM technique was used to detect the morphology and the size of the synthesized Ag-NCs. A JEOL JEM-2100 transmission electron microscope operated at an accelerating voltage f 200 keV. TEM grids were prepared by placing a drop of the particle solution on a carbon-coated copper grid and drying under lamp [24] and the image was assessed with ImageJ software to measure the particle size distribution.

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3. *In vivo* analysis

3.1. Experimental animals

Male albino Wistar rats (*Rattusnorvegicus*) weighing 140 ± 10 gm for T1DM were used in this study. The rats were obtained from the National Research Center (NRC, Dokki, Giza). They were grouped and housed in polyacrylic cages (six animals per cage) in the well-ventilated animal house of the Zoology Department, Faculty of Science, Cairo University. Rats were given food and water *ad libitum*. Rats were maintained in a friendly environment of a 12 hr/12 hr light-dark cycle at room temperature (22– 25 °C). They were acclimatized to laboratory conditions for seven days before the commencement of the experiment.

3.2. Experimental design

Induction of T1DM by mean intraperitoneal injection of 60 mg/kg of streptozotocin (STZ) dissolved in 0.1mol/l sodium citrate buffer at pH. Blood glucose levels were measured 72 hr after injection of STZ using a blood glucose meter device. Rats were starved but had access to drinking water for 6 hrs before blood glucose measurement. Fasting plasma glucose concentrations ≥ 300 mg/100 ml were considered T1DM in this experiment [25]. Rats were divided into 3 groups with 6 rats in each. Control group: after a single dose of citrate buffer (0.1 mol/l, i.p), the rats received 1 ml (dist. water, orally) daily for 4 weeks. Diabetic group: after a single dose of STZ (60 mg/kg, i.p), the rats received 1 ml (dist. water, orally) daily for 4 weeks with no treatment. Ag-NCs group: after a single dose of STZ (60 mg/kg, i.p), the rats received Ag-NCs as a treatment (0.25 mg/Kg body weight, orally) daily for 4 weeks [26]. Confirmation of induced Diabetes was done after three days through measuring blood level of glucose in the blood samples which is obtained from the tail vein with the One Touch Ultra Glucometer.

3.3. Blood collection

After the end of all experiments, the rats were fully anesthetized with 3% sodium pentobarbital, and the chest was opened. A needle was inserted through the diaphragm into the heart. Negative pressure was gently applied once the heart had been punctured, and the needle was repositioned as required until blood flowed into the syringe. The blood collected from the rats was separated by centrifugation (3000 rpm, 15 min) to obtain sera which were stored at −80 °C for the biochemical measurements. Kidneys were removed and were immediately blotted using a filter paper to remove traces of blood. Part of the kidneys was stored at −80 °C for biochemical analysis. The kidneys were suspended in 10% formal saline for fixation preparatory to histopathological examination.

3.4. Determination of blood glucose and insulin levels

The blood glucose levels were carried out by the commercial kit Biodiagnostic Company (Dokki, Giza, Egypt) by the method of Freund *et al*. [27] And plasma insulin level was measured using ELISA kit SinoGeneClon Biotech Co.,Ltd with Catalog No.: SG-20161 following the manufacturer's instructions [28] .

3.5. Determination of kidney functions

The blood creatinine [29], urea [30], uric acid [31] statuses were examined with the aid of commercial colorimetric assay kits according to the manufacturer's instructions using Biodiagnostic kits (Giza, Egypt).

3.6. Kidney homogenate preparation

 Renal tissue was homogenized (10% w/v) in ice-cold 0.1 M Tris–HCl buffers (pH 7.4). The homogenate was centrifuged at 860 ×g for 15 min. at 4 ℃ and the resultant supernatant was used for the biochemical analyses.

3.7. Estimation of oxidative stress markers

MDA level is an index of lipid peroxidation and it was estimated by Ohkawa et al [32], glutathione reduced (GSH) [33], nitric oxide (NO) [34], superoxide dismutase (SOD) [35] and catalase [36] were determined in the kidney homogenate supernatant according to the manufacturer's instructions using Biodiagnostic kits (Giza, Egypt).

3.8. Histopathological examination

Kidneys were fixed in 10% neutral-buffered formalin. The fixed specimens were washed, dehydrated, and embedded in paraffin wax. The tissues were sectioned at a thickness of 4–5 μm and stained with hematoxylin and eosin (H&E) according to Bancrof and Stevens [37]**,** as routine procedures for histopathological examination.

3.9. Statistical analysis

Values were expressed as means ± SE. The comparisons within groups were evaluated utilizing one way analysis of variance (ANOVA) with Duncan post hoc test was used to compare the group means and p b 0.05 was considered statistically significant. SPSS, for Windows (version 15.0) was used for the statistical analysis.

4. Results

4.1. Characterization of Ag-NCs

The synthesis of Ag-NCs was observed by the colour change from golden yellow colour to brown colour. The absorption spectrum of the represented Ag-NCs suspension showed a Surface Plasmon Absorption band with maximum peak at 440 nm, which indicates the formation of Nano-sized particles (Figure 1) as *Regiel-Futyra etal* assessed the measurement of Ag-NCs [21].
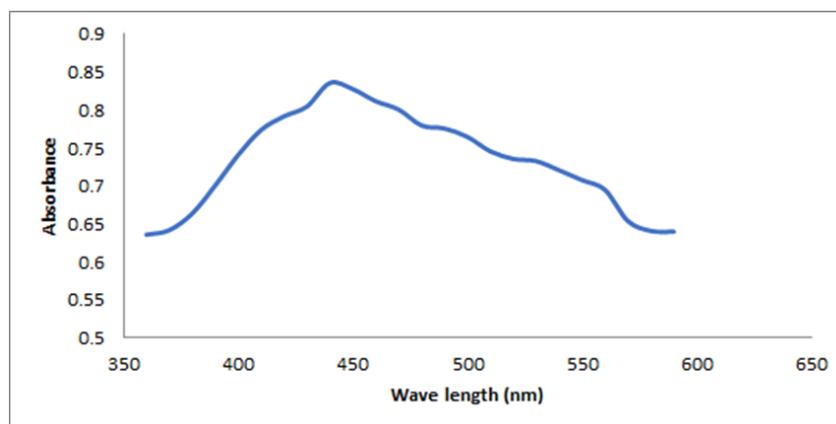
Figure 1 UV–Visible absorption spectrum of Ag-NC

The analysis of the crystalline size and structure of the Ag-NCs was carried out by XRD as shown in Figure 2. The numbers 38.12°, 44.32°, 64.51° and 77.35° of Bragg reflections with 2θ values indicated that the Ag-NCs were spherical and crystalline.
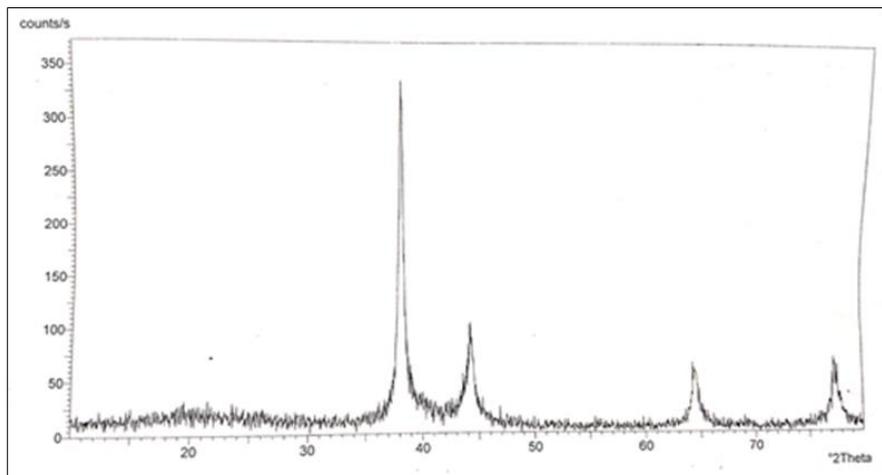
Figure 2 X-ray diffractometer (XRD) analysis of Ag-NCs

The TEM analysis of Ag-NCs showed that the nanoparticles had spherical shape and the size was 35.26±15.0 nm (Figure 3).
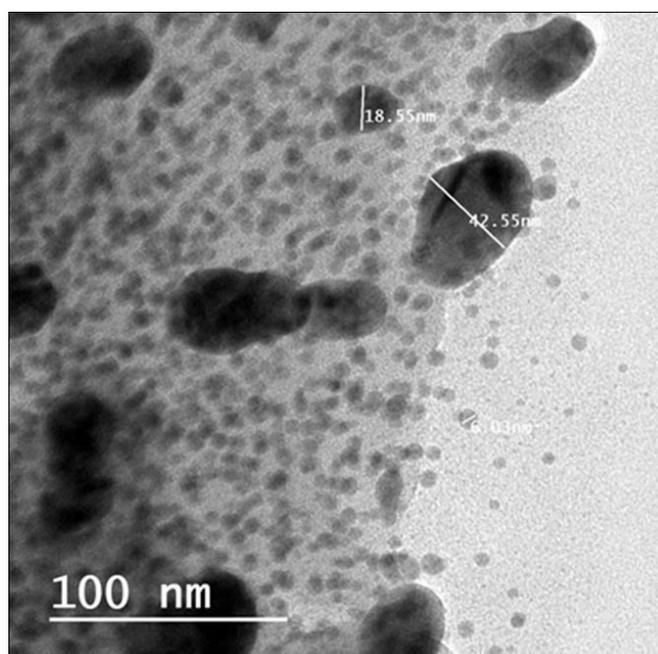
Figure 3 TEM micrographs of Ag-NCs

4.2. Biochemical parameters

4.2.1. Diabetic markers

Significant elevation (P <0.05) in glucose concentration was observed in T1DM rats, while insulin concentration were decreased compared to the control group (Table 1). Ag-NCs treatment significantly (p < 0.05) reduced the glucose concentration, while it increased insulin concentration compared with the diabetic group.

Table 1 The curative effect of Ag-NCs on diabetic markers of diabetic rats

Parameter	Groups		
	Control	DM	DM+AgNCs
Glucose (mg/dl)	95.00±2.77[a]	354.60±34.87[c]	174.00±20.67[b]
Insulin (µIU/ml)	6.70±0.69[a]	2.86±0.25[c]	4.01±0.37[b]

Values are means ± se (n = 6 per group). Each value not sharing a common letter superscript is significantly different (P <0.05).

4.2.2. Kidney function bioindicators

Concentrations of creatinine, urea and uric acid of T1DM rats showed a general increase compared to the control group (Table 2). On the other hand, oral administration of Ag-NCs produced a significant decrease (p<0.05) in concentrations of creatinine, urea and uric acid compared with T1DM rats.

Table 2 The curative effect of Ag-NCs on kidney function bioindicators of diabetic rats

Parameter	Groups		
	Control	DM	DM+AgNCs
Creatinine (mg/dl)	0.72±0.03[a]	1.09± 0.06[b]	0.84± 0.04[a]
Urea (g/dl)	24.00±1.81[a]	48.83±1.35[c]	30.67±2.36[b]
Uric acid (mg/dl)	3.47±0.08[a]	5.23±0.23[b]	4.03±0.26[a]

Values are means ± se (n = 6 per group). Each value not sharing a common letter superscript is significantly different (P <0.05).

4.2.3. Oxidative stress markers

Significant decreases (p < 0.05) in renal GSH, SOD, and CAT levels were observed in the diabetic group, while MDA and NO concentrations increased compared to the control group (Table 3). Ag-NCs treatment caused significant increases (p < 0.05) in kidney GSH, SOD, and CAT levels while MDA and NO concentrations decreased compared to the diabetic group.

Table 3 The curative effect of Ag-NCs on oxidative stress parameters of diabetic rats

Parameter	Groups		
	Control	DM	DM+ Ag-NCs
MDA (nmol /tissue)	2.74±0.19[a]	4.18±0.47[b]	3.10±0.21[a]
CAT (U/ tissue)	1.45±0.07[b]	1.08±0.09[a]	1.30±0.06[ab]
GS (mg/ tissue)	25.08±1.31[a]	18.62±1.26[a]	24.50±3.86[a]
NO (mg/ tissue)	194.00±14.00[a]	324.84±11.31[c]	265.09±21.43[b]
SO (U/ tissue)	46.83±3.54[b]	26.31±2.31[a]	35.02±2.98[a]

Values are means ± se (n = 6 per group). Each value not sharing a common letter superscript is significantly different (P <0.05).

4.3. Histopathology of kidneys

Histopathological examination of kidneys sections of rats from the control group exhibited normal histological structure of both renal cortex and medulla (Fig. 4 A&B). Diabetic group showed focal interstitial nephritis (Fig. 4C) represented by the presence of focal aggregations of mononuclear inflammatory cells within the renal cortex. Perivascular edema and mononuclear inflammatory cells infiltration were frequently observed as well (Fig. 4D). Degeneration and necrosis of the epithelial lining of the renal tubules were also detected (Fig. 4E). Focal aggregations of mononuclear inflammatory cells were observed in the renal medulla of some individuals (Fig. 4F). Administration of the Ag-NCs succeeded to protect the renal tissue (Fig. 4G&H) that appeared apparently normal.
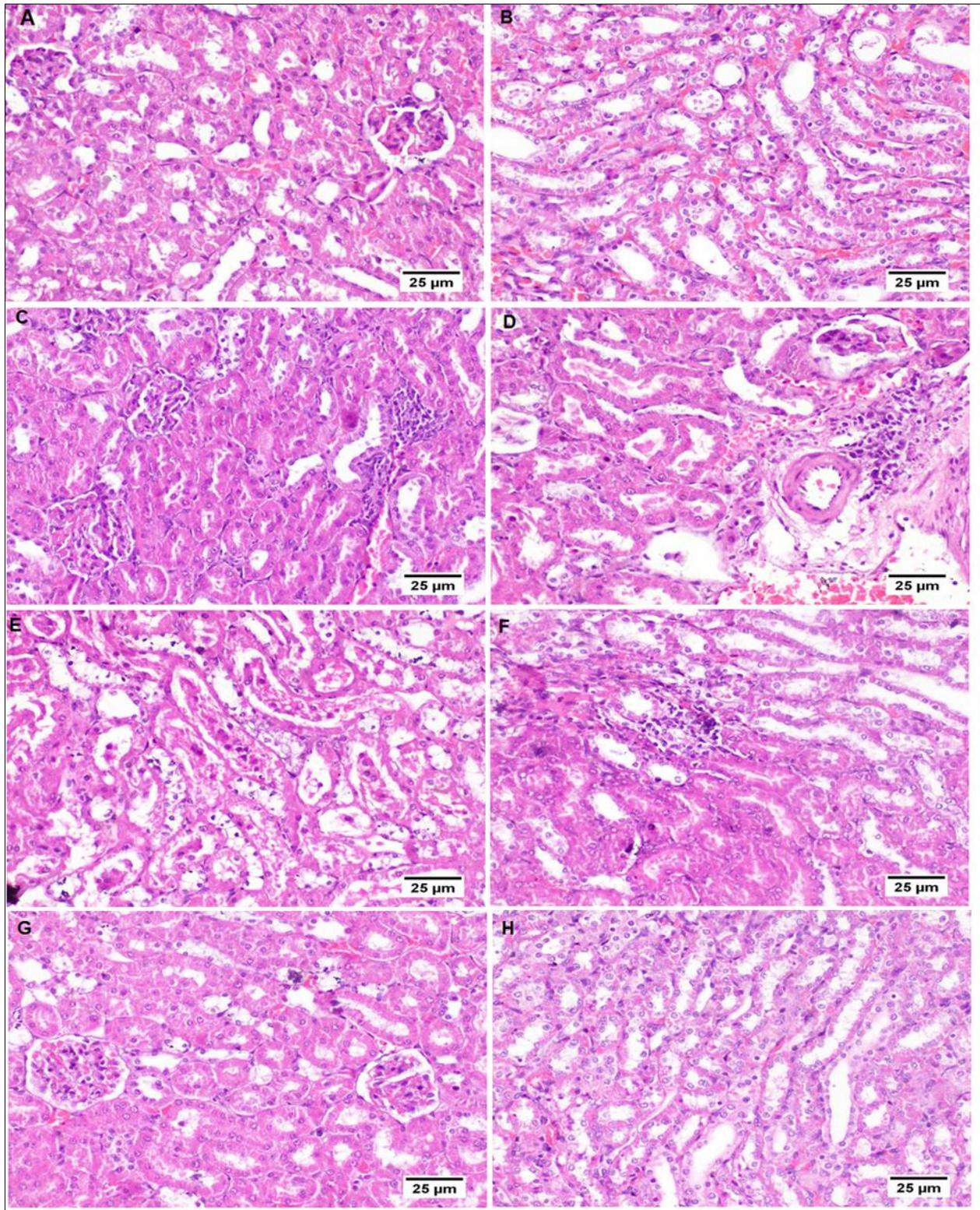
Figure 4 Photomicrograph of haematoxylin and eosin stained kidney sections from control rats; diabetic rats and Ag-NCs -treated rats

5. Discussion

Type 1 diabetes mellitus (T1DM) is autoimmune syndrome in which deterioration of pancreatic β-cells causes insulin deficiency which increases blood glucose level and stimulates ketoacidosis [38]. In early diagnosis of T1DM, the function of specific organs such as kidneys declines and renal tissue abnormalities occur due to the hyperglycemic condition which has an obvious role in initiating abnormal homeostasis in blood flow and causing vascular permeability in the glomerulus leading to glomerular hyperfiltration and hypertrophy and glomerular basement membrane thickening associated with diffuse or nodular glomerulosclerosis [39,1] . Excess blood glucose over the normal level promotes one of the major diabetic complications known as diabetic nephropathy that further directs into end stage renal disease. Diabetic nephropathy (DN) is a significant diabetic microvascular complication. The progress in the incidence of DN has expanded with the increased number of diabetic patients around the world [40] and has become a leading cause of chronic kidney disease in developed countries [41] .

 A previous study proposed the mechanism of hyperglycemia mediated diabetic nephropathy through the activation of the expression of transporting growth factor beta (TGF- β) gene and its receptors. Also, hyperglycemia enhances glucose transporters (GLUT), angiotensin II and platelet derived growth factor (PDGF), polyol pathway, protein kinase C pathway, hexosamine pathway, increases advanced glycation end products (AGE) and increases oxidative stress. These effects increase TGF- β proteins which further cause thickening of glomerular basement membrane (GBM) and glomerulosclerosis [42]. Currently, manufacturing anti-diabetic drugs with improved pharmacokinetic properties is considered as one of future challenges to achieve good control of blood glucose concentrations in insulin dependent diabetes [43].

In this study, experimental T1DM induction in rat models by streptozotocin (STZ) infusion led to destruction of the β cells of the islets of Langerhans [44] as showed in the present histopathological investigation and classical symptoms of the disease appeared such as hyperglycemia and hypoinsulinemia in the model rats. Ag-NCs treated rats showed a significant decrease in serum glucose and improvement of serum insulin activity. This therapeutic action may occur by AgNPs in combined nanocomposites were designed as anti-diabetic agents according to previous literature because of their ability to the increase of serum insulin level, hepatic glucose transporter-2 (GLUT-2) gene activation that facilitates glucose transport across the hepatocyte plasma membrane under insulin regulation and liver glucokinase (GK) activity that enhance hepatic glycolysis [45]. Furthermore, biopolymer chitosan in nanocomposites was capable of accelerating β-cell proliferation and defending β-cells against apoptosis [46] that confirmed from the histopathological finding observed in the present study. Also, chitosan regulates glucose metabolism in the liver through two routes; suppressing gluconeogenesis and stimulating glycogen synthesis [47].

Kidneys are main organs responsible for critical body functions as blood filtration and waste elimination and subsequently play an important role in the clearance of nanoparticles *in vivo* [48]. Therefore, early pathological effect of diabetes takes place on kidneys through disturbed regulation of angiotensin and prostaglandin secretion, thus structural abnormalities appears such as glomerular basement membrane and mesangial thickening [49].Fernandes, *et al.* demonstrated that hyperglycemia reinforced the progression of diabetic nephropathy evidenced by intense ROS and focal changes in renal histology leading to hyperplasia and hypertrophy of the tubular area [50]as shown in the histopathological examination of diabetic rats. Additionally, based on biochemical analysis; high serum creatinine, serum urea and serum uric acid levels were observed in model rats which are noticeable markers of renal injury [51]. Interestingly, the daily administration of Ag-NCs has ameliorated the renal function via correcting serum creatinine, uric acid and urea near to normal levels and proliferating damaged renal tissues in treated rats by means of two suggested pathways including AgNPs which act as lowering blood glucose agent in *in vivo* as well as alleviating the development of diabetic nephropathy by regulating key genes involved in insulin signaling pathway that inhibit inflammatory cytokines, TGF-β and tumor necrosis factor-a (TNF-a) [52]. On the other hand, chitosan directly improves renal structure and function during diabetic nephropathy development through reducing renal fibrotic protein expression which is defined as transforming growth factor-β1 (TGF-β1), renal tubular injury markers, and pro-inflammatory cytokine production [53]. In addition to ascorbic acid in nanocomposites was assessed as a powerful anti-oxidant against highly oxidative stress produced by diabetes disease that causes renal tissue destruction and performs as a key role in lowering the uric nitrogen in blood, serum creatinine, and the excretion rate of albumin in urine, as well as increasing the creatinine clearance rate and protecting from renal lesions [54,55].

Oxidative stress is a serious condition in which the balance between free reactive species and anti-oxidant enzymes (superoxide dismutase [SOD], glutathione reductase and catalase) is disturbed. In diabetes mellitus, hyperglycemia stimulates oxidative stress consisting of the formation of advance glycation end products (AGEs), an increase in the production of reactive oxygen species (ROS), and the polyol pathways, moreover, oxidative stress integrated with hyperglycemia to evolve diabetic nephropathy through generating defective effects in structure and function of the

kidney, endothelial cell dysfunction, mesangial cell injury, and, the increase in profibrotic cytokines (TGF-β) [56]. Malondialdehyde (MDA) is end product of peroxidized polyunsaturated fatty acids and considered as a marker of lipid peroxidation which characterized by invasion of free radicals to membrane lipids, generating excess amounts of reactive species (toxic hydrogen peroxide [H_2O_2], super oxide [O_2^-] ,and hydroxyl radical [^-OH]). Therefore, concentration of MDA elevated in diabetic onset with pathogenesis of nephropathy [57].The reduction in the defense system that provided by SOD, catalase and glutathione reduced against reactive species yields degenerated renal tissue, also, previous reports claimed that catalase deficiency accelerated renal injury in diabetes via peroxisomal dysfunction [58,59,56].

Nitric oxide (NO) is lipophilic gas generated in almost all tissues and plays a critical physiological role in kidneys involving the control of renal and glomerular hemodynamics, the dilation of both the afferent and the efferent arteriole, and the enhancement of natriuretic and diuretic rates. So that, the abnormalities in NO concentration resulting from diabetes disease can badly affect renal filtration rate [60]. However, in DN, the production of NO is increased that may relate to hyperfiltration and the kidney lesions [61].

In the present work, kidney tissue analysis displayed in diabetic rats that increase in concentrations of MDA and NO, and decrease in GSH concentration, SOD activity and catalase activity. In contrast, the treated group showed decrease concentrations of MDA and NO, and elevation in GSH concentration, SOD activity and catalase activity, this anti-oxidant action exhibited by Ag-NCs though 3 components;1) AgNPs has antioxidant and scavenging activity against hydrogen peroxide, hydroxyl radicals, and superoxide [62],lipid peroxidation and reducing of DNA damage [63]. 2) Chitosan has the ability to regulate secretion of cytokine (TGF-β1) and improve activities of anti-oxidative enzymes and has anti-inflammatory properties that prevent renal injury caused by inflammation, fibrosis and oxidative stress [64]. 3) Ascorbic acid is excellent anti-oxidant supplement that suppresses oxidative damage by scavenging oxidative stress, reducing the lipid peroxidation and increased activity of the antioxidant enzymes such as SOD and catalase, as well as decreasing the albuminuria and glomerular basement membrane thickness [56].

6. Conclusion

Oral administration of Ag-NCs exhibited antidiabetic activity by alleviating hyperglycemia and improving insulin secretion, anti- inflammatory and anti-oxidant characteristics that will be a promising candidate in clinical treatment of human chronic kidney disease.

Compliance with ethical standards

Disclosure of conflict of interest

All author declare that no conflict of interest.

Statement of ethical approval

The Cairo University approved experimental protocols and procedures used in this study, Faculty of Science, Institutional Animal Care and Use Committee (IACUC) (Egypt) (CUFS/F/67/19). All the experimental procedures were carried out following international guidelines for the care and use of laboratory animals.

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