Histological and biochemical effects of *Cinnamomum cassia* nanoparticles in kidneys of diabetic Sprague-Dawley rats

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ABSTRACT

This study investigated the antidiabetic activity of *Cinnamomum cassia* (*C. cassia*, Cc) silver nanoparticles (CcAgNPS) and effects of *C. cassia* on the kidneys of rats with induced type 2 diabetes. Twenty-four Sprague-Dawley rats weighing 250 ± 20 g were induced with diabetes by intraperitoneal injection of streptozotocin (STZ, 60 mg/kg). Animals were randomly assigned to one of four groups (n = 6) and treated for eight weeks with normal saline (control, group A), 5 mg/kg of CcAgNPs (group B), 10 mg/kg of CcAgNPs (group C), or 200 mg/kg of Cc (group D). Body weight and fasting blood glucose (FBG) was measured weekly and fortnightly, respectively. At the end of experiments animals were euthanized, blood and kidney tissue samples were collected for biochemistry (oxidative stress markers and renal function parameters) and kidneys were harvested for histology (PAS and H&E staining). Body weight was significantly higher in group B and C vs. control ($p < 0.05$), while no significant differences were observed in the kidney-to-body weight ratio between groups. FBG, glutathione, malondialdehyde, alanine aminotransferase, aspartate aminotransferase, serum urea and creatinine were significantly lower in group B, C and/or D vs. control (all $p < 0.05$). In group A, severe distortion of the glomerular network was observed, marked by the loss of capsular integrity, thickened basement membrane, tubular cells with pyknotic nuclei, vacuolization, and interstitial space with infiltrations. These adverse effects were mitigated by 5 mg/kg and 10 mg/kg of CcAgNPs. Our study confirms structural and functional damage to kidneys caused by diabetes. CcAgNPs have a regenerative potential in diabetes-induced kidney damage and may be used as an antidiabetic agent.

KEY WORDS: *Cinnamomum cassia*; silver nanoparticles; diabetes; histomorphology
DOI: https://dx.doi.org/10.17305/bjbms.2019.3481 *Bosn J Basic Med Sci. 2019;19(2):138-145. © 2019 ABMSFBIH*

INTRODUCTION

Diabetes is no longer a disease of predominantly rich nations and the prevalence of diabetes is steadily increasing in the middle-income countries, including African countries [1]. Despite the introduction of various antidiabetic and hypoglycemic agents, diabetes and associated secondary complications continue to be a major health problem worldwide [2]. Antidiabetic drugs currently in use do not completely control blood glucose levels, moreover, they cause side effects and insufficient response after prolonged use. Therefore, efforts are being made to find new medicines and therapeutic interventions to treat this debilitating condition [2]. Satyanarayan et al. [3] emphasized the need for alternative therapies in patients with diabetes, with a special focus on different indigenous plants. Compared to conventional drug formulations, nanotechnology-based drug carriers are more advantageous for medical use [4] and may be used for the treatment of various diseases.

Nanobiotechnology is a rapidly developing discipline at the intersection of nanotechnology and biology, utilized for the exploration and transformation of biological systems and pharmaceutics. Targeting of nanocarriers can be improved through conjugation with various active bio-ligands such as biotin [4]. Moreover, core-shell and multi-layered nanoparticles can be loaded with several drugs and modified to enable controlled drug release. The integration of such nanocarriers

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Submitted: 13 April 2018/Accepted: 02 October 2018

in the treatment of diabetic nephropathy appears to be very promising [4].

Over the past few years, the success with the so-called green synthesis of biocompatible silver nanoparticles (AgNPs) has gained much attention for its application in biomedical sciences [5]. NPs are particles with a diameter of 100 nm or less that possess unique physical, chemical and biological properties, which make them potent diagnostic and therapeutic tools. Silver (Ag) is the metal of choice for the application of NPs in biology and medicine. AgNPs have been used as anticancer, antiangiogenic, and antibacterial agents [6-8]. Moreover, they can be used to treat diseases that require a sustained release of a drug at several anatomical sites [9]. Due to the ultra-small size of AgNPs (1–100 nm), they exhibit unique physical, chemical, magnetic and optical properties [10,11]. Furthermore, they enhance solubility, bioavailability, pharmacological activity and stability of drugs, and protect from their toxicity [12]. Incorporation of herbal extracts into novel formulation systems such as nanosized drug delivery systems has captured the attention of major pharmaceutical corporations, due to the ability of those systems to overcome multiple dosage and lower absorption as major problems [12]. Natural products such as medicinal plants have, therefore, been a very good source of green NPs.

Cinnamomum cassia (*C. cassia*) of the plant family Lauraceae, also known as Chinese cassia or Chinese cinnamon, is an evergreen tree originating from southern China and widely cultivated in southern and eastern Asia. Nowadays, *C. cassia* is also found in Southern Africa. *C. cassia* extracts have shown to have antidiabetic effects *in vitro* [6]. In addition, it has been reported that *C. cassia* lowers blood glucose, blood pressure and serum cholesterol levels, exerts antioxidant and free radical scavenging activity, and has hepatoprotective effects *in vivo* [13,14]. However, in contrast to other medicinal plants that have been used as antidiuretic and antihyperlipidemic remedies [15], there is a lack of data on *C. cassia* effects on the kidneys in animal models with diabetes. In this study, we investigated the antidiabetic activity of *C. cassia* silver nanoparticles (CcAgNPs) and effects of *C. cassia* on the kidneys of rats with induced type 2 diabetes.

MATERIALS AND METHODS

Collection of plant material

Pure *C. cassia* powder was obtained from Warren Chem Specialities (Pty), Cape Town (Reference 492733) and silver nitrate ($AgNO_3$) was obtained from Capital Laboratory (Pty), KwaZulu-Natal.

Preparation of aqueous plants extract

The aqueous extract of *C. cassia* was prepared by adding a fine powder of the plant (10 g) to 300 mL of double-distilled water, which was allowed to boil for 10 minutes [16]. The resulting mixture was filtered and stored in a refrigerator at 4°C until analyzed.

Synthesis of *Cinnamomum cassia* silver nanoparticles (CcAgNPs)

An aqueous solution of $AgNO_3$ was dissolved in 250 mL Erlenmeyer flasks and added dropwise to 100 mL of the plant extract while stirring and heated at 45°C. This solution was stirred constantly at room temperature for 120 minutes, using a magnetic stirrer. A color change from light brown to dark brown indicated the formation of AgNPs.

Structural analysis of CcAgNPs

The CcAgNPs solution was centrifuged in an Eppendorf Centrifuge (Model: 5804/5804 R, USA). The treated solutions were transferred separately into Eppendorf tubes that were pre-weighed and subjected to purification for 2 hours at 5000 rpm and 4°C.

The bioreduction of Ag^1 to Ag^0 was evaluated using a SHIMADZU UV-2600 UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan), at a range of 120–900 nm and with a resolution of 1 nm. UV-Vis analysis was performed by taking a baseline measurement with the solvent (distilled water) at different wavelengths from 190 nm to 900 nm, at room temperature and pH 3.96 [17]. An aqueous component of *C. cassia* was sampled. The component was scanned over 190 nm to 900 nm wavelength range.

To evaluate the bioreduction and capping functional groups of AgNPs, infrared spectra of the extracts and their corresponding biosynthesized AgNPs were obtained on a Fourier transform infrared (FTIR) spectrophotometer with universal attenuated total reflectance (ATR) sampling accessory (Perkin Elmer Spectrum 100, USA) [17]. The size and morphology of AgNPs were examined using transmission electron microscopy (TEM). Powdered AgNPs were mixed with the pellet and the analysis was immediately carried out using the Perkin Elmer-Spectrum 100 (Perkin Elmer) [17].

Transmission electron microscopy (TEM) was also used to obtain selected area electron diffraction (SAED) patterns of AgNPs to evaluate their crystallinity. For TEM measurements, solutions of synthesized AgNPs were sonicated using a sonication bath (Soniclean, England) and evenly dispersed AgNPs were coated onto carbon-coated TEM grids and placed under a lamp to evaporate the solvent before analysis.

Animal handling and ethics approval

Twenty-four pathogen-free, male, Sprague-Dawley rats weighing 250 ± 20 g were selected for the experiments; they were kept and maintained under laboratory conditions at a

temperature of 21.5°C to 2.2°C, humidity (60 ± 1%), and 12 hour light/dark cycle. They were allowed free access to food (standard pellets) and water, and were fed *ad libitum*.

Animals were allowed to acclimatize in their respective cages for seven days prior to the commencement of experiments. The institutional Ethics Committee of the University of KwaZulu-Natal approved the experimental protocols and procedures used in this study (Ethics references number AREC/074/016 D).

Induction of type 2 diabetes

Hyperglycemia was induced by injecting nicotinamide [100 mg/kg of body weight] (Sigma Aldrich, South Africa) and streptozotocin (STZ, 60 mg/kg, intraperitoneal injection [I.P]). Nicotinamide was administered 15 minutes before STZ that was dissolved in phosphate buffer citrate at pH 4.5 immediately before use [18].

Animals were then kept under observation for confirmation of diabetes induction. Blood glucose was tested and monitored on days 0, 7, 14, 28, 42, and 56. The animals were tested using Accu-chek Active 50 (Roche) blood glucose test strips, purchased from Dis-Chem, South Africa. Blood was collected from the tail vein of the rats at 10 am daily. The animals with a fasting blood glucose (FBG) level >9 mmol/L were considered diabetic.

Experimental design

Twenty-four STZ-induced diabetic Sprague-Dawley rats were randomly assigned to four groups (six animals per group). In control group A, rats received normal saline (1 mL) orally once per day. Group B rats were orally administered with CcAgNPs at low dose (5 mg/kg), once per day [7]. Group C rats were orally administered with CcAgNPs at high dose (10 mg/kg), once per day [7]. Group D rats were administered with *C. cassia* plant extract at 200 mg/kg. CcAgNPs and *C. cassia* plant extract were dissolved in normal saline and administered orally once daily at 10 am for 56 consecutive days, using a rat gavage needle [17]. FBG was measured on days 0, 14, 28, 42 and 56. Body weight was recorded every week between 8:00 am and 10:00 am using an electronic balance (Zeiss, West Germany (Pty) Ltd; 0.000 g calibration). At the end of the experiment, all rats were euthanized with an overdose of Halothane anesthesia. Blood was immediately withdrawn via cardiac puncture and stored in 10 ml EDTA heparinized tubes. After that, it was centrifuged at 3000 rpm for 10 minutes and serum was collected using Eppendorf tubes and stored at -80°C in a deep freezer.

After laparotomy, organs were harvested for histology. Kidneys were immediately washed with phosphate buffer solution (PBS) to remove blood and then dissected. Blood and kidney tissue samples were used for biochemistry (oxidative stress biomarkers: glutathione [GSH], malondialdehyde [MDA], alanine aminotransferase [ALT], aspartate aminotransferase [AST], and albumin [ALB]; renal function parameters: serum urea, creatinine, and uric acid). In addition, the weight of the kidneys was recorded.

Measurement of MDA levels in rat kidney tissue

Kidney tissue was homogenized in 0.2 M sodium phosphate buffer (pH 7.8) and then centrifuged for 15 minutes at 12000 rpm to separate the supernatant from the tissue extract, which is done under high speed. The supernatant was then collected and used for the measurement of lipid peroxidation. The procedure was carefully performed according to a previous method with slight modifications [19]. This method indicated the complex formed between MDA and thiobarbituric acid (TBA). Two hundred microliters of MDA standard series (0, 7.5, 15, 22.5, and 30 µL) was added to a Pyrex screw-capped test tube containing the assay mixture of 200 µL of 8.1% sodium dodecylsulfate (SDS), 750 µL of 20% acetic acid (pH 3.5), 2 mL of 0.25% TBA, and 850 µL of distilled water. The mixture was heated at 95°C for 60 minutes in a sand bath, cooled down to room temperature, and the absorbance was read at 532 nm using the spectrophotometer Synergy HTX multi-mode reader (Vacutec, USA). The concentration of TBA reactive substances (TBARS) in the samples was extrapolated from the MDA standard curve.

Measurement of GSH levels in rat kidney tissue

Glutathione concentration was assessed in rat renal tissue. The sample was first precipitated with 10% trichloroacetic acid (TCA) and then centrifuged at 2000 rpm for 10 minutes at 25°C. This technique was not used to separate the supernatant from the tissue extract but to separate the deproteinized supernatant from the extracted sample (tissue extract). The low centrifugation speed was also used to separate protein sediment according to the following steps: precipitated sample was inserted into the centrifuge, centrifuged, and as a result a supernatant and precipitate were obtained. This reaction mixture contained 100 µL of supernatant, 50 µL of 0.5 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and 150 µL of 0.2 M sodium phosphate buffer (pH 7.8). After 15 minutes incubation at 25°C, the absorbance was measured at 412 nm using a spectrophotometer (Synergy HTX multi-mode reader, VACUTEC, USA). GSH concentration was extrapolated from a standard GSH curve.

Histopathological examination of rat kidney tissues

Kidneys were washed in saline and fixed in 10% neutral buffered formalin for 24 hours. The samples were transferred

to 70% ethanol solution [20]. Ascending grades of alcohol were then used to dehydrate the samples and xylene was used as a clearing agent. The samples were immersed in molten paraffin wax at 58°C to 62°C. The prepared blocks were cut into slices of 5 µm) using a microtome (Microtome HM 315, Walldorf, Germany) and stained with hematoxylin and eosin (H&E) stain.

Additionally, the periodic acid-Schiff (PAS) staining technique was used to detect the presence of polysaccharides (e.g., glycogen) and mucosubstances (e.g., glycoproteins, glycolipids, and mucins) in rat kidney tissues. The PAS technique is most commonly used to evaluate the thickness of glomerular basement membrane (GBM) in renal disease [21]. The sections were viewed and photographed using an Olympus light microscope (Olympus BX, Tokyo, Japan) with an attached camera (Olympus E-330, Olympus Optical Co. Ltd., Tokyo, Japan).

Statistical analysis

All results are presented as the mean ± standard error of the mean (SE). One-way analysis of variance (ANOVA) followed by Turkey's post-hoc test was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, La Jolla, California, USA). Comparisons with $p < 0.05$ were considered statistically significant.

RESULTS

Synthesis of CcAgNPs

The aqueous solution of biosynthesized AgNPs from *C. cassia* powder showed a dark brown color indicating the formation of AgNPs (150 mg).

Effects of CcAgNPs on rat body and kidney weight

The oral treatment of diabetic rats with a low (5 mg/kg) and high (10 mg/kg) dose of CcAgNPs significantly increased the body weight of experimental animals compared to saline-treated diabetic controls ($p < 0.05$). In addition, the kidney-to-body weight ratio was not significantly different between groups [$p > 0.05$] (Table 1).

Effects of CcAgNPs on FBG levels

A significant reduction in FBG levels was observed in group B (5 mg/kg CcAgNPs), group C (10 mg/kg CcAgNPs), and group D (200 mg/kg *C. cassia* extract) of diabetic rats compared to diabetic control rats treated with saline [$p < 0.05$] (Figure 1).

Effects of CcAgNPs on renal function parameters

Diabetic rats treated with 5 mg/kg (group B) and 10 mg/kg (group C) doses of CcAgNPs showed significantly reduced levels of serum urea and creatinine compared to saline-treated diabetic rats ($p < 0.05$). In addition, this effect of CcAgNPs on the renal function parameters of diabetic rats was higher in group B compared to group C (Figure 2).

Effects of CcAgNPs on the histology of kidneys in diabetic rats

H&E-stained kidney sections of diabetic rats in control group showed a severe distortion of the glomerular architecture, with atrophic and necrotic changes, pyknotic nuclei as well as infiltrations of mesangial spaces and interstitium.

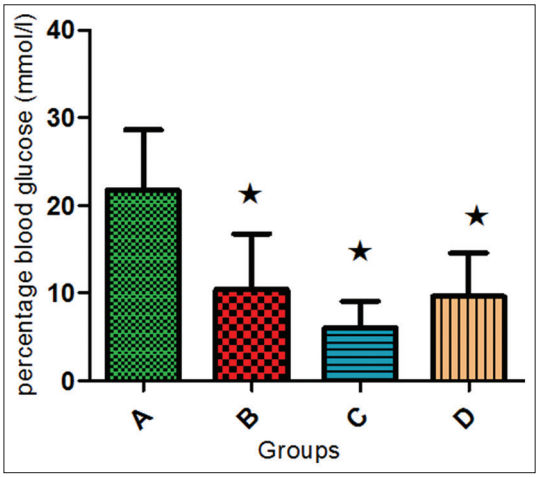

FIGURE 1. Comparison of FBG levels (mmol/L) between different groups of streptozotocin-induced diabetic rats. A significant reduction in FBG levels was observed in group B (5 mg/kg CcAgNPs), group C (10 mg/kg CcAgNPs), and group D (200 mg/kg *C. cassia* extract) of diabetic rats compared to diabetic rats treated with saline (group A) [*$p < 0.05$]. FBG: Fasting blood glucose; CcAgNPs: *Cinnamomum cassia* silver nanoparticles.

TABLE 1. Effects of *Cinnamomum cassia* silver nanoparticles (CcAgNPs) on body weight of streptozotocin-induced diabetic rats

Group	BWI (g)	BWF (g)	W gain/Loss (g)	KW (g)	KBWR
Control (saline)	240±21	210±19	Loss	2.7±0.81	0.89±0.48
5 mg/kg CcAgNPs	290±16*	300±19*	Gain	2.4±0.26	0.87±0.23
10 mg/kg CcAgNPs	220±14	230±14*	Gain	2.1±0.34	0.93±0.13
200 mg/kg *C. cassia* extract	220±12*	230±16*	Gain	1.9±0.36*	0.89±0.077

Results are expressed as mean ± standard error of the mean. *Statistically significant at $p < 0.05$. BW: Body weight; BWI: Initial body weight; BWF: Final body weight; W gain/loss: Difference between control diabetics and treated groups, in body weight; KW: Kidney weight; KBWR: Kidney-to-body weight ratio

Many of the proximal and distal convoluted tubules showed hypertrophic cells and vacuolization (Figure 3A). H&E-stained sections of diabetic rats in group B (5 mg/kg CcAgNPs) showed moderate degenerative changes in the glomeruli. The mesangium and interstitium were essentially normal with cellular components (Figure 3B). In group C (10 mg/kg CcAgNPs), the kidney sections (Figure 3C) showed glomeruli with Bowman's space and capillary tufts, which appeared normal compared to controls. The kidney sections of diabetic rats in group D (200 mg/kg *C. cassia* extract) looked normal with clearly visible glomeruli, basement membrane of the capsular wall, and mesangial tissue (Figure 3D). The interstitium appeared normal and with no infiltrations.

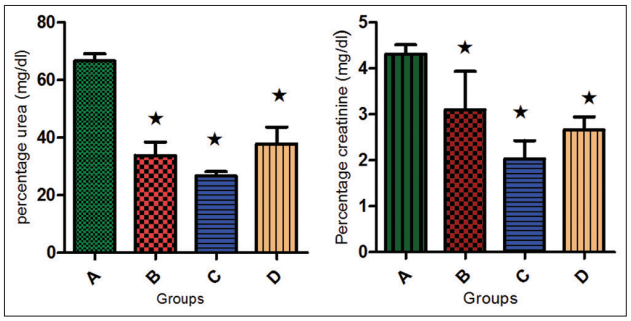

FIGURE 2. Comparison of serum urea and creatinine levels (mg/dL) between different groups of streptozotocin-induced diabetic rats. Diabetic rats treated with 5 mg/kg (group B) and 10 mg/kg (group C) doses of CcAgNPs showed significantly reduced levels of serum urea and creatinine compared to saline-treated diabetic rats (group A) [*$p < 0.05$]. CcAgNPs: *Cinnamomum cassia* silver nanoparticles.

PAS-stained kidney sections of diabetic control rats (group A) showed glomerular distortions and ruptured capsular wall (stained purple), thickened basement membranes, and hypocellularity in the interstitial components. In group B and C, the PAS-staining intensity for glycogens was stronger; moreover the capsular walls of the glomeruli were evident in group B. The mesangial tissues were clearly delineated and interstitial tissue did not indicate any abnormalities. The kidney sections of group D (200 mg/kg *C. cassia* extract) showed PAS-positive glomerular capsular basement layers stained purple, indicating a normal glomerular tuft/capillary meshwork. The proximal and distal convoluted tubular cells with no abnormalities and purple-stained (glycogen) membranes of tubules were clearly visible (Figure 4A-D).

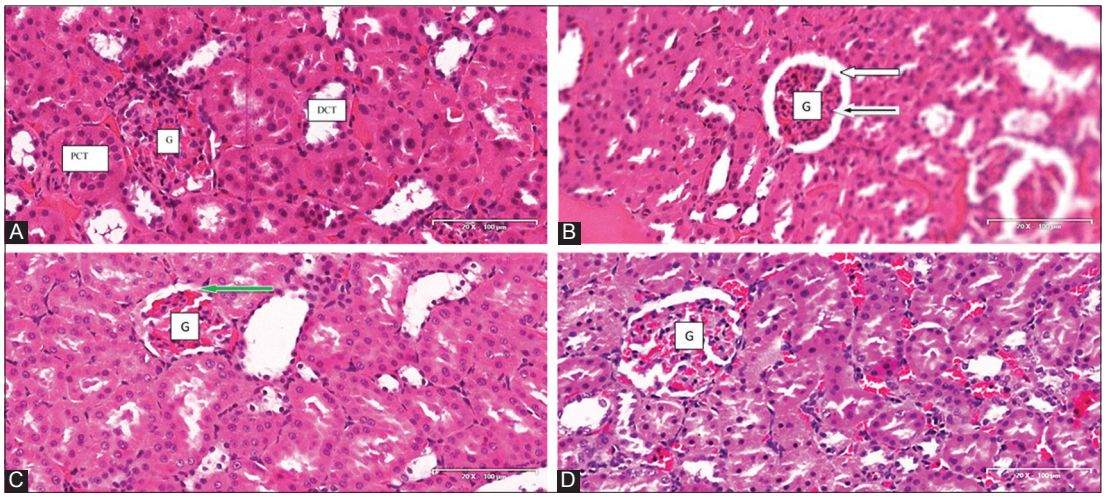

FIGURE 3. Hematoxylin and eosin (H&E)-stained kidney sections of streptozotocin-induced diabetic rats in different groups. A) In control group A (saline), the sections showed a severe distortion of the glomerular architecture, with atrophic and necrotic changes, pyknotic nuclei as well as infiltrations of mesangial spaces and interstitium. Many of the proximal and distal convoluted tubules showed hypertrophic cells and vacuolization. B) In group B (5 mg/kg CcAgNPs), there were moderate degenerative changes in the glomeruli. The mesangium and interstitium were essentially normal with cellular components. C) In group C (10 mg/kg CcAgNPs), the kidney sections showed glomeruli with Bowman's space and capillary tufts, which appeared normal compared to controls. D) The kidney sections of diabetic rats in group D (200 mg/kg *C. cassia* extract) looked normal with clearly visible glomeruli, basement membrane of the capsular wall, and mesangial tissue. G: Glomerulus; DCT: Distal convoluted tubules; PCT: Proximal convoluted tubules; White arrow: Bowman's space; Black arrow: Mesangial cells; Green arrow: Parietal layer; CcAgNPs: *Cinnamomum cassia* silver nanoparticles.

TABLE 2. Effects of *Cinnamomum cassia* silver nanoparticles (CcAgNPs) on oxidative stress markers in streptozotozin-induced diabetic rats

Group	GSH (mg/L)	MDA (mmol/L)	ALT (U/L)	AST (U/L)	ALB (g/dL)
Control (saline)	0.084±0.004	1.4±0.22	89±0.0	46±14	6.6±1.6
5 mg/kg CcAgNPs	0.035±0.004*	0.60±0.15*	87±0.28*	20±1*	5.4±0.18
10 mg/kg CcAgNPs	0.027±0.005*	0.83±0.07*	88±0.25	9.5±4.4*	5.6±0.19
200 mg/kg *C. cassia* extract	0.042±0.003*	0.81±0.09	88±0.25	30±9.4	6.05±0.31

Results are expressed as mean ± standard error of the mean. *Statistically significant at $p < 0.05$. GSH: Glutathione; MDA: Malondialdehyde; ALT: Alanine transaminase; AST: Aspartate transaminase; ALB: Albumin

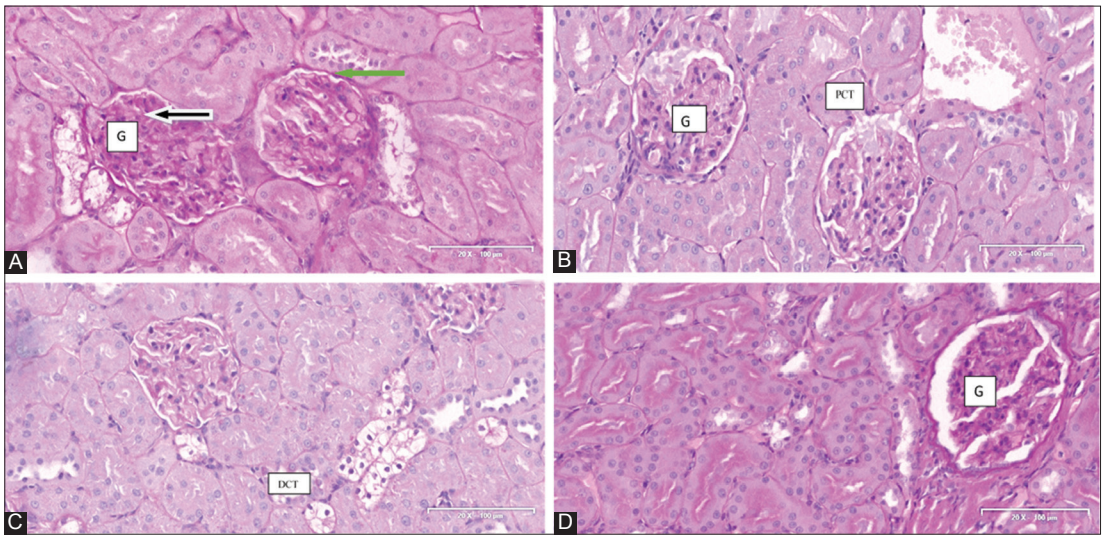

FIGURE 4. Periodic acid-Schiff (PAS)-stained kidney sections of streptozotocin-induced diabetic rats in different groups. A) The sections in control group A (saline) showed glomerular distortions and ruptured capsular wall (stained purple), thickened basement membranes, and hypocellularity in the interstitial components. B-C) In group B (5 mg/kg CcAgNPs) and C (10 mg/kg CcAgNPs), the PAS-staining intensity for glycogens was stronger; moreover the capsular walls of the glomeruli were evident in group B. The mesangial tissues were clearly delineated and interstitial tissue did not indicate any abnormalities. D) The kidney sections of group D (200 mg/kg *C. cassia* extract) showed PAS-positive glomerular capsular basement layers stained purple, indicating a normal glomerular tuft/capillary meshwork. The proximal and distal convoluted tubular cells with no abnormalities and purple-stained (glycogen) membranes of tubules were clearly visible. Note the thickening of basement membranes in A part. G: Glomerulus; DCT: Distal convoluted tubules; PCT: Proximal convoluted tubules; White arrow: Bowman's space; Black arrow: Mesangial cells; Green arrow: Parietal layer; CcAgNPs: *Cinnamomum cassia* silver nanoparticles.

Effects of CcAgNPs on oxidative stress biomarkers

GSH levels were significantly lower in groups B, C, and D compared to control ($p < 0.05$). MDA and AST levels were significantly lower in groups B and C compared to control ($p < 0.05$). ALT levels were significantly lower in group B compared to control ($p < 0.05$). On the other hand, no significant differences were observed in ALB levels between experimental groups and control (Table 2).

DISCUSSION

Diabetes and its vascular complications are associated with different biochemical disorders such as hyperlipidemia and oxidative stress [22]. In our study, the treatment of diabetic rats with high and low dose of CcAgNPs resulted in weight gain, probably due to changes in glucose and lipid metabolism. The body weight in STZ-induced diabetic rats was reduced partly due to the increased rate of proteolysis and lipolysis in the diabetic state, which leads to muscle wasting and consequent loss of adipose tissue [23]. An increase in body weight in rats treated with CcAgNPs might also be due to higher fluid intake and decreased urinary glucose excretion.

Previous studies have shown the ability of cinnamon to control hyperglycemia in diabetes [13]. In our study, diabetic rats treated with CcAgNPs increased their body weight by decreasing blood glucose levels. Similar findings were reported previously, which showed that oral administration of biosynthesized *Cassia fistula* gold nanoparticles improves body weight in rats with STZ-induced diabetes [17]. The proposed underlying mechanisms by which plant extracts control high blood glucose levels are the stimulation of pancreatic insulin secretion from β-cells in the islets of Langerhans and increased transport of blood glucose to peripheral tissues [24]. *C. cassia* extracts (nanoparticle conjugates) might have direct effects on residual beta cell secretion and probably increase peripheral utilization of glucose, as postulated by Hassan et al. [25]. *Gynura procumbens* water extract showed hypoglycemic effect by promoting glucose uptake through muscles, and *C. cassia* might have a similar mechanism of action. Nevertheless, in this study, we did not perform assays with pancreatic tissue of diabetic rats to confirm these assumptions.

In most of our cases, we observed lesions in the kidney sections similar to those seen in glomerulosclerosis in human patients. Zafar et al. [26] showed glomerular membrane thickening and early hypertrophy of glomeruli in the kidney sections of STZ-induced diabetic rats. Glomerular hypertrophy in diabetes represents an early event in the progression of glomerular pathology, in the absence of mesengial expansion [26,27].

Serum urea and creatinine are useful indices for evaluating the status of renal function. An increase in the level of serum urea may imply impaired renal excretion [28]. In our study, the serum levels of urea and creatinine increased in saline-treated diabetic control rats (Figure 2) but showed a concomitant decline in the groups treated with CcAgNPs, suggesting

that renal dysfunction and possible kidney injury occurred in control diabetic rats due to severe hyperglycemia [29]. The observed increase in serum urea and creatinine levels in control diabetic rats was corroborated by the histomorphological changes indicative of early diabetic nephropathy, including mesangial expansion, diffuse GBM thickening and increased mesangial cellularity [29]. On the other hand, these effects were mitigated by 5 mg/kg and 10 mg/kg of CcAgNPs in group B and C, respectively.

In our study, increased ALT and AST levels indicated hepatic dysfunction due to hyperglycemia in STZ-induced diabetic rats (Table 2). ALT and AST are released when the injury involves organelles such as the mitochondria. Transaminases catalyze a transamination reaction and are vital markers of liver injury in clinical diagnostics [30]. A decrease in the level of serum urea after 56 days of administration of CcAgNPs observed in our study might be attributable to reduced amino acid degradation by the liver; moreover, this finding correlates with a decline in ALB levels in CcAgNPs-treated groups. Previously, studies showed that serum creatinine levels in animal model were significantly reduced suggesting enhanced glomerular clearance or impaired muscle metabolism [31,32].

Tissue antioxidant status is suggested to be an important factor in the development of diabetic complications [33]. Medicinal plants used to synthesize AgNPs, including *C. cassia*, exhibit strong antioxidant and free radical scavenging properties [34]. The antioxidant defense system is comprised of several enzymes, such as catalase and GSH, which are responsible for the maintenance of redox homeostasis and prevention of oxidative stress [35]. In hyperglycemia, MDA levels increase [18] due to increased oxidant production, which impairs the antioxidant defense system via multiple pathways [36].

Diabetes mellitus is a leading cause of kidney disease globally and the assessment of renal functional parameters remains an essential tool in the diagnosis of diabetes. In this study, in addition to urea and creatinine, we have measured serum AST, ALP and ALB levels and showed that increased ALP levels reflected renal insufficiency in saline-treated control diabetic rats, while CcAgNPs-treatment attenuated this effect.

Albuminuria is a known prognostic marker of diabetic nephropathy [37]. Although serum ALB levels in our study were lower in CcAgNPs-treated groups they were not significantly different compared to saline-treated group. The presence of albuminuria in patients with diabetes usually indicates an underlying structural renal damage, as demonstrated by histological studies [38]. While *C. cassia* essential oil is known to contain several active components, such as cinnamaldehyde, flavonoids and coumarins, the specific component responsible for its antidiabetic effects is yet to be identified [39].

CONCLUSION

Our study showed that *C. cassia* silver nanoparticles (CcAgNPs) produced using the green synthesis approach are able to attenuate the histomorphological changes following STZ-induced diabetes, possibly acting via oxidant/antioxidant pathways.

ACKNOWLEDGMENTS

We acknowledge the College of Health Sciences, UKZN for their financial support to the doctoral student, Koffi Kouame. This work is supported in part by the National Research Foundation of South Africa to the senior author (OOA) Grant U99053 and Dr. Roshila Moodley (Grant No. 94041). We also thank the School of Chemistry and Physics, UKZN (Westville Campus) especially the support of Judie Magura and Bongisiwe Shelembe. The authors also acknowledge the UKZN Nanotechnology platform.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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