Evaluation of tenofovir disoproxil fumarate loaded silver nanoparticle on testicular morphology in experimental type-2 diabetic rats

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ABSTRACT
Reproductive derangement and metabolic disorders in human immunodeficiency virus (HIV) infected persons require a nanoparticle delivery system to convey antiretroviral drugs to the anatomical sanctuary such as testis. This study investigated the effects of tenofovir disoproxil fumarate (TDF) loaded silver nanoparticles (AgNPs) on the testicular oxidative stress, inflammatory cytokines and histology in male diabetic rats. Thirty-six Sprague-Dawley rats weighing 230±20 g were randomly divided into diabetic and non-diabetic groups (n=18). Diabetes was induced using the fructose-streptozotocin (Frt-STZ) rat model. Both groups were further divided into three (n=6) and administered distilled water, TDF, or TDF-AgNP. Results obtained with the TDF-AgNP administration showed a significant increase (p<.05) in the reduced glutathione and catalase levels. Tumour necrosis factor-alpha and interleukin 6 were reduced in diabetic rats administered TDF-AgNP. More so, administration of TDF-AgNP to diabetic rats improved testicular histoarchitecture in diabetic rats. In addition, diabetic rats administered TDF-AgNP showed a significant reduction (p<.05) in blood glucose levels. TDF-AgNP to diabetic rats enhanced testicular antioxidant enzyme, reduced testicular inflammation, and alleviated structural derangements in the testis. Thus, the application of AgNP to deliver TDF may alleviate testicular toxicity and subsequently cater for neglected reproductive dysfunction during the management of HIV infection.

Introduction
In the last four decades, tremendous advancement in antiretroviral drugs (ARVDs) has improved the quality of life of people infected with the human immuno-deficiency virus (HIV) and sustenance in their life expectancy [1]. Consequently, different choices of ARVDs are now available in the effective management of this infection, either from nucleoside reverse transcriptase inhibitors (NRTI) or non-reverse transcriptase inhibitors (NNRTI) or other classes of the antiretroviral drugs.

Among these available ARVDs is tenofovir disoproxil fumarate (TDF), the first-line drug in managing HIV infection, which belongs to the class of antiretroviral agents known as nucleotide reverse transcriptase inhibitor (NRTI). With strict compliance, the effectiveness of TDF as preexposure prophylaxis and in managing different variants of HIV and hepatitis B virus infection has been demonstrated in both men and women [2–4]. However, long-term administration is required for these therapeutic agents, which has been a significant concern despite increasing drug options and attempts to eradicate HIV infection.

As a result of continuous administration of these drugs, adverse effects such as the development of drug resistance, unfavourable drug interactions, systemic drug toxicity and reproductive dysfunction have been reported [5–8]. More so, TDF has been associated with severe metabolic acidosis, bone adverse effects, Fanconi’s syndrome (characterized by polyuria), impaired renal function [9–11]. Emerging evidence has revealed a significant link between metabolic acidosis, chronic renal failure, and diabetes mellitus. The urea accumulation in the blood and metabolic acidosis as a result of severe renal impairment increases the level of parathyroid hormones, defect insulin binding, defect glucose metabolism and subsequently cause insulin resistance [12,13].

Consequently, growing evidence has implicated hyperglycaemia in oxidative stress and cellular inflammation induction, which promotes testicular dysfunction and infertility [14]. It is noteworthy that inflammation causes oxidative stress, resulting in a reduced cellular antioxidant system containing catalase, superoxide dismutase and glutathione. Furthermore, reduction in the level of these enzymes and increase in the marker of lipid peroxidation such as malondialdehyde (MDA) contributes...
to the failure of the testicular biological system and subsequent testicular dysfunction [14]. Previous studies have described the role of inflammatory cytokines such as tumour necrosis factor-alpha (TNF-\(\alpha\)) and Interleukin 6 (IL-6) in metabolic regulations [15,16]. Also, the role of increased plasma concentration of inflammatory cytokines such as IL-6 and TNF-\(\alpha\) in the development of type II diabetes mellitus (T2DM) and obesity has been described. This surge was delineated to have detrimental consequences on the action of insulin by interfering with the insulin signalling pathway [17].

The predisposing factors to the adverse effects observed in the management of HIV were attributed to viral latent reservoir sites and biological barriers such as the blood-testis barrier. Furthermore, the cluster of differentiation 4 T cells (CD4) and macrophages of the lymphocyte serves as latent reservoir sites for HIV and host for viral replications and production of viral mutant genes, thereby making completed eradication of this virus difficult [18,19]. Asides from blood, anatomical sanctuary sites [20], especially testis, harbour HIV and represent another obstacle to the complete eradication of this HIV infection [21]. Testis exhibits the blood-testis barrier (BTB), a physiological barrier that separates the seminiferous tubule and vascular compartments and regulates the uptake of substances, drugs, and molecules [22].

Given these challenges, there is a growing interest in developing novel nanoparticle delivery vehicles to manage and completely eradicate HIV infection and HIV therapeutic adverse effects. In this regard, tenofovir disoproxil fumarate (TDF) encapsulated nanoparticles (NPs) has been delineated as a new curative modality in infectious diseases, especially (HIV) infection getting to the anatomical virus reservoirs [23]. These new nano-loaded ARVDs delivery systems promise to eliminate the virus from these anatomical sanctuary sites. The efficiency of these drug delivery systems in enhancing standard management of HIV was reported by a previous study [24]. Several of these nanoparticles are currently being investigated due to the efficacy of these nanoparticle-TDF formulations to ensure targeted and sustained delivery.

Silver nanoparticles (AgNPs) are among the most promising metallic nanoparticles that have received wide research attention and application in delivery of therapeutic agents due to their unique characteristics. The properties that give AgNPs an edge in drug delivery include a large surface area to volume ratio, targeted delivery, sustained release, efficient-density surface molecules attachment, and adjustable shape. In addition, AgNPs exhibit tuneable size, antiviral property, high loading efficiency, antimicrobial property, and the ability to protect the loaded drugs from degradation [25–28]. Despite the extensive application of AgNPs to load some therapeutic agents, there is a dearth of data on employing AgNPs to load TDF (TDF-AgNPs) and their effect on the testis as a nano-drug that may cater for reproductive dysfunctions while managing HIV infection.

Hence, there is a need to exploit the unique characteristics of AgNPs, reduce the burden of multiple pill administration, ensure the therapeutic efficiency of TDF to the anatomical sanctuary sites and manage reproductive dysfunction as well as metabolic disorders during the management of HIV infection. This study was designed to examine the interaction of this nano conjugation (TDF-AgNPs) on the testicular oxidative stress markers, inflammatory cytokines and testicular histology of fructose-streptozotocin-induced diabetic rats.

**Materials and method**

**Ethical approval and care of experimental animals**

This research was approved by the University of KwaZulu-Natal, Animal Research Ethics Committee (AREC) with the approval number AREC/043/019D. All animal protocols were carried out as specified in the Guide for the Care and Use of Laboratory Animals (8th edition National Academies Press).

**Chemicals and therapeutic agent**

Tenofovir disoprophil fumarate (TDF), a drug from the nucleoside reverse transcriptase inhibitors (NRTI) classes of antiretroviral drugs with a human equivalent dose of 300 mg, was purchased from Dis-Chem Pharmacy, Ballito, South Africa. Other reagents such as silver nitrate (AgNO\(_3\)\), sodium hydroxide, trisodium citrate (TSC), and streptozotocin (STZ) of analytical grade were sourced from Sigma-Aldrich Company, Johannesburg, South Africa. The inflammatory cytokines kits, TNF-\(\alpha\) and IL-6, were purchased from BIOCOM Africa (ElabScience Biotechnology Co., Ltd., Houston, TX).

**Synthesis of AgNPs and preparation of TDF-AgNP**

The AgNPs were synthesised by the chemical reduction process using trisodium citrate as a reducing and stabilising agent as described in the Turkevich method [29]. The AgNO\(_3\) crystals were oven-dried at 100°C, 0.3 g of the dried crystals were weighed and poured into a 500 mL volumetric flask, dissolved, and made to the mark with distilled water. The concentration of the AgNO\(_3\) stock solution was 0.03 mol/dm\(^3\). An aqueous stock solution of 2 mol/dm\(^3\) trisodium citrate was prepared by dissolving 58.8 g in a 100 mL volumetric flask and made to the mark with distilled water. The resultant solution was continuously stirred for 5 min at 90°C, at pH 10.5 of sodium hydroxide (NaOH) for 90 min. A colour change from colourless to amber yellow was observed. The synthesised AgNPs were cool at room temperature, centrifuged at 12,000 rpm for 15 min, and oven-dried at 40°C overnight. Then, 100 mL of 0.35 M TDF solution was mixed with 100 mL of synthesised AgNPs in double distilled. The solution was stirred continually in ultrasonication to ensure proper reaction of the components (TDF and AgNP). The tenofovir silver nanoparticle (TDF-AgNPs) was centrifuged at 4500 rpm at 40°C, at 40 min to discontinue the unincorporated drug. The resultant supernatant was analysed using an Ultra Visible (UV) spectrophotometer at a wavelength of 364 nm to calculate the amount of unincorporated drug (W1) from the total quantity of drug loaded with AgNPs (W2). The percentage of drug incorporation efficiency (% IE) was computed by employing the below equation [30]:

\[
\% \text{IE} = \left( \frac{TDFW2 - TDFW1}{TDFW2} \right) \times 100\%.
\]

TDFW2 is the total amount of Tenofovir disoproxil fumarate loaded with the silver nanoparticle, and TDFW1 represent
the quantity of unincorporated Tenofovir disoproxil fumarate. This percentage incorporated efficiency for the TDF-AgNP synthesised was calculated to be 85.00 ± 0.0%.

**Characterisation of AgNPs and TDF-AgNPs**

AgNPs and TDF-AgNPs formations were confirmed using ultraviolet-visible (UV-Vis) spectroscopy (Shimadzu MultSpec-1501, Shimadzu Corporation, Tokyo, Japan) and Fourier transform infra-red (FTIR) spectroscopy (Perkin-Elmer Universal ATR spectrometer, USA). The morphology and size of the NPs and nanoconjugates were assessed using a high-resolution transmission electron microscope (HR-TEM, JEOL 2100, Japan) performed at a voltage of 200 kV, and the field emission scanning electron microscope (FESEM, Carl Zeiss, Germany) performed at a voltage of 5 kV. Also, elemental compositions were observed with energy dispersive x-ray (EDX, Aztec Analysis Software, UK). Ultraviolet-visible spectroscopy demonstrated an absorption peak from 325 to 328 nm. Fourier-transform infra-red spectroscopy confirmed the conjugation of TDF to AgNP with the presence of C-N and O-H functional groups on TDF-AgNP. The Microscopic images revealed spherical particles of AgNP. Furthermore, the mean particle sizes were 12 nm to 22 nm. Energy-dispersive X-ray spectroscopy confirmed the presence of silver in the TDF-AgNP.

**Experimental design**

Thirty-six, male, adult Sprague-Dawley rats weighing between 230 and 250 g were used for this research. The experimental rats were housed in well ventilated plastic cages (3 rats in a cage of dimensions 52 cm × 36 cm × 24 cm length, wide and high respectively). The rats were maintained under standardised animal house conditions with temperature ranges from 23 °C to 25 °C with 12 h of natural light per day. The experimental rats were fed with standard animal pellets bought from Meadow feeds, division of Astral Operations Limited, Durban, South Africa and water were given *ad libitum*. The rats were randomly assigned into diabetic (n = 18) and non-diabetic (n = 18) groups.

**Type II diabetes mellitus induction**

The rats allotted to the diabetic group received fructose (Frt) and streptozotocin (STZ) to induce type II diabetes mellitus (T2DM) following the protocol previously described by Wilson and Islam [31]. Briefly, rats in the diabetic group were administered 10% fructose dissolved in drinking water *ad-libitum* for two weeks. After that, the rats were injected (i.p) with 40 mg/kg body weight of STZ freshly prepared in 0.1 M citrate buffer. Besides, the rats in the non-diabetic group received the same volume of 0.1 M citrate buffer intraperitoneally. Rats with a fasting blood glucose level ≥200 mg/dL and above were considered diabetic and included in the study.

**Grouping and animal treatment**

The diabetic and non-diabetic groups were further divided into three (3) groups each (n = 6) as follows and treated as follows:

- **Group 1**: Non-Diabetic Control (NDC) rats received 1 mL distilled water daily.
- **Group 2**: Non-Diabetic TDF (NDT) rats were administered 26.8 mg/kg/body weight daily (p.o).
- **Group 3**: Non-Diabetic Nanoparticles TDF (NDNT) rats received 6.7 mg/mL/body weight (6/7 days i.p).
- **Group 4**: Diabetic control (DC) rats 1 mL received distilled water daily.
- **Group 5**: Diabetic TDF (DT) rats treated with 26.8 mg/kg/body weight daily (p.o).
- **Group 6**: Diabetic Nanoparticle TDF (DNT) rats administered 6.7 mg/mL/body weight (6/7 days i.p).

Tenofovir disoproxil fumarate, TDF (300 mg) in tablets form, were crushed to powder, and adequate amounts weighed out following the recommended human doses. The animal dose was calculated from human equivalent dose using the below formula and following the United States Food and Drug Administration recommendation [32–34];

\[
\text{Human Equivalent Dose} = \frac{\text{Animal NOAEL (mg/kg)} \times \text{Animal Weight (kg)}}{\text{Human weight (kg)}}^{1.067}
\]

**Bodyweight measurement**

The body weights of all the experimental rats were adequately observed and recorded twice a week throughout the 56 days of the experiment using a sensitive weighing balance (Metler, Greifensee, Switzerland).

**Measurement of blood glucose level**

The sterile lancet pins were used to obtain blood samples from the tail vein by pinprick, and the blood glucose levels were checked once a week throughout the 56 days of administration using a Glucometer (Acucheck®, Boehringer-Mannheim, Germany).

**Measurement of relative testicular weight**

The relative testicular weights (RTW) were estimated for the testes using the following formula: [35]

\[
RTW = \frac{\text{Testicular weight}}{\text{Total rat body weight}} \times 100.
\]

**Tissue preparation for oxidative stress markers and inflammatory cytokines**

The quantity of 0.5 g of each harvested testes were homogenised in 5 mL sodium phosphate buffer with 1% Triton X-100.
of 50 mM at pH 7.5. Testicular tissue homogenates were then centrifuged for 10 min at 20,000 g at temperature of 4°C using Centrikon H-401 (Germany) centrifuge. Thereafter, the supernatants were collected, decanted into 2 mL Eppendorf tubes, labelled, and used for oxidative stress and inflammation analysis. The concentration of Malondialdehyde (MDA) [36], reduced glutathione (GSH) [37], Superoxide Dismutase (SOD) [38] and catalase (CAT) [39] were analysed using the protocol previously described.

**Determination of TNF-α and IL-6**

The inflammatory cytokine concentrations (TNF-α & IL-6) were evaluated from the testicular homogenate using their respective Enzyme-Linked Immunosorbent Assay kit (ElabScience Biotechnology Co., Ltd., Houston, TX) according to the manufacturer’s protocol. Absorbance was estimated by the microplate reader, SPECTRO star Nano spectrophotometer (BMG Labtech, Ortenburg, LGBW, Germany).

**Histological analysis of testis**

The testes were excised, weighed, and fixed immediately in Bouin’s fixative for 24 h before tissue processing. The testicular tissues were sectioned at 4 μm thickness using Leica RM 2255 microtome, embedded in paraffin blocks, and stained with haematoxylin & Eosin (H&E), Periodic Acid Schiff (PAS) and Masson’s Trichrome (MT). The slides were cover-slipped using distyrene, plasticiser and xylene (DPX) mounting glue straight on the tissue slides to ensure that air bubbles were not trapped. Following this, the slides were left overnight to dry for examination under a light microscope. Finally, the tissue sections were examined using a binocular Olympus microscope and a digital image camera (Nikon Eclipse 50i, Tokyo, Japan).

**Statistical analysis**

The statistical analyses for the obtained data were performed using one-way analysis of variance (ANOVA) to determine the mean differences between the groups. Also, Tukey’s multiple comparisons posthoc tests were performed using Graph pad prism®, statistical software version 7.0. The results were visually displayed and expressed as mean ± standard error of the mean (SEM) at 95% confidence level (p < .05).

**Results**

**Effect of TDF-AgNP on body weight, blood glucose and relative testicular weight**

As shown in Figure 1(a–c), diabetic control (DC) rats showed a significant reduction (p < .05) in body weight vs non-

![Figure 1](image)
diabetic control (NDC) rats. The body weight changes in diabetic rats treated with TDF-AgNP were not different from that of diabetic control rats. However, diabetic rats treated with TDF (DT) showed a significant increase ($p < .05$) in body weight vs DC.

Diabetic control (DC) rats showed a significant increase ($p < .05$) in blood glucose level vs non-diabetic control (NDC) rats, while diabetic rats treated with silver nanoparticles loaded Tenofavir (DNT) showed a significant reduction ($p < .05$) in blood glucose level vs DC rats. Also, diabetic control (DC) rats showed a significant decrease ($p < .05$) in relative testicular weight vs non-diabetic control (NDC) rats. At the same time, administration of TDF-AgNPs and TDF caused no significant change in the relative testicular weight in non-diabetic and diabetic rats (Table 1 and Figure 1).

**Effect of TDF-AgNP on malondialdehyde, reduced glutathione and antioxidant enzymes**

As shown in Figure 2(a–d), diabetic control (DC) rats showed a significant decrease ($p < .05$) in GSH SOD and CAT as well as a significant increase ($p < .05$) in the levels MDA vs non-diabetic control (NDC) rats. However, administration of silver nanoparticle loaded Tenofavir (DNT) to diabetic animals significantly increased ($p < .05$) GSH and CAT with a significant decrease ($p < .05$) in MDA level vs diabetic control (DC) rats.

### Table 1. Bodyweight, blood glucose, and relative testicular weight.

| Parameters groups | Bodyweight (g) | Blood glucose level (mg/dL) | Relative testicular weight (%) |
|-------------------|----------------|-----------------------------|-------------------------------|
| NDC               | 357 ± 11.8     | 75 ± 2.40                   | 1.2 ± 0.08                    |
| NDT               | 367.5 ± 21.4   | 75.8 ± 3.20                 | 1.1 ± 0.06                    |
| NDNT              | 339.7 ± 13.6   | 67.8 ± 1.50                 | 0.9 ± 0.02                    |
| DC                | 251 ± 6.90*    | 407.7 ± 30.1*               | 0.6 ± 0.03*                   |
| DT                | 307 ± 7.20*    | 350.5 ± 9.70                | 0.7 ± 0.03                    |
| DNT               | 283.7 ± 17.9   | 345.7 ± 13.0*               | 0.6 ± 0.07                    |

Data are expressed as mean ± SEM ($n = 6$), * $p < .05$ vs NDC; # $p < .05$ vs DC.

Key: NDC: Non-diabetic control; NDT: Non-diabetic Tenofovir; NDNT: Non-diabetic nano tenofovir; DC: diabetic control; DT: Diabetic tenofovir; DNT: Diabetic nano tenofovir.

**Figure 2.** Effect of treatment on testicular (A) Reduced glutathione (GSH) (B) Superoxide dismutase (SOD); (C) Catalase (CAT) and (D) Malondialdehyde (MDA). NDC: Non-diabetic control; NDT: Non-diabetic Tenofovir; NDNT: Non-diabetic nano tenofovir; DC: diabetic control; DT: Diabetic tenofovir; DNT: Diabetic nano tenofovir. Data are expressed as mean ± SEM ($n = 6$). * $p < .05$ vs NDC; # $p < .05$ vs DC. The diabetic control (DC) rats showed a significant decrease in GSH SOD and CAT and a significant increase in the levels of MDA vs non-diabetic control (NDC) rats. However, TDF-AgNPs (DNT) administration to diabetic animals significantly increased GSH and CAT, decreasing MDA level vs diabetic control (DC) rats. Also, there was a substantial reduction in testicular SOD and CAT levels in the non-diabetic rats administered TDF (NDT) vs NDC.
Also, there was a significant reduction ($p < .05$) in testicular SOD and CAT levels in the non-diabetic rats administered TDF (NDT) vs NDC.

**Effect of TDF-AgNP on tumour necrotic factor-$\alpha$ and interleukin-6**

As presented in Figure 3(a-b), diabetic rats showed a significant ($p < .05$) increase in testicular TNF-$\alpha$ and IL-6 vs non-diabetic control rats. However, administration of silver nanoparticle loaded tenofovir to diabetic (DNT) rats significantly reduced TNF-$\alpha$ and IL-6 vs DC rats.

**Effects of HAART-AgNP on the testicular histology**

The non-diabetic control (NDC) rats showed a normal histological structure of testis with well-populated seminiferous tubule (ST) with numerous immotile spermatozoa in the lumen. ST appeared well circumference and had an excellent alignment of the interstitial spaces with no distortion. In contrast, the diabetic control rats (DC) showed a marked atrophied ST, distorted lumen, widely sparse interstitial spaces, thickened basement membrane and sparse luminal spermatic. The ST of the diabetic rats administered with TDF demonstrated mid atrophy, mid widened interstitial spaces and few dispersed Leydig cells. However, diabetic rats administered TDF-AgNPs showed an improved histological architecture with mild tubular disorientation compared to DT and DC. More so, treatment with TDF and TDF-AgNPs in non-diabetic rats showed a collagen fibre almost like non-diabetic control rats (Figure 4(b)).

The non-diabetic control showed a normal thickness of the seminiferous tubule and the basement membrane that contained positive PAS-stained peritubular interstitial tissues and Leydig cells. Also present are the PAS-stained acrosome in the NDC. In contrast, the Leydig cells and peritubular interstitial tissues of diabetic control rats displayed a weakly PAS reaction. However, diabetic rats treated with TDF-AgNP (DNT) showed a normal peritubular interstitial tissue and the interstitial cells (Leydig) PAS reaction better than the DT. In addition, the non-diabetic rats treated with both TDF and TDF-AgNP demonstrated a normal PAS-stained peritubular interstitial tissue and the interstitial cells (Leydig) (Figure 4(c)).

**Discussion**

Although various available antiretroviral drugs have transformed the narrative of HIV from deadly to manageable chronic infection, notwithstanding some adverse effects, such as insulin resistance, type-2 diabetes mellitus, and reproductive dysfunction, poses a significant threat in managing HIV infection. Hence, this study investigated the interaction of TDF-AgNP on the testicular oxidative stress markers, inflammatory cytokines and testicular histology.

The increase in body weights observed with TDF administration in diabetic rats suggested a weight gain potential of TDF in diabetic conditions. This finding corroborates the previous research that revealed body weight gain at TDF initiation [40]. The exact mechanism for weight gain at TDF initiation is unclear, although some evidence suggested faster viral control, drug-specific impact, and off-target biological interplay. A study described the modulation of the leptin signalling pathway, which controls the receptors responsible for
Figure 4.  (a) Photomicrograph of testicular tissues for groups NDC, NDT, NDNT, DC, DT, and DNT (H&E – Scale bar -50 μm). NDC shows the normal histarchitecture with seminiferous tubules (ST) well preserved and populated by the layers of the spermatogonia series. The lumen (L) shows numerous immotile spermatozoa. Slides of the DC group show ST atrophy with widespread distorted spermatogenetic layers. DT slide shows moderate ST atrophy with reduced spermatogenic cells in the tubule. Slides of the DNT group exhibited remarkable improvement in the qualitative histological architecture compared to DC and DT groups. (b) Photomicrograph of testicular sections stained with Masson’s Trichrome for groups NDC, NDT, NDNT, DC, DT, and DNT (Scale bar-50μm). Histoarchitecture of the NDC group displayed a well preserved, blue-stained connective tissue capsule (of collagen) with clear delineated seminiferous tubular linings and interstitial spaces showing no obvious pathology (white arrow). Various degrees of disorganisation in seminiferous tubular integrity alongside capsular distortions were visible in DC and DT groups. In some areas appears to have some detachment/fenestrated appearance. The capsular integrity in the DNT groups appeared normal with seminiferous tubular components. (c) Photomicrograph of the testicular sections stained with Periodic-Acid Schiff (PAS) for groups NDC, NDT, NDNT, DC, DT, and DNT (Scale bar-50 μm). Weak PAS-staining is noted in the DC treated animals indicating possible tubular atrophy and interstitial hypoplasia (Black star *).
caloric intake for a possible mechanism for weight gain, especially in antiretroviral drugs [41,42].

Interestingly, the significant reduction in the blood glucose levels in the diabetic rats administered with TDF-AgNPs indicates glycaemic control, which may be attributed to silver nanoparticles. Previous studies have documented an improved antidiabetic activity of AgNPs loaded natural products based on the intrinsic property of AgNPs to hinder α-glucosidase and α-amylase [43,44]. The α-glucosidase and α-amylase represent the most abundant carbohydrate hydrolysing enzymes in the gastrointestinal tract. Their inhibition leads to a reduction in the postprandial surge in blood glucose levels [45]. Remarkably, frt-STZ induced diabetic rats displayed a significant decrease in relative testicular weight. Recently, a substantial reduction in the relative testicular weight of diabetic rats was delineated as an indication of metabolic derangement resulting from enormous tissue catabolism [46]. The testicular weight loss caused by diabetes mellitus has been linked with atrophy of testis and subsequent testicular and reproductive dysfunction [47].

Furthermore, the significant decrease in testicular GSH, SOD and CAT, and increased MDA in the frt-STZ diabetic rats suggest the role of reactive oxygen and nitrogen species (ROS, NOS) in the pathogenesis of diabetes-induced testicular injury [48,49]. Interestingly, administration of TDF-AgNP alleviates oxidative damage in diabetic rats, suggesting that AgNPs exert some antioxidant effect. Previously, Chandrasekhar and Vinay [50] reported the potential of AgNPs to scavenge free radicals, which may be attributed to the presence of a bioreductant substance in AgNP. It is imperative to state that an increase in these antioxidant enzymes and a decrease in MDA level as observed in diabetic rats treated with TDF-AgNP may boost the testicular antioxidant defence system and alleviate testicular toxicity.

Furthermore, a significant reduction in testicular SOD and CAT observed among the non-diabetic rats treated with TDF, signifying a compromised antioxidant system due to chronic exposure to TDF. Abraham and Co-workers revealed that TDF causes mitochondrial impairment excessive production of reactive oxygen species and subsequently weakens the antioxidant enzyme defence mechanism [51]. This finding implies that chronic administration of TDF posed a significant threat to the testicular antioxidant defence system.

Tumour necrosis factor-alpha, a potent pro-inflammatory cytokine released by the immune system, is a principal constituent of an inflammatory response, which its release prompts the release of interleukin-6 [52,53]. Studies have reported the significant role of TNF-α and IL-6 in the pathogenesis of T2DM and diabetic complications [54,55]. Furthermore, a case-controlled male cohort demonstrated reproductive dysfunction and increased seminal inflammatory cytokines as an adverse effect of metabolic disorders [56].

In this study, the increased TNF-α and IL-6 in the testicular homogenate of diabetic control rats suggest significant inflammation. A similar study reported an increase in the levels of TNF-α, IL-6 and other cytokines in diabetic subjects [17,57]. The increased concentrations of these cytokines in the male reproductive system has a deleterious implication on spermatozoa quality and quantity, linked with reproductive dysfunction [58].

Moreover, the reduction in testicular TNF-α and IL-6 after administering TDF-AgNPs in diabetic rats suggests some inhibitory activities of the TDF-AgNPs nanoconjugate. The characterisation and properties of the AgNPs used in this study may account for the significant inhibition of TNF-α and IL-6 in diabetic rats. Previous studies have documented the anti-inflammatory potential of AgNPs [59,60]. Wong and colleagues [59] investigated the mechanism of anti-inflammatory activity of AgNP in both in-vitro and in vivo models. Their findings show that AgNP exerts its anti-inflammatory response by interrupting the signalling pathway of inflammation but not through apoptosis.

The atrophy of seminiferous tubules, distortion of the lumen, abnormal large interstitial space, and thickened basement membrane observed in the testicular section of diabetic control rats indicate testicular damage. Similar results on testicular histological toxicity have been reported in the literature [61]. These damages in testicular histology are implicated in reproductive dysfunction. However, few histological alterations seen in diabetic rats administered with TDF-AgNPs could result from progression in the diabetic condition. A study has shown successful coupling and delivery of nanoparticles such as liposomes, polymeric, and metallic as effective therapeutic modalities in HIV infection [62]. Also, Naidu et al. [63] described size, shape, method of synthesis, route of administration, choice stabilising and reducing agents as factors that determine the safety or toxicity of AgNPs. The ability of TDF-AgNPs to improve the testicular toxicity caused by diabetes in this study may be due to the physicochemical properties, small size and spherical shape of the formulated AgNPs.

In addition, a significant increase and distortions in collagen fibres observed in the diabetic control rats indicates a decrease in collagen metabolism, suggesting an implication of oxidative stress. In a similar study, oxidative stress was linked to increased collagen fibres with a detrimental reduction in collagen metabolism [64]. Also, a mild increase in collagen fibres noticed in TDF treated rats treated with TDF suggested testicular toxicity. This finding implies that TDF administration to diabetic rats may aggravate the diabetic condition. Their report and results in this study indicate that treatment with TDF in diabetic rats may worsen the situation, suggesting that administration of TDF in diabetic rats alters testicular histology and may contribute to some level of reproductive dysfunction.

**Conclusion**

This study has shown a beneficial impact of TDF-AgNP on the testicular antioxidant system, anti-inflammatory cytokines and testicular cytoarchitecture. This impact was demonstrated through an increased testicular GSH, CAT and reduced MDA levels. Also, lowering testicular TNF-α and IL-6 concentrations improved testicular histological architecture in diabetic rats and significantly reduced blood glucose levels in diabetic rats. Hence, these beneficial effects of TDF-AgNP...
conjugate on testicular antioxidant enzymes, inflammatory biomarkers and morphology may be a tempting candidate to cater for the neglected reproductive dysfunction during the management of HIV infection.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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