

Article

Carbon Dot Nanotherapeutics Modulating the Polyol Pathway and Targeting Infection Pathogens Associated with Diabetic Complications

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Abstract

Background: Diabetes mellitus is a global health challenge associated with chronic complications like diabetic nephropathy and diabetic foot infections. Diabetic nephropathy, mediated by hyperglycemia-induced activation of the polyol pathway, represents a primary cause of end-stage renal disease. Additionally, infections caused by multidrug-resistant bacteria like *Enterococcus faecalis* lead to amputations and contribute to morbidity in diabetic patients. **Methods:** In this study, we synthesized nitrogen-doped carbon dots (N-CDs) using succinic acid with either hexamethylenediamine (N-HCD) or ethylenediamine (N-ECD) and evaluated their potential therapeutic applications. **Results:** Both N-HCD and N-ECD demonstrated a significant reduction in aldose reductase (AR) and sorbitol dehydrogenase (SDH) *in vitro*, with a substantial reduction in polyol pathway enzymatic activity. Furthermore, these N-CDs exhibited antibacterial activity against *E. faecalis in vitro*. **Conclusions:** Taken together, our findings suggest that N-HCD and N-ECD represent promising candidates for addressing diabetes-related complications and warrant further investigation for potential drug delivery applications.

Keywords: nitrogen-doped carbon dots; aldose reductase; sorbitol dehydrogenase; *Enterococcus faecalis*



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1. Introduction

As diabetes reaches global epidemic levels, there is an urgent need for effective and reliable treatments for its complications, including microvascular and macrovascular diseases. Chronic hyperglycemia triggers multiple biochemical pathways that contribute to microvascular and macrovascular complications through mechanisms involving glucose toxicity and oxidative stress [1]. The polyol pathway, while extensively studied as a

therapeutic target for diabetic complications, remains a subject of ongoing debate regarding its precise contribution to disease pathogenesis and the clinical efficacy of its inhibition [2].

The polyol pathway represents a secondary route of glucose metabolism in which aldose reductase (AR) catalyzes the reduction of glucose to sorbitol, under hyperglycemic conditions. Sorbitol produced in this reaction is transformed into fructose by sorbitol dehydrogenase [3,4]. Beyond osmotic stress from sorbitol accumulation in tissues such as kidneys, eyes, and peripheral nerves, this pathway disrupts cellular redox balance. It depletes NADPH through the AR reaction and generates NADH through the SDH reaction, thereby altering the NADH/NAD⁺ ratio and promoting oxidative stress [5]. This redox imbalance and metabolic disruption contributes significantly to diabetic nephropathy, which remains a leading cause of end-stage renal disease.

On the other hand, diabetic patients also face substantial risk from infectious complications, particularly diabetic foot infections that can progress to lower limb wounds. Poor glycemic control, compromised immune function, and impaired wound healing create conditions favorable to bacterial colonization and chronic infection. Gram-positive bacteria, particularly *Staphylococcus* and *Enterococcus* genera, colonize diabetic wounds and may develop multidrug resistance and biofilm formation, further complicating treatment [6]. The dual burden of metabolic dysregulation and infectious complications in diabetes requires innovative therapeutic approaches that can address multiple pathological mechanisms.

Recent advances in nanomedicine offer promising avenues for addressing complex disease challenges. Carbon dots (CDs), a class of carbon-based nanomaterials typically less than 10 nm in diameter, have attracted attention due to their properties including excellent biocompatibility, low toxicity, tunable optical properties, facility of synthesis, and potential for surface functionalization [7]. Nitrogen doping of CDs can enhance their physicochemical properties and biological activities, including potential antibacterial effects and interactions with biological macromolecules. Due to their small size, biocompatibility, and modifiable surface chemistry, nitrogen-doped CDs represent attractive candidates for therapeutic intervention in diabetes complications.

In this study we attempted to test both N-HCD (Nitrogen-doped Hexamethylenediamine) and N-ECD (Nitrogen-doped Ethylenediamine) for their antidiabetic and antibacterial possible effects to counteract the dual challenges of hyperglycemia and diabetic foot infections. Specifically, we evaluated their capacity to inhibit the key polyol pathway enzymes AR and SDH, as well as their antibacterial activity against *Enterococcus faecalis*, a pathogen commonly isolated from diabetic foot infections. This work explores the potential of rationally designed nanomaterials to target multiple pathological mechanisms relevant to diabetes complications.

2. Materials and Methods

2.1. Hydrothermal Synthesis of N-Doped Carbon Dots

The synthesis procedures for N-HCD and N-ECD are illustrated in Figure 1. In a typical preparation, 10 mL of deionized water was used to dissolve an equimolar mixture of succinic acid (0.198 g, 3 mmol) and either hexamethylenediamine (0.18 g, 3 mmol) or ethylenediamine (0.18 g, 3 mmol) Fluka (Buchs, Switzerland). under sonication. The resulting homogeneous solution was transferred to a sealed autoclave and heated at 200 °C for 18 h. After cooling, the crude product was purified by dialysis (molecular weight cut-off ≈ 3.0 kDa) for 24 h to remove unreacted species and low-molecular-weight by-products. Finally, solvent removal followed by vacuum drying yielded the N-HCD and N-ECD [8].

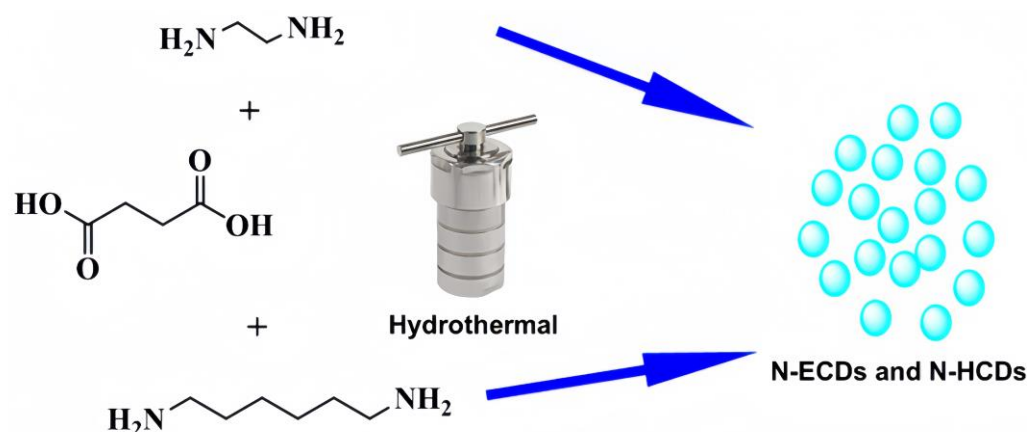


Figure 1. Schematic representation of the synthesis of the CDs.

2.2. Structural and Spectroscopic Characterization of N-Doped Carbon Dots

ATR-FTIR spectroscopy was used to analyze the N-ECD and N-HCD samples. The obtained spectra exhibited characteristic absorption bands at specific wavenumbers, reflecting the presence of different functional groups. In particular, a strong absorption around 3310 cm^{-1} , observed in both N-ECD and N-HCD powders, is attributed to N–H and O–H stretching vibrations. (Figure 2A) [9]. A weaker peak at 2954 cm^{-1} is attributed to C–H stretching, while a faint band at 1672 cm^{-1} indicates the presence of C=O stretching vibrations, characteristic of carbonyl groups [10]. The peak at 1450 cm^{-1} is assigned to C–N stretching vibrations [11], and the peak at 1265 cm^{-1} corresponds to C–O stretching vibrations [12]. Additionally, the band at 1510 cm^{-1} , attributed to N–C=O bond formation, suggests effective reactions among hexamethylenediamine, ethylenediamine, and succinic acid [13].

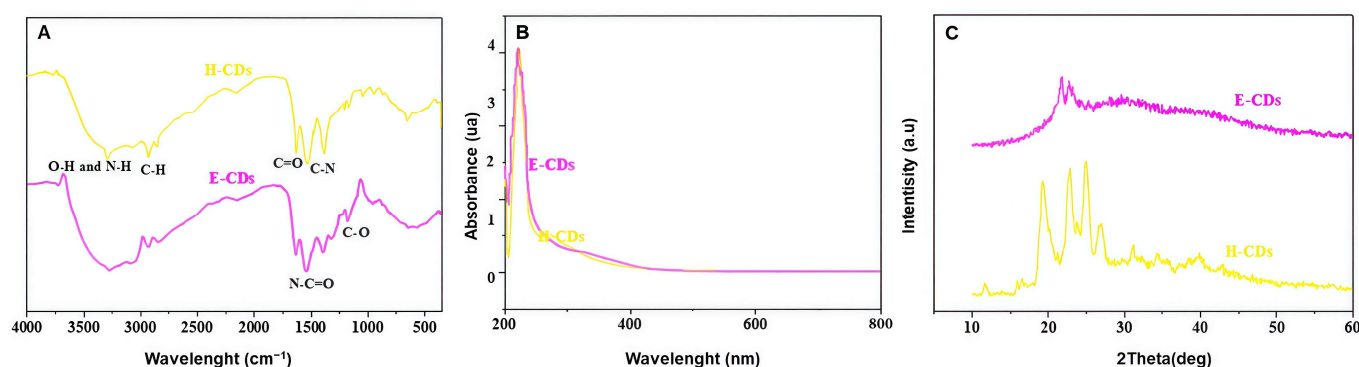


Figure 2. Characterization of N-ECD and N-HCD, showing (A) ATR-FTIR spectra, (B) UV-Vis absorption spectra, and (C) X-ray diffraction patterns.

The UV-Vis spectra of the N-ECD and N-HCD samples (Figure 2B) showed two prominent absorption bands. The first, at 253 nm , is associated with $\pi-\pi^*$ electronic transitions, while the second, at 297 nm , corresponds to $n-\pi^*$ transitions [13]. Specifically, π -electrons are excited to π^* orbitals, and non-bonding electrons (n) are promoted to π^* orbitals. In combination with the FTIR results, the $\pi-\pi^*$ absorption at 253 nm is likely linked to C=O bonds, whereas the $n-\pi^*$ absorption at 297 nm can be attributed to N–H or O–H functionalities [14].

XRD analysis, which provides insight into the carbon dot (CD) core structures, further differentiates N-ECD and N-HCD. As illustrated in Figure 2C, N-HCD samples display notably higher crystallinity compared to N-ECD, as indicated by their sharper and more intense diffraction peaks. In contrast, N-ECD shows relatively weak peaks at $2\theta = 20^\circ$

and $2\theta = 22^\circ$, suggesting smaller crystalline domains. This reduced order may result from structural irregularities caused by the non-repetitive ethylenediamine side chains. In contrast, N-HCD shows four distinct peaks at $2\theta = 19^\circ$, 22° , 25° , and 27° [15]. This improved crystallinity in N-HCD is likely due to the more regular and homogeneous structure of hexamethylenediamine, promoting crystal growth. Furthermore, the rightward shift in peak positions for N-HCD compared to N-ECD consistent with the Bragg equation indicates smaller interplanar spacing, reflecting a tighter and more compact crystal structure in N-HCD [16].

2.3. Determination of Antibacterial Activity via Agar Well Diffusion

The antibacterial effects of the four bacterial strains—*Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*—were examined using the agar well diffusion method, following previously reported procedures [17–19]. Bacterial suspensions (10^8 CFU/mL) were evenly spread onto Petri dishes containing 20 mL of Mueller-Hinton agar (MHA) BLOKAR Diagnostics (Allonne, France). Wells measuring 4 mm in diameter were created, and 10 μ L of each test compound, prepared at 50 μ g/mL in 10% DMSO, was added to the wells. Plates were then incubated at 37 $^\circ$ C for 24 h, after which the zones of inhibition were recorded.

2.4. Microdilution Method

The broth microdilution technique was carried out to determine the MIC of the CD. A total of 50 μ L of each bacterial strain (10^8 CFU) was spread in a sterile 96-well microtiter plate containing 50 μ L of MHB. Then, 10 μ L of each molecule dissolved in DMSO (10%) Sigma-Aldrich (St. Louis, MO, USA). was added at a concentration of 50 μ g/mL and was serially diluted. The incubation was done under optimal conditions for 24 h at 37 $^\circ$ C.

A resazurin solution was prepared at a concentration of 0.01% (*w/v*); then, each well was filled with 30 μ L. The microtiter plates were incubated at a temperature of 37 $^\circ$ C for 4 h. Control wells were performed with a culture medium, bacterial suspension, DMSO (10%) solution, and molecules [20,21].

2.5. In Silico Methods

Computational docking was used to assess the interaction between CDs and the aldose reductase enzyme. The crystal structure of human aldose reductase (1IEI) was uploaded from the protein data bank <https://www.rcsb.org/structure/1IEI> (accessed on 15 August 2025). Ligands and their analogs were designed using ChemDraw 12.0, and the structures corresponding to the synthesized CDs were saved in PDB format. These files were subsequently converted to PDBQT format, and docking was carried out using AutoDock Vina (1.5.6). For the 1IEI structure, the grid box was centered at coordinates $x = 5.611$, $y = 1.861$, and $z = 0.083$, with dimensions of $80 \times 80 \times 110$ Å. The docking results were analyzed using Discovery Studio (2021).

2.6. Experimental Validation of Bioinformatic Data

2.6.1. Experimental Induction of Diabetes in Rats

Male Sprague-Dawley rats weighing 200–230 g were housed in an air-conditioned animal facility maintained at 25 $^\circ$ C with 55% relative humidity and a 12/12 h light/dark cycle. Food and water were provided *ad libitum*.

Following a one-month acclimation period, diabetes in rats was induced by intraperitoneal injection of a freshly prepared streptozotocin (STZ) Sigma-Aldrich (St. Louis, MO, USA) solution in a citrate buffer 0.1 M with pH 4.5 at a dose of 60 mg/kg. Generally, 2-Deoxy-2-(methylnitrosourea)-D-glucopyranose, commonly known as STZ, is taken up by cells via the GLUT2 transporter (Figure 3). At 48 h post-injection, rats displaying clinical

signs of diabetes (polydipsia and polyuria) were assessed for hyperglycemia using a blood glucose meter. Animals with blood glucose levels ≥ 300 mg/dL were classified as diabetic. Diabetes was fully established one week after STZ administration, at which time organs were collected for biochemical analyses.

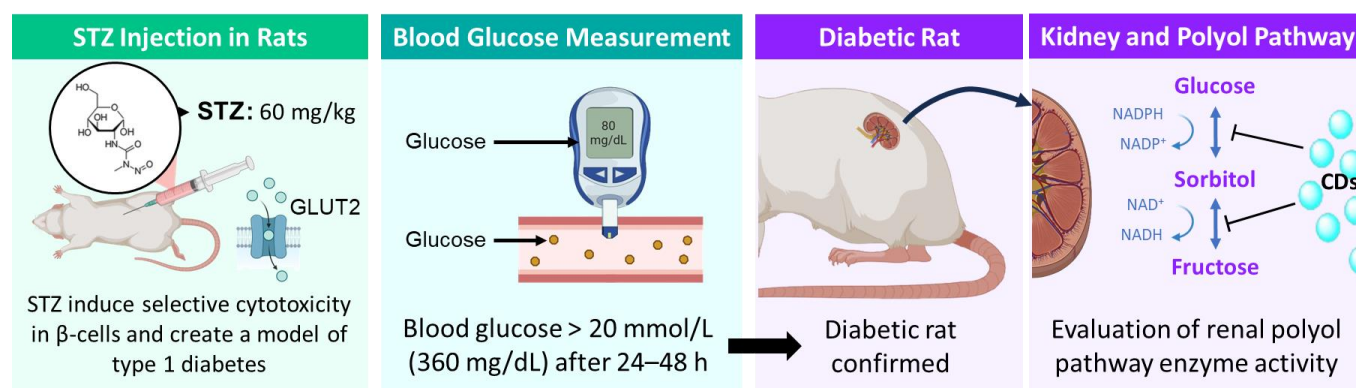


Figure 3. Experimental design illustrating the induction of diabetes in rats using streptozotocin (STZ), confirmation of hyperglycemia, and subsequent assessment of renal polyol pathway enzyme activity to evaluate the impact of carbon dots (CDs).

2.6.2. *Ex Vivo* Evaluation of CD Inhibitory Activity on Polyol Pathway Enzyme Activity

Kidney tissues were promptly removed and placed in an ice-cold homogenization buffer. Tissues were homogenized in a Potter-Elvehjem homogenizer with a motor-driven Teflon pestle (10 strokes at highest speed) with 1:3 *w/v* of homogenization buffer. The homogenates were centrifuged at $19,000 \times g$ for 30 min at 4 °C. The supernatants were collected and used for the assessment of protein content and polyol pathway enzyme activities. All the chemicals used were of high-quality analytical grade chemicals and were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The activity of aldose reductase (AR) was assessed based on a previously described protocol [22]. Sorbitol dehydrogenase (SDH) activity was measured according to established methods. The 1 mL reaction mixture consisted of 100 mM Tris-HCl buffer (pH 8.9), 40 mM sorbitol, 1.5 mM NAD⁺, and rat kidney preparation. To evaluate the inhibitory potential of CDs, both AR and SDH activities were tested at concentrations of 10, 20, and 40 μ g/mL (Figure 3). The reaction was carried out for 1 min, and enzyme activity was monitored spectrophotometrically by following the change in absorbance at 340 nm corresponding to the NAD⁺ reduction.

2.7. Statistical Analysis

All experiments were carried out at least in triplicate. The differences between groups were tested for statistical significance using one-way ANOVA at a $p < 0.05$ significance level using GraphPad Prism 8.0.2 software for Windows (GraphPad Software Inc., San Diego, CA, USA).

3. Results and Discussion

3.1. Antibacterial Activity of CDs Against *Enterococcus faecalis* Associated with Diabetic Foot Infections

Infectious diseases pose a serious concern for the public health and still cause high rates of deaths worldwide. The developed antibiotics were expected to heal or attenuate different infectious severities; however, their overuse led to the emergence of drug-resistant strains constituting a limiting problem [23].

Bacteria have the capacity to evade the inhibitory action of antimicrobial agents through mobile genetic elements that can be acquired via horizontal transfer. They can resist diverse classes of antibiotics via different mechanisms [24].

In response to this problematic, it is of great importance to find out novel molecules in order to tackle drug the resistance phenomenon and resolve the treatment failure of wound management in diabetic patients. Due to their distinctive physicochemical properties and bioactivities, carbon dot (CD)-based materials have captivated favorable attention. Here, we explored the antibacterial properties of two CDs synthesized from succinic acid and either hexamethylenediamine (N-HCD) or ethylenediamine (N-ECD). The inhibitory effects of CDs were assessed against four pathogenic strains related to foot diabetic infections.

The pathogenic strains were resistant to antibiotics, especially penicillin. The well diffusion assay showed that N-ECD and N-HCD have the ability to inhibit the growth of *Enterococcus faecalis* with inhibition zone diameters of 11.5 ± 0.707 , and 13 ± 0.0 mm respectively. They exhibited this inhibitory activity at a concentration of $50 \mu\text{g/mL}$ with a bacteriostatic effect. However, they showed no inhibition against the other pathogenic bacteria in either the well diffusion test or the microdilution assay. It is noteworthy that the growth inhibition activity of the two synthesized molecules was observed against a Gram-positive bacterium. This may be due to the difference between bacterial cell wall compositions, as Gram-negative species own a rigid outer membrane rich in lipopolysaccharides, making the penetration of the antibacterial components more difficult. In contrast, as a Gram-positive bacterium, *E. faecalis* is generally more susceptible to antibacterial agents, allowing them to bind more effectively to the cell wall, which has a thick peptidoglycan layer, thereby causing its disruption. Consequently, this can cause osmotic imbalance, energy failure, and ultimately cell lysis [25].

E. faecalis was reported to show resistance against several antibiotics, including erythromycin, ciprofloxacin, quinolones, and fluoroquinolones. This may be due to its ability to harbor genes encoding different aminoglycoside modifying enzymes, suppressing their inhibitory effects. It is necessary to note that diabetic wounds majorly attract polymicrobial species that undergo mutual and synergistic evolution, thereby interrupting the wound healing process. This can result in serious chronic infections, constituting a real concern, which may eventually lead to lower limb amputation. Moreover, the severity of infection can be increased by the ability of bacteria to develop biofilms which increase the persistence of the disease as well as the mortality rate [25].

Tetracycline inhibits bacterial growth by interfering with protein synthesis, while colistin acts through the permeabilization of the bacterial outer membrane [26]. In this study, the synthesized molecules were more effective against *E. faecalis* in comparison with the tested antibiotics in terms of inhibition diameters (Table 1). Previous studies have reported that CDs exhibit low cytotoxicity in mammalian cells, maintaining more than 80% cell viability at concentrations up to $100 \mu\text{g/mL}$. In our study, the CDs showed significant inhibitory activity against *E. faecalis* with a minimal inhibitory concentration of $50 \mu\text{g/mL}$ regarding *in vitro* antibacterial assay, thereby necessitating further *in vivo* investigations to confirm their promising potential.

CDs are good biocompatible agents applied in the biomedical sector; they possess fascinating antibacterial properties against different bacteria. It has been reported that CDs from levofloxacin hydrochloride showed a minimum inhibitory concentration (MIC) of $64 \mu\text{g/mL}$ against *Pseudomonas aeruginosa* and *Escherichia coli*, in addition to a MIC of $128 \mu\text{g/mL}$ against *Staphylococcus aureus* and *Bacillus subtilis*. Another study reported that diamines reduced the number of *Bacillus subtilis* viable cells at a concentration of 0.2 mg/mL . Their positively charged end groups ($-\text{NH}_3^+$) strongly adhered to the negatively charged bacterial surface and exhibited a bactericidal effect [27]. The inhibitory

action of bacterial growth is displayed through adhesion to the cell membrane altering its structure and permeability, as well as the promotion of reactive oxygen species production, and perturbation of cell functions [28]. Oxidative damage is the most common mechanism; it causes morphological changes in the bacterial cell membrane, leakage of cytoplasmic constituents, and degradation of DNA [29].

Table 1. Antibacterial activity of molecules against four pathogenic strains.

Molecules	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Enterococcus faecalis</i>
N-ECD	-	-	-	11.5 ± 0.70 ^a
N-HCD	-	-	-	13 ± 0.0 ^b
Penicilline	R	R	R	R
Tetracycline	14 ± 0.0 ^a	10 ± 0.0 ^a	10 ± 0.0 ^a	10 ± 0.0 ^c
Colistine	18 ± 0.0 ^b	14 ± 0.0 ^b	16 ± 0.0 ^b	12 ± 0.0 ^a

Inhibition zone diameters are reported in millimeters (mm), R indicates resistant strains, columns labeled with different superscript letters are significantly different ($p < 0.05$).

Furthermore, Varghese and Balachandran reported that the effectiveness of CDs relies on their surface charge impacting the electrostatic attraction to the bacterial cell, as well as the type and sensitivity of the pathogenic strains [29].

3.2. In Silico Analysis of CD Interaction with Aldose Reductase Active Site

Here, we investigated the use of N-ECD and N-HCD, new nanomaterials candidates, for their possible interaction with aldose reductase. The antidiabetic effect of CDs was assessed in silico. The binding affinities of CDs towards aldose reductase, a key enzyme related to the polyol pathway, were evaluated and the results are presented in Table 2.

Table 2. Binding affinities and interactions of carbon dot major constituents and reference inhibitor with aldose reductase (AR).

Enzyme	Ligands	Binding Affinity (kcal/mol)
AR (PDB id: 1IEI)	N-ECD	−7.8
	N-HCD	−7.0

Docking analyses were used as a qualitative, hypothesis-generating approach to explore possible interactions between representative surface functional groups of carbon dots towards residues within the AR active site. The N-ECD fragment is accommodated within the enzyme's catalytic pocket. It showed predicted contacts with HIS¹¹⁰, TYR³⁰⁹, TRP¹¹¹, and LYS⁷⁷ through conventional hydrogen bonding, suggesting a favorable orientation relative to catalytically relevant residues (Figure 4A). Further, the presence of van der Waals interactions reinforces the stability in the pocket. These interactions suggest that surface functionalities present on N-ECD could engage catalytically relevant residues of enzyme.

Additionally, the N-HCD fragment is positioned within the active pocket and TRP¹¹¹, LYS⁷⁷, ASP⁴³, and GLN¹⁸³ helped form conventional hydrogen bonds, showing a favorable orientation (Figure 4B). Moreover, N-HCD shows a good binding profile with hydrogen bonds targeted at catalytic residues, suggesting possible binding. The presence of GLN¹⁸³ and ASP⁴³ in the interactions could also play a stabilizing role.

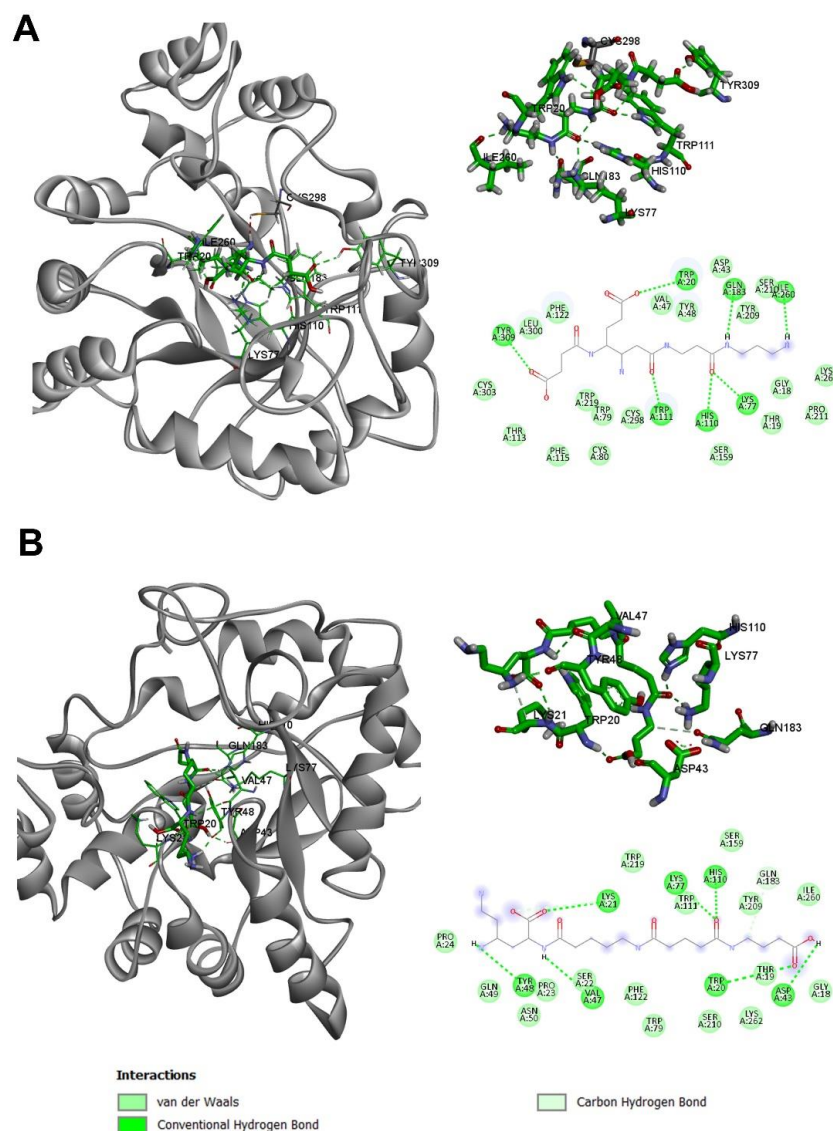


Figure 4. Incorporation of carbon dots fragments within the active sites of aldose reductase. (A) N-ECD and (B) N-HCD. The specific residues involved in each interaction are highlighted in the magnified views and 2D diagrams.

Both CD-derived fragments showed favorable interaction patterns with the AR active site. While N-ECD shows greater complementarity with several key catalytic residues compared to N-HCD, these observations should be interpreted qualitatively rather than as evidence of true binding strength or inhibitory potency. This is reflected in its binding affinity (-7.9 kcal/mol).

It is important to highlight that our docking simulations employed simplified molecular fragments rather than complete carbon dot nanoparticles. While this approach provides qualitative insight into potential functional group interactions with enzyme active sites, it cannot capture the full complexity of CD–enzyme interactions in biological systems. The reported binding affinities are indicative of interaction potential rather than quantitative predictions.

3.3. *In Vitro* Validation of CDs as Modulators of Polyol Pathway Enzyme Activity

Here, we have evaluated the effect of N-ECD and N-HCD on polyol pathway enzymes, which involved two enzymatic reactions. As expected, in STZ-treated animals, aldose reductase and sorbitol dehydrogenase in the kidney increased twofold compared to control

(Figure 5A,B). However, addition of N-ECD or N-HCD significantly reduced the activity of both enzymes in a dose-dependent manner. Functional groups such as COOH, OH, and NH, and aromatic portions are known to interact with proteins and can influence enzyme activity through various mechanisms. CDs may have the ability to interact with enzymes and modify their activity through different mechanisms. These interactions depend on the core composition and chemical surface groups [30]. They may have different types of enzyme inhibition such as competitive inhibition, non-competitive inhibition, or mixed inhibition (CDs can be bound to both the active site and an allosteric site), and inhibition by steric obstruction (CDs can block the substrate's access to the active site of the enzyme by attaching to it or by forming bulky complexes nearby). However, our experimental design does not distinguish between direct enzyme inhibition and indirect effects such as antioxidant activity or redox modulation. Cell-free enzyme assays with purified AR and SDH, along with kinetic studies, would be required to confirm direct inhibition mechanisms.

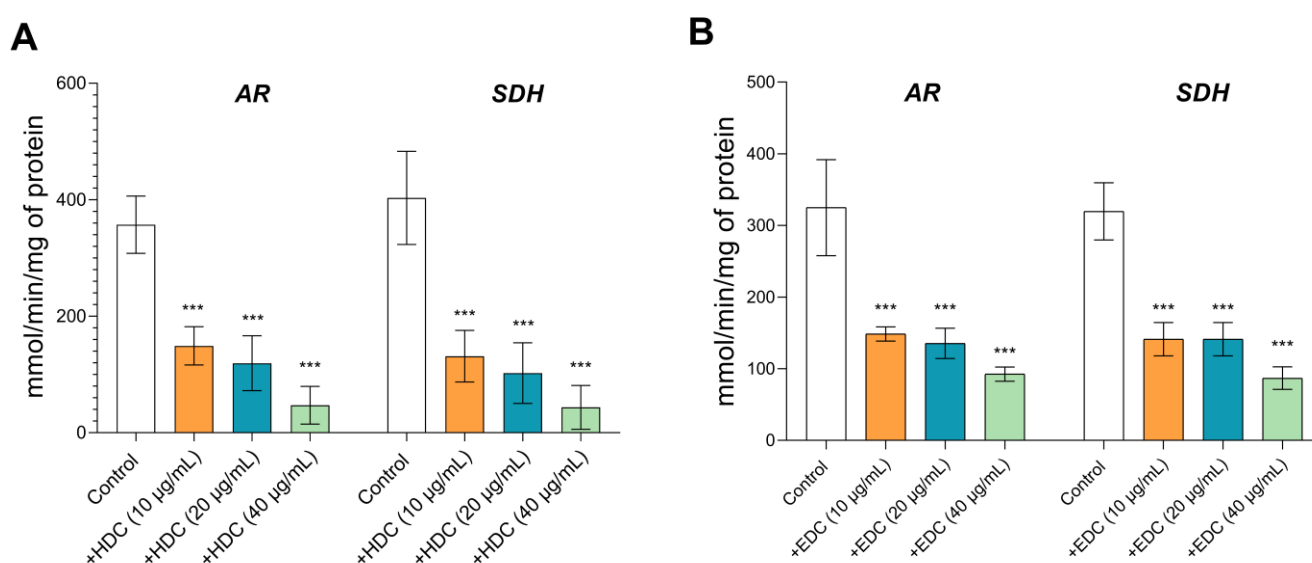


Figure 5. Dose-dependent effects of (A) N-HCD and (B) N-ECD on aldose reductase (AR) and sorbitol dehydrogenase (SDH) activities in kidney tissue from STZ-diabetic rats. Data are represented as mean $\pm$ SD ($n = 3$). *** $p < 0.05$ vs. control (one-way ANOVA with Dunnett's post hoc test). Enzyme activities are expressed as nmol/min/mg of protein. Data are presented as mean $\pm$ SD from three independent experiments ($n = 3$). Statistical significance was determined by one-way ANOVA followed by Dunnett's post hoc test comparing treated groups to control. *** $p < 0.05$ vs. untreated control. Both carbon dot formulations demonstrate concentration-dependent enzyme reduction.

CDs are good biocompatible agents; applied in biosensors, bioimaging, and drug delivery, they possess fascinating antioxidant properties [31]. It has been reported that metformin CDs showed a significant reduction in blood sugar levels compared to the group receiving only metformin. Moreover, histopathological examinations of the liver tissue indicate improved liver health in the group treated with metformin CDs compared to the group receiving metformin alone [32]. Furthermore, it is known that diabetic hyperglycemia is associated with the generation of free radicals, damaging nucleic acids, protein glycation, and oxidative degeneration leading to the development of other diseases [33]. Free radical-induced oxidative stress disbalances the protein turnover, thereby accumulating damaged proteins instead of their degradation. Thus, targeting the endogenous antioxidant system seems a promising therapeutic approach. Several studies reported that the CDs synthesized through a biogenic approach using coriander, garlic, tomato, various plants, and microorganisms have antioxidant properties [34].

N-ECD and N-HCD were associated with a significant, dose-dependent reduction in AR and SDH, along with significant growth inhibition of *E. faecalis* using disk diffusion assays. These preliminary findings suggest the potential for future therapeutic applications. However, several critical limitations must be addressed: (i) the mechanism of action (direct inhibition vs. indirect antioxidant effects) remains non-described, (ii) downstream metabolic consequences such as tissue sorbitol accumulation were not measured, and (iii) comprehensive toxicity and biocompatibility studies were not performed. Future research should include cell-free enzyme assays, kinetic analyses, the quantification of polyol pathway metabolites, the assessment of diabetic complication markers, in vivo antibacterial infection models, and systematic toxicity evaluation before clinical translation can be considered.

4. Conclusions

In this study, we synthesized N-ECD and N-HCD using simple, low-cost methods. Bioinformatic analysis identified AR and SDH, which are implicated in diabetic complications, as potential therapeutic targets in the polyol pathway. Both N-ECD and N-HCD demonstrated inhibitory activity against the tested strain *E. faecalis* and showed a dose-dependent reduction in AR and SDH enzyme activities in *ex vivo* kidney tissue from STZ-induced diabetic rats.

Although challenges remain in CD synthesis, including yield optimization, improvement of optical and electrochemical properties, and ensuring biocompatibility, our study contributes to the progressing research on carbon nanomaterials. The dual antibacterial and enzyme-modulating properties observed in vitro and ex vivo in this study suggest that CDs warrant further investigation as potential multifunctional agents. However, in vivo studies are essential to validate therapeutic efficacy, assess downstream metabolic effects like sorbitol accumulation and oxidative stress markers, and evaluate functional renal outcomes before clinical translation can be considered. Future research combining CDs with natural bioactive compounds may offer opportunities to develop novel nanoplateforms, provided that the fundamental questions of mechanism, efficacy, and safety are adequately addressed through rigorous preclinical studies.

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Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

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References

- Giri, B.; Dey, S.; Das, T.; Sarkar, M.; Banerjee, J.; Dash, S.K. Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: An update on glucose toxicity. *Biomed. Pharmacother.* **2018**, *107*, 306–328. [\[CrossRef\]](#)
- Sun, W.; Oates, P.J.; Coutcher, J.B.; Gerhardinger, C.; Lorenzi, M. A selective aldose reductase inhibitor of a new structural class prevents or reverses early retinal abnormalities in experimental diabetic retinopathy. *Diabetes* **2006**, *55*, 2757–2762. [\[CrossRef\]](#) [\[PubMed\]](#)
- Chbel, A.; Lafnune, A.; Nait Irahah, I.; Bourhim, N. Macromolecules from mushrooms, venoms, microorganisms, and plants for diabetes treatment—Progress or setback? *Biochimie* **2024**, *227*, 119–128. [\[CrossRef\]](#)
- Singh, M.; Kapoor, A.; Bhatnagar, A. Physiological and Pathological Roles of Aldose Reductase. *Metabolites* **2021**, *11*, 655. [\[CrossRef\]](#)
- Yan, L.J. Redox imbalance stress in diabetes mellitus: Role of the polyol pathway. *Anim. Models Exp. Med.* **2018**, *1*, 7–13. [\[CrossRef\]](#)
- Keogh, R.A.; Huyvaert, S.; Moore, G.D.; Horswill, A.R.; Doran, K.S. Virulence characteristics of Gram-positive bacteria isolated from diabetic foot ulcers. *FEMS Microbes* **2024**, *5*, xtae013. [\[CrossRef\]](#)
- Li, P.; Sun, L.; Xue, S.; Qu, D.; An, L.; Wang, X.; Sun, Z. Recent advances of carbon dots as new antimicrobial agents. *SmartMat* **2022**, *3*, 226–248. [\[CrossRef\]](#)
- Thoume, A.; Kellal, R.; Zertoubi, M.; Elmakssoudi, A.; Safi, Z.; Wazzan, N.; Al-Qurashi, O.S.; Left, D.B. Efficient enhancement of corrosion resistance of steel in HCl environment using N-doped succinic acid-based carbon quantum dots: A comprehensive quantum and electrochemical study. *J. Ind. Eng. Chem.* **2025**, *147*, 381–396. [\[CrossRef\]](#)
- Cui, M.; Ren, S.; Xue, Q.; Zhao, H.; Wang, L. Carbon dots as new eco-friendly and effective corrosion inhibitor. *J. Alloys Compd.* **2017**, *726*, 680–692. [\[CrossRef\]](#)
- Genc, R.; Alas, M.O.; Harputlu, E.; Repp, S.; Kremer, N.; Castellano, M.; Colak, S.G.; Ocakoglu, K.; Erdem, E. High-Capacitance Hybrid Supercapacitor Based on Multi-Colored Fluorescent Carbon-Dots. *Sci. Rep.* **2017**, *7*, 11222. [\[CrossRef\]](#)
- He, H.; Shuang, E.; Ai, L.; Wang, X.; Yao, J.; He, C.; Cheng, B. Exploiting machine learning for controlled synthesis of carbon dots-based corrosion inhibitors. *J. Clean. Prod.* **2023**, *419*, 138210. [\[CrossRef\]](#)
- Cen, H.; Chen, Z.; Guo, X. N, S co-doped carbon dots as effective corrosion inhibitor for carbon steel in CO₂-saturated 3.5% NaCl solution. *J. Taiwan Inst. Chem. Eng.* **2019**, *99*, 224–238. [\[CrossRef\]](#)
- Ye, Y.; Yang, D.; Chen, H. A green and effective corrosion inhibitor of functionalized carbon dots. *J. Mater. Sci. Technol.* **2019**, *35*, 2243–2253. [\[CrossRef\]](#)
- Long, W.-J.; Li, X.-Q.; Zheng, S.-Y.; He, C. A novel effective carbon dots-based inhibitor for carbon steel against chloride corrosion: From inhibition behavior to mechanism. *Carbon* **2024**, *218*, 118708. [\[CrossRef\]](#)
- Sattariazar, S.; Nejad Ebrahimi, S.; Arsalani, N. Enhancing the properties of electrospun polyvinyl Alcohol/Oxidized sodium alginate nanofibers with fluorescence carbon Dots: Preparation and characterization. *Int. J. Pharm.* **2023**, *644*, 123358. [\[CrossRef\]](#)
- Zeng, Y.; Kang, L.; Wu, Y.; Wan, S.; Liao, B.; Li, N.; Guo, X. Melamine modified carbon dots as high effective corrosion inhibitor for Q235 carbon steel in neutral 3.5 wt% NaCl solution. *J. Mol. Liq.* **2022**, *349*, 118108. [\[CrossRef\]](#)
- Chbel, A.; Abdelhakim, E.; Manuel, R.-M.; Barja, J.L.; Aniq, F.O.; Abdelaziz, S.; Khalfi, B.E. Comparative Study of Essential Oil Composition, Anti-bacterial And Antioxidant Activities of the Aerial Parts of *Thymus vulgaris* Grown in Morocco and France. *J. Essent. Oil Bear. Plants* **2022**, *25*, 380–392. [\[CrossRef\]](#)
- Thoume, A.; Nait Irahah, I.; Dahib, Z.; Chbel, A.; Loukhmi, Z.; Abdou-Allah, F.; Achagar, R.; Zertoubi, M.; Benmessaoud Left, D.; Bourhim, N.; et al. Bioactive assessment of hexahydroquinoline derivatives prepared via a biochar/Fe₃O₄@APTMS magnetic catalyst: Focus on antidiabetic and antibacterial properties. *RSC Med. Chem.* **2025**, *16*, 5702–5716. [\[CrossRef\]](#)
- Kadiri, F.; Zahid, B.; Thoume, A.; Irahah, I.N.; Bennis, F.; Chegani, F.; Blaghen, M. Characterization and valorization of a biosurfactant produced by *Aeromonas salmonicida* isolated from the marchika lagoon of Nador. *Biocatal. Agric. Biotechnol.* **2022**, *45*, 102523. [\[CrossRef\]](#)
- Thoume, A.; Irahah, I.N.; Benzbiria, N.; Left, D.B.; Achagar, R.; Elmakssoudi, A.; El foulani, A.A.; Dakir, M.; Azzi, M.; Bourhim, N.; et al. In vitro and in silico antibacterial and anti-corrosive properties of *Persea americana* leaves extract as an environmentally friendly corrosion inhibitor for carbon steel in a hydrochloric acid medium. *Colloids Surf. A Physicochem. Eng. Asp.* **2023**, *674*, 131848. [\[CrossRef\]](#)
- Ousaid, F.E.-Z.; Guenaou, I.; Nait Irahah, I.; Lahlou, F.A.; Errami, A.; Ait Si, L.; Abdou-Allah, F.; Ridaoui, K.; Zouheir, Y.; Hmimid, F.; et al. The chemical composition, in vitro, and in silico studies of *Lavandula mairei* essential oil. *Sci. Afr.* **2024**, *23*, e02062. [\[CrossRef\]](#)
- Irahah, I.N.; Guenaou, I.; Lahlou, F.A.; Hmimid, F.; Bourhim, N. Syzygium aromaticum bud (clove) essential oil is a novel and safe aldose reductase inhibitor: In silico, in vitro, and in vivo evidence. *Hormones* **2022**, *21*, 229–240. [\[CrossRef\]](#) [\[PubMed\]](#)

23. Chbel, A.; Rodriguez-Castro, J.; Quinteiro, J.; Rey-Méndez, M.; Delgado, A.S.; Soukri, A.; Khalfi, B.E. Isolation, Molecular Identification and Antibacterial Potential of Marine Bacteria from Deep Atlantic Ocean of Morocco. *Avicenna J. Med. Biotechnol.* **2022**, *14*, 206–215. [\[CrossRef\]](#)
24. Gauba, A.; Rahman, K.M. Evaluation of Antibiotic Resistance Mechanisms in Gram-Negative Bacteria. *Antibiotics* **2023**, *12*, 1590. [\[CrossRef\]](#)
25. Shettigar, K.; Bhat, D.V.; Satyamoorthy, K.; Murali, T.S. Severity of drug resistance and co-existence of *Enterococcus faecalis* in diabetic foot ulcer infections. *Folia Microbiol.* **2018**, *63*, 115–122. [\[CrossRef\]](#)
26. Andrade, F.F.; Silva, D.; Rodrigues, A.; Pina-Vaz, C. Colistin Update on Its Mechanism of Action and Resistance, Present and Future Challenges. *Microorganisms* **2020**, *8*, 1716. [\[CrossRef\]](#)
27. Abu Rabe, D.I.; Al Awak, M.M.; Yang, F.; Okonjo, P.A.; Dong, X.; Teisl, L.R.; Wang, P.; Tang, Y.; Pan, N.; Sun, Y.P.; et al. The dominant role of surface functionalization in carbon dots' photo-activated antibacterial activity. *Int. J. Nanomed.* **2019**, *14*, 2655–2665. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Liang, J.; Li, W.; Chen, J.; Huang, X.; Liu, Y.; Zhang, X.; Shu, W.; Lei, B.; Zhang, H. Antibacterial Activity and Synergetic Mechanism of Carbon Dots against Gram-Positive and -Negative Bacteria. *ACS Appl. Bio Mater.* **2021**, *4*, 6937–6945. [\[CrossRef\]](#)
29. Varghese, M.; Balachandran, M. Antibacterial efficiency of carbon dots against Gram-positive and Gram-negative bacteria: A review. *J. Environ. Chem. Eng.* **2021**, *9*, 106821. [\[CrossRef\]](#)
30. Barros, M.R.; da Silva, L.P.; Menezes, T.M.; Garcia, Y.S.; Neves, J.L. Efficient tyrosinase nano-inhibitor based on carbon dots behaving as a gathering of hydrophobic cores and key chemical group. *Colloids Surf. B Biointerfaces* **2021**, *207*, 112006. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Innocenzi, P.; Stagi, L. Carbon dots as oxidant-antioxidant nanomaterials, understanding the structure-properties relationship. A critical review. *Nano Today* **2023**, *50*, 101837. [\[CrossRef\]](#)
32. Camlik, G.; Bilakaya, B.; Küpeli Akkol, E.; Velaro, A.J.; Wasnik, S.; Muhar, A.M.; Degim, I.T.; Sobarzo-Sánchez, E. Oral Active Carbon Quantum Dots for Diabetes. *Pharmaceuticals* **2024**, *17*, 1395. [\[CrossRef\]](#)
33. Asmat, U.; Abad, K.; Ismail, K. Diabetes mellitus and oxidative stress—A concise review. *Saudi Pharm. J.* **2016**, *24*, 547–553. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Sahu, V.; Sahoo, S.K. Biogenic synthesis of carbon dots with inbuilt biological activity. *Next Nanotechnol.* **2024**, *5*, 100034. [\[CrossRef\]](#)

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