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Polydatin-Chitosan Nanoparticles Alleviate Diabetic Renal-Cardiac Complications via Oxidative Stress and Inflammation Modulation

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Abstract:Background: Renal and cardiovascular complications stand as the primary contributors to both mortality and morbidity among individuals with diabetes. Polydatin (Pd) has garnered attention for its potential roles as an anti-inflammatory, anti-oxidant, and protector of the kidneys and heart. Nanoparticles are being developed to aid in the early diagnosis of diabetes through imaging contrast agents. The aim of this research was to explore the defensive properties of Pd and Polydatin-Chitosan nanoparticles (Pd-NPs) in mitigating diabetic renal-cardiac complications, modulation of oxidative stress and inflammation plays a crucial role. The rats were segregated into four groups: normal, diabetic, diabetic + Pd (diabetic rats treated with 50 mg/kg b. wt of Pd), and diabetic + Pd-NPs (diabetic rats treated with Pd-NPs at a dose of 50 mg/kg b. wt). These treatments were administered orally on a daily basis for a duration of four weeks.

Results: Diabetic rats treated with Pd or Pd-NPs resulted in a noteworthy enhancement in renal markers such as creatinine and urea, as well as improvements in heart function indicators by reducing serum levels of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and troponin I activities when compared to the diabetic control group. The treatment agents not only inhibited the elevated levels of malonaldehyde (MDA) and nitric oxide (NO) but also increased the levels of glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities in the diabetic-treated rats. Additionally, both Pd and Pd-NPs reduced the concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-18 (IL-18), interleukin-23 (IL-23), atrial natriuretic peptide (ANP), tumor necrosis factor-alpha (TNF-α), and nuclear factor-kappa β (NF-kβ), while elevating the levels of interleukin-10 (IL-10) and interleukin-35 (IL-35).

Conclusions: The novel Pd-NPs formulation demonstrated greater efficacy than Pd in ameliorating diabetic cardiomyopathy and nephropathy by their anti-inflammation and anti-oxidant activities.

Introduction

Diabetes mellitus (DM) is a metabolic group of disorders characterized by chronic hyperglycemia (Quazi et al., 2022). It is possible for it to result in a wide variety of implications if it is not adequately treated or managed (Rahman et al., 2022). Heart disease and chronic renal disease are examples of some of the most prevalent acute complications of DM (Mitra et al., 2022). Hyperglycemia, resulting from deficiencies in insulin action, secretion, or both, is a hallmark of DM (Mohammed and Tajuddeen, 2022). Type1 diabetes is characterized by hyperglycemia resulting from the insufficient production of insulin by pancreatic β-cells. In contrast, type 2 diabetes is caused by poor insulin secretion due to insulin resistance or damaged β-cells (Tran et al., 2020). Diabetic cardiomyopathy refers to structural and functional changes in the heart induced by diabetes, typically recognized when there is cardiac dysfunction independent of coronary artery disease (Bugger and Bode, 2015). Cardiovascular complications contribute to poorer clinical outcomes in patients with diabetes, encompassing ischemic heart disease, hypertension, and heart failure (Jia et al., 2018 and Viigimaa et al., 2020).

Despite the availability of numerous synthetic anti-diabetic drugs, their use is limited due to unwanted side effects (Chaudhury et al., 2017). Therefore, herbal products have gained considerable demand for diabetes management owing to their affordability, widespread availability, and minimal side effects (Liyanagamage et al., 2020). Medicinal plants encompass a diverse array of botanical species utilized for various medicinal purposes (Jamshidi-Kia et al., 2018). In the past two decades, there has been a significant global increase in the importance of medicinal plants. According to the World Health Organization, medicinal plants are utilized by 80% of the world's population (Gopal et al., 2014).

Polydatin (Pd) known as resveratrol-3-O-β-mono-D-glucoside, is extracted from the perennial herb *Polygonum cuspidatum*as a naturally occurring compound (Du et al., 2013). Pd is a polyphenol readily obtainable from peanuts, grapes, and red wine, exhibits antiglycation, antioxidant, and anti-inflammatory effects (Kim et al., 2022). Pd has demonstrated diverse biological and pharmacological actions across various disease models (Du et al., 2013). Natural product-derived nanoformulations, for instance, may potentially be more effective and have fewer adverse effects compared to synthetic nanoformulations (Kashyap et al., 2021). Pd-NPs have demonstrated anti-diabetic and cardioprotective effects (Mostafa et al., 2021). Therefore, this study was conducted to explore the potential protective benefits of Pd and Pd-NPs in mitigating diabetic renal-cardiac complications through the regulation of oxidative stress and inflammation.

Materials and methods

2.1 Experimental animals

This study employed 40 male Wistar albino rats, weighing between 100 and 140 g. The animals were housed in plastic cages under a normal 12-hour light/dark cycle, with adequate aeration, at a temperature of 25°C, humidity of 55%, and standard atmospheric conditions. A two-week acclimatization period was implemented prior to the experiment to mitigate potential intercurrent infections. Throughout the study, the rats had ad libitum access to standard laboratory food and water.

2.2 Chemicals

Streptozotocin (STZ), nicotinamide (NA), and polydatin (Pd) were procured from Sigma Aldrich Co., MO, USA. Chitosan with a medium molecular weight (average molecular weight 200 kDa) was obtained from Techno Pharmchem Co., Delhi, India.

2.3 Preparation and Characterization of Pd-NPs

Detailed techniques for Pd-NPs preparation and characterization were discussed in our earlier paper by (Abdel-Moneim et al., 2020). These techniques include TEM, Zetasizers and zeta potential measurements, XRD, FTIR spectroscopy, and ultraviolet-visible spectrometery. The release of Pd from chitosan and EE were also examined in this work.

2.4 Experimental Design and Animal Grouping

The study comprised four groups, each consisting of ten rats. Group 1 served as the normal control and received no treatment. The remaining three groups underwent the following protocol: after an overnight fast, they received streptozotocin (STZ) injections (50 mg/kg body weight, dissolved in cold citrate buffer 0.1 M, pH 4.5) following intraperitoneal injection of nicotinamide (NA) (110 mg/kg body weight, prepared in normal saline) after 15 minutes [18]. Subsequently, an oral glucose load (10% in water) was administered after 4 hours to prevent hypoglycemia. Fasting blood glucose levels were monitored seven days post-STZ/NA injection using a glucometer (CERA-CHECKTM 1070, Korea), with blood drawn from the tail vein. Group 2 served as the untreated diabetic control and received 1% carboxymethylcellulose (CMC). Diabetic rats in Group 3 (Diabetic + Pd) were administered 50 mg/kg body weight of Pd dissolved in 1% CMC. Diabetic rats in Group 4 (Diabetic + Pd-NPs) received 50 mg/kg body weight of Pd-NPs dissolved in 1% CMC (Abdel-Moneim et al., 2020). All treatments were administered daily via gastric intubation for one month starting seven days post-STZ/NA administration **(Fig. 1).**

2.5 Sampling of blood and tissue

After completion of the experiment, the animals in each group were euthanized under mild diethyl ether anesthesia. Blood samples were collected from the jugular vein and placed in tubes with serum-separating gel. After centrifugation at 3000 rpm for 15 minutes at 30°C, serum was withdrawn into Eppendorf tubes and stored at -30°C until chemical analysis.

Biochemical analyses

Troponin I levels in serum were detected using the Elisa kit MBS727624 (Competitive ELISA) purchased from MyBioSource.com. The activity of CK-MB was assessed using reagent kits obtained from Spectrum Diagnostics (Egypt). BioSystem Company (Spain) provided reagent kits for detecting AST in serum via a kinetic method. LDH activity in serum was determined using a kinetic technique with reagent kits purchased from Human (Germany). Creatinine and urea levels were estimated using reagent kits acquired from Diamond Diagnostics (Egypt).

ANP, NF-κB, IL-10, TNF-α, IL-1β, and IL-6 were assessed using Sandwich ELISA kits sourced from R&D Systems (USA), following the manufacturer's instructions. IL-18 was evaluated with an ELISA kit from Boster Bio-technology Co., Ltd., China. Additionally, IL-23 and IL-35 levels were measured using ELISA Kits from RayBiotech Life, Inc., USA.

All protocols were conducted in strict accordance with the provided manufacturer's instructions.

2.6 Detection of oxidative stress and anti-oxidant status estimation

Activities of SOD, CAT, GSH, MDA, and NO were measured following the manufacturer's instructions of the kits purchased from Bio-diagnostic (Dokki, Giza, Egypt).

2.7 Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences (SPSS) version 20 for Windows, developed by SPSS Inc., Chicago, IL, USA. Statistical analyses included one-way ANOVA, followed by Duncan's post hoc test for comparison between groups. Results were reported as mean ± standard error (SE), with significance set at a P value of 0.05

Results and Discussion

3.1 Effect of Pd and Pd-NPs on cardiac biomarkers

Troponin I, CK-MB, AST, and LDH are cardiac biomarkers utilized for diagnosing myocardial dysfunction. Elevated levels of serum troponin I, CK-MB, AST, and LDH commonly indicate heart damage. In our study, we observed increased levels of troponin I and activities of CK-MB, AST, and LDH in the serum of diabetic rats compared to normal rats. Treatment with Pd or Pd-NPs significantly reduced troponin I levels and CK-MB, AST, and LDH activities in the treated rats **(Fig. 2).** Pd-NPs demonstrated greater effectiveness than free Pd in reducing cardiac biomarkers.

3.2 Effect of Pd and Pd-NPs on renal functions

The diabetic group showed significantly elevated serum creatinine and urea levels compared to the control group. Administration of Pd and Pd-NPs led to a marked decrease in these levels, nearly restoring them to normal levels observed in the control group **(Fig. 3).**

3.3 Effect of Pd and Pd-NPs on ANP and NF-κB

The diabetic rats exhibited elevated levels of ANP and NF-κB compared to the normal group. Treatment with Pd and Pd-NPs resulted in a significant decrease in the levels of ANP and NF-κB compared to the diabetic group, as shown in **(Fig. 4).**

3.4 Effects of Pd and Pd-NPs on the levels of pro- and anti –inflammatory cytokines

In **Fig. 5,** we depict the impact of both Pd and Pd-NPs on serum levels of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, IL-18, and IL-23, as well as anti-inflammatory cytokines IL-10 and IL-35. Pro-inflammatory cytokines showed a significant increase in diabetic rats, particularly TNF-α, IL-1β, IL-6, IL-18, and IL-23, compared to non-diabetic rats. Treatment with both Pd and Pd-NPs resulted in a notable decrease in these pro-inflammatory cytokine levels compared to the diabetic group. Additionally, diabetic rats exhibited reduced levels of the anti-inflammatory cytokines IL-10 and IL-35 compared to non-diabetic rats, which were restored following administration of Pd and Pd-NPs. Interestingly, the Pd-NPs group demonstrated a more pronounced anti-inflammatory effect compared to the free Pd group.

3.5 Effect of Pd and Pd-NPs on oxidative stress and anti-oxidant biomarkers:

In contrast to normal rats, diabetic rats exhibited a significant increase in MDA and NO levels, along with a decrease in GSH content and the activities of SOD and CAT. Administration of both Pd and Pd-NPs resulted in decreased production of MDA and NO, while increasing GSH content and the activities of SOD and CAT compared to the diabetic rat group. Consequently, Pd and Pd-NPs effectively mitigated oxidative stress and enhanced the expression of antioxidant markers in diabetic rats **(Fig. 6).**

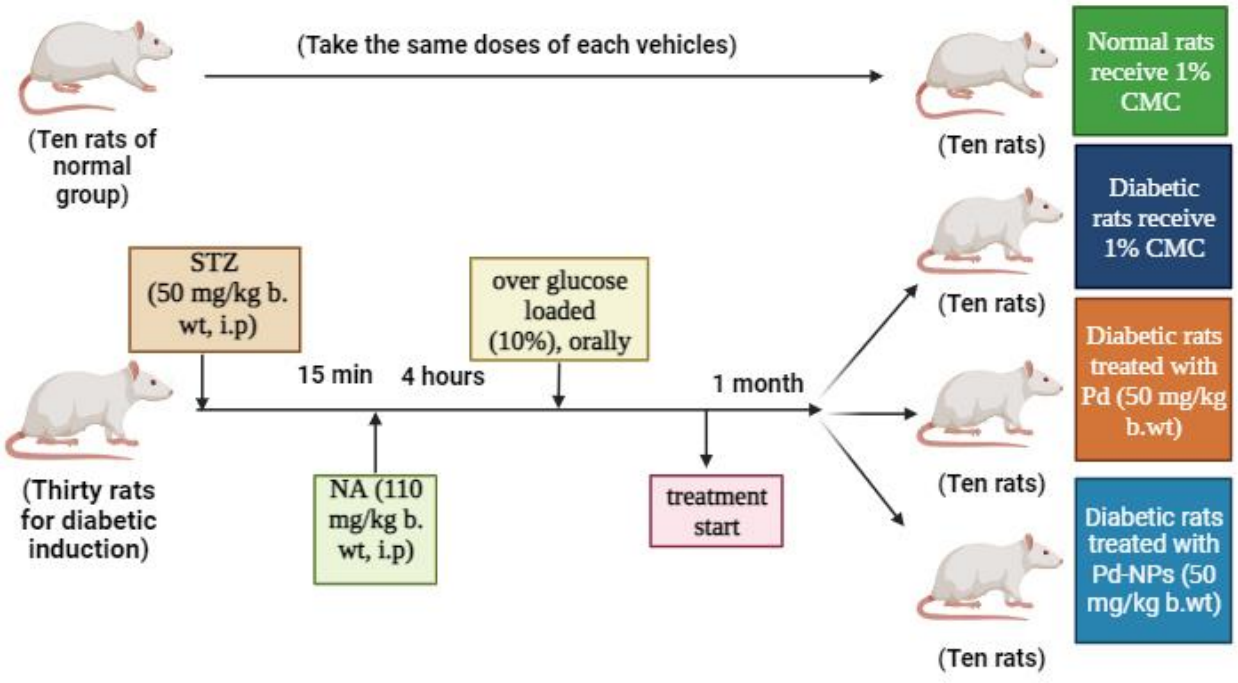

Figure 1. Schematic of the experimental design and animal grouping

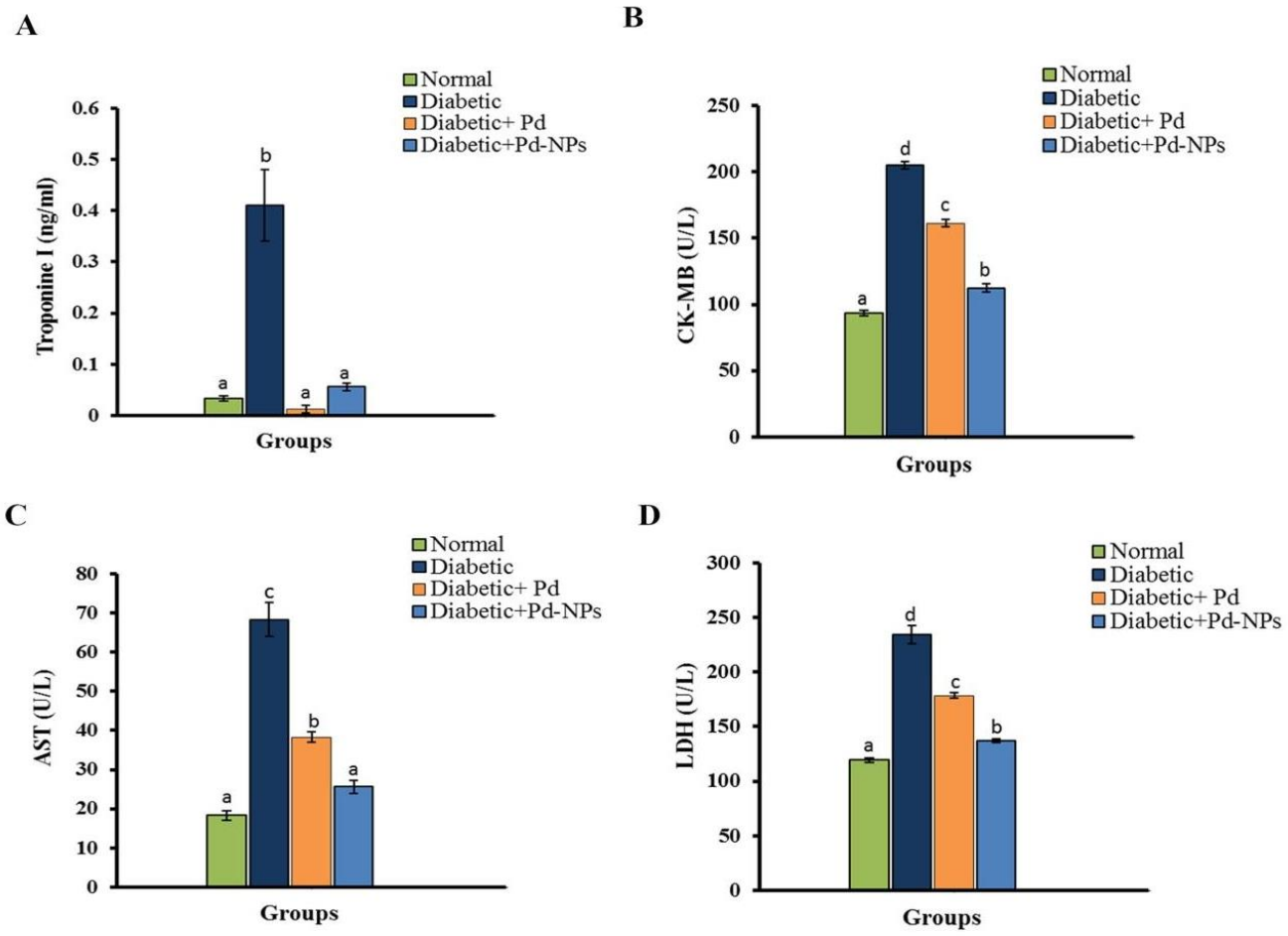

Figure 2. The impact of Pd or Pd-NPs on serum levels of (A) Troponine I, (B) CK-MB, (C) AST, and (D) LDH in diabetic rats is illustrated in Figure. Data are presented as means ± SE (n=6). Significantly different symbols indicate means with a p-value < 0.05.

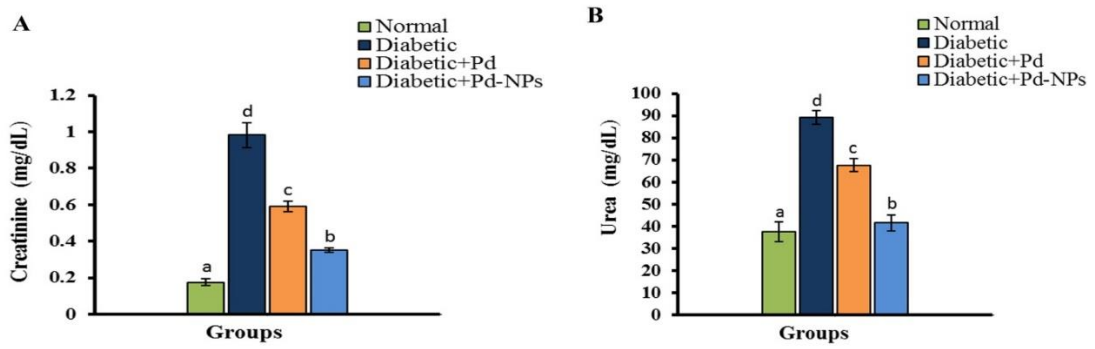

Figure 3. The impact of Pd or Pd-NPs on serum levels of (A) creatinine and (B) urea in diabetic rats is illustrated in Figure. Data are presented as means ± SE (n=6). Significantly different symbols indicate means with a p-value < 0.05.

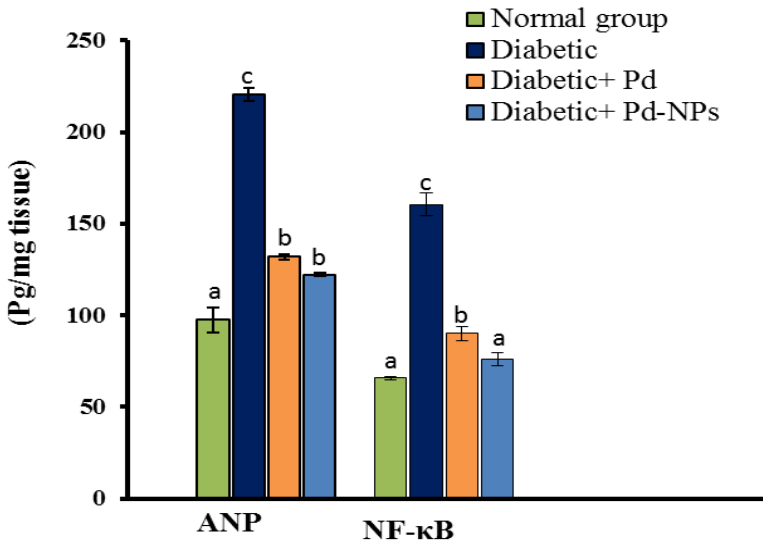

Figure 4. The impact of Pd or Pd-NPs on serum levels of of ANP and NF-κB in diabetic rats is illustrated in Figure. Data are presented as means ± SE (n=6). Significantly different symbols indicate means with a p-value < 0.05.

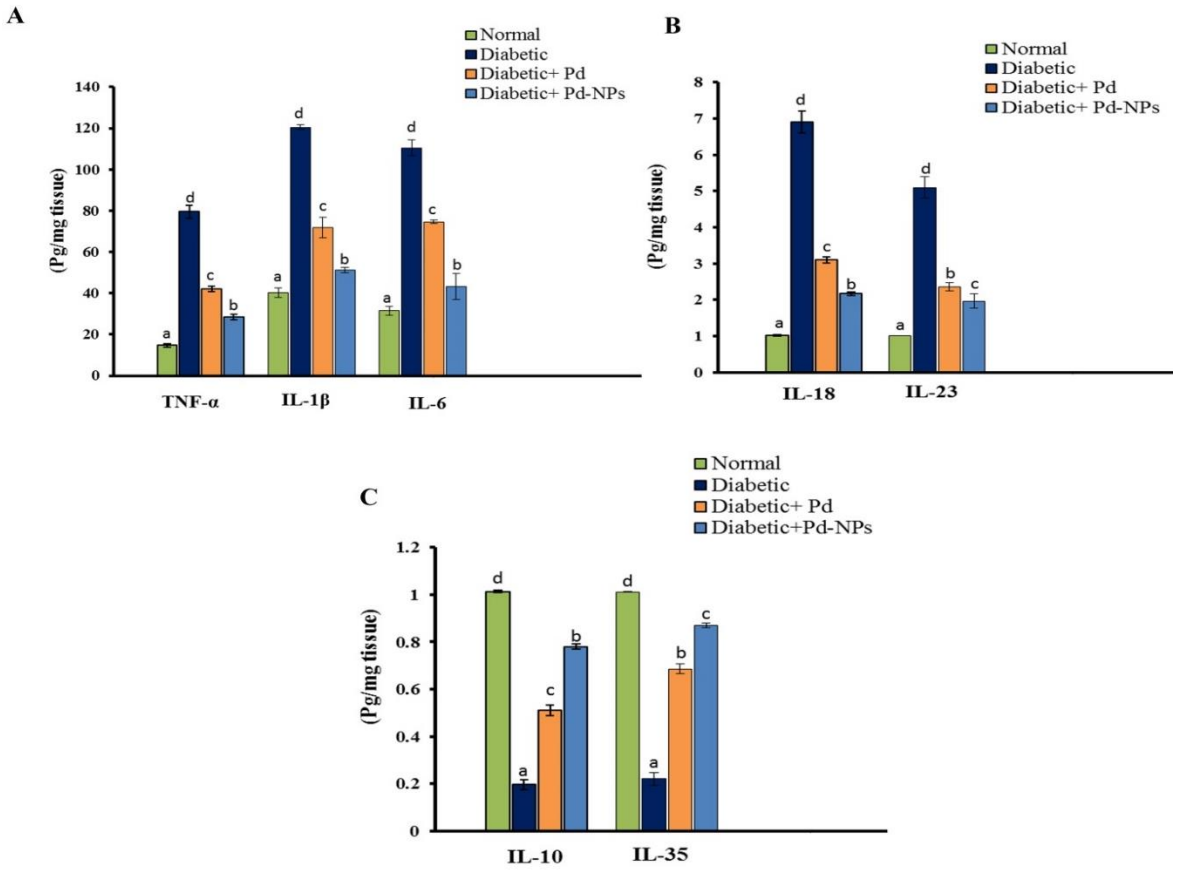

Figure 5. The impact of Pd or Pd-NPs on serum levels of (A) TNF-α, IL-1 β, IL-6, (B) IL-18, IL-23, and (C) IL-10 and IL-35 in diabetic rats. Data are presented as means ± SE (n=6). Significantly different symbols indicate means with a p-value < 0.05.

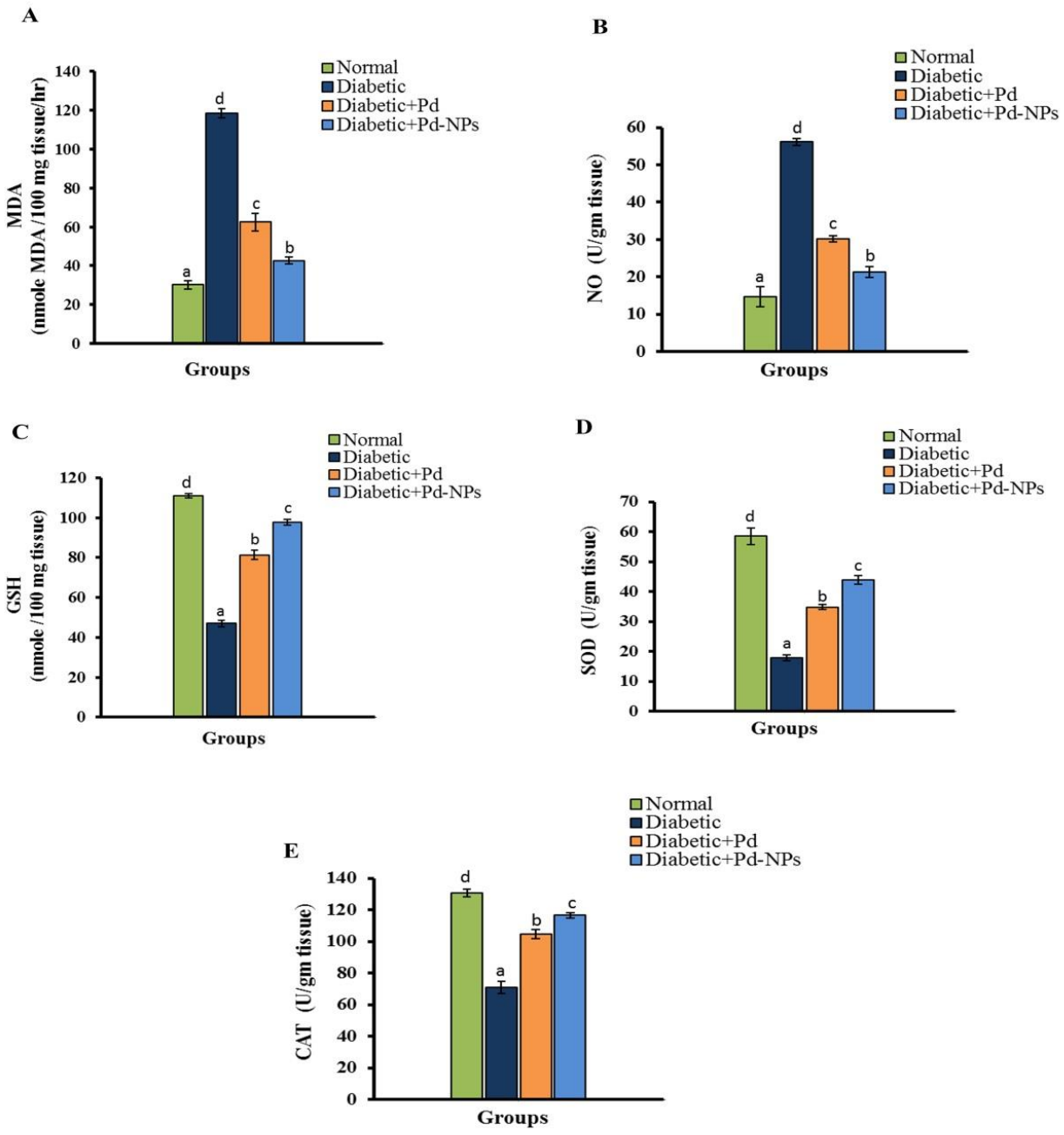

Figure 6. The impact of Pd or Pd-NPs on heart levels of (A) MDA (B) NO (C) GSH (D) SOD and (E) CAT in diabetic rats. Data are presented as means ± SE (n=6). Significantly different symbols indicate means with a p-value < 0.05.

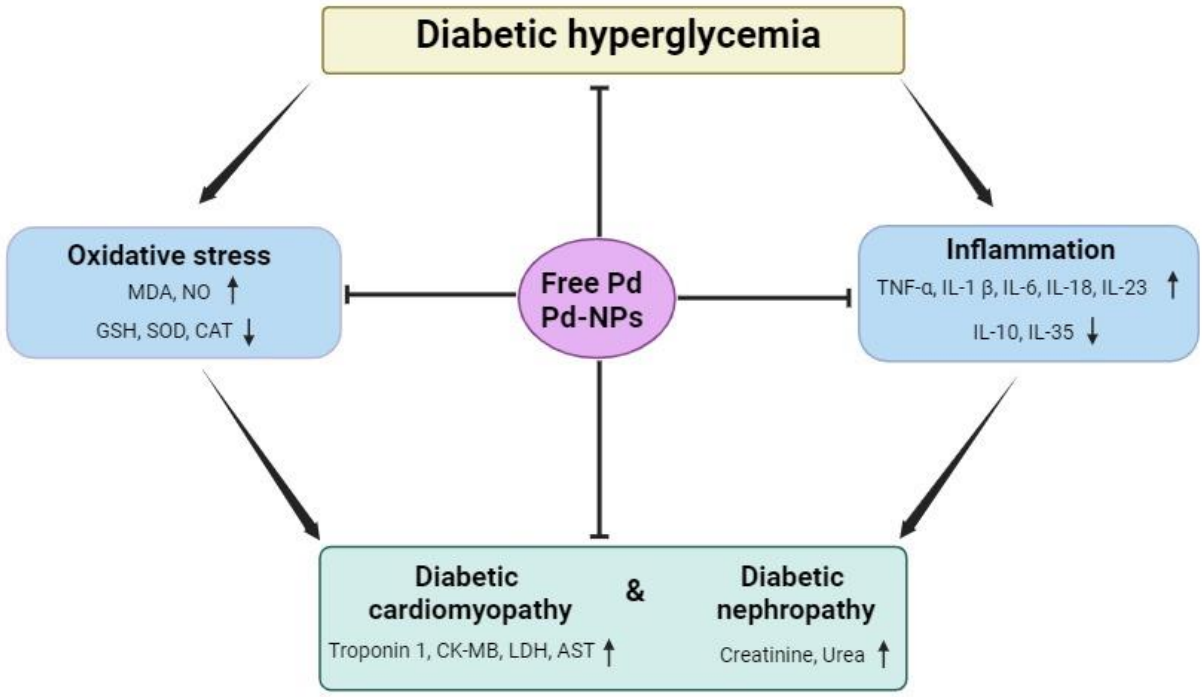

Figure 7. The pathways of free Pd and Pd-NPs in improving diabetic cardiomyopathy and nephropathy by reducing inflammation and oxidative stress.

Diabetes is a chronic metabolic disorder associated with significant complications, notably heart disease and kidney damage. It is a well-established risk factor for cardiovascular diseases due to elevated blood glucose levels that contribute to the development of atherosclerosis. Additionally, diabetes can lead to diabetic nephropathy, a kidney disease characterized by impaired kidney function and proteinuria, which may progress to end-stage renal disease (Ducluzeau et al., 2023 and Jyotsna et al., 2023).

In diabetes mellitus, oxidative stress and multiple inflammatory pathways are activated, contributing to the development of diabetic cardiomyopathy and nephropathy (Tan et al., 2020). This study aimed to explore the protective effects of Pd and Pd-NPs against diabetic renal-cardiac complications through the regulation of oxidative stress and inflammation.

The markers of cardiac myopathy, such as troponin I, CK-MB, AST, and LDH, significantly increased due to diabetes, consistent with findings reported in references (Mostafa et al., 2021 and Othman et al., 2017). Elevated levels of these biomarkers indicate deteriorative and necrotic changes in heart tissue (Mostafa et al., 2021 and Khanra et al., 2015), reflecting alterations in cardiomyocyte plasma membrane integrity and permeability (Koti et al., 2013). Treatment with Pd and Pd-NPs improved the impaired heart function biomarkers. These findings are consistent with previous studies (Yu et al., 2018), which have reported the therapeutic effects of Pd on cardiac function regulation (Dong et al., 2015). Pd-NPs may prolong drug circulation in vivo and enhance oral bioavailability (Wang et al., 2016).

Our findings revealed significantly elevated levels of urea and creatinine in the diabetic group, consistent with previous studies such as those by (Abouzed et al., 2018) and (Abd El-Hameed, 2020). Elevated urea levels in diabetes are associated with renal injury, attributed to muscle wasting and metabolic disturbances in uncontrolled diabetes, leading to increased purine release, a major precursor of urea formation (Abd El-Hameed, 2020). Several studies have demonstrated that both Pd-NPs and free Pd can decrease creatinine and

urea levels, suggesting potential amelioration of renal dysfunction. This effect may be attributed to reduced glucose levels, leading to decreased non-enzymatic glycation reactions (Abd El-Hameed, 2020).

It is well-established that oxidative stress plays a significant role in the pathogenesis of various diseases, including diabetic cardiomyopathy and nephropathy (Ducluzeau et al., 2023). Oxidative stress arises from an imbalance between reactive oxygen species (ROS) production and the antioxidant defense system within cells, leading to the accumulation of ROS and subsequent cellular damage (Volpe et al., 2018). Various abnormalities in antioxidant enzymes are linked to diabetes, and enhancing these enzymes is crucial for protecting cells against reactive oxygen species (ROS)-induced cardiac and renal damage (Giacco and Brownlee, 2010). In diabetic rats, levels of MDA and NO are significantly elevated, likely due to reduced enzymatic antioxidants (such as GSH, SOD, and CAT), which contribute to increased free radical production and lipid peroxidation (Mostafa et al., 2021 and Khanra et al., 2015).

Furthermore, oxidative stress can diminish the antioxidant capacity of cells by promoting the oxidation of antioxidant enzymes (Wilson et al., 2015). Oral administration of Pd and Pd-NPs decreased levels of MDA and NO products while increasing the activities of SOD and CAT, along with the content of GSH in diabetic rats. These findings indicate that Pd and Pd-NPs effectively mitigate oxidative stress in these rats by reducing superoxide accumulation (Yu et al., 2018). Pd's antioxidative actions have been linked to enhancing mitochondrial function, restoring balance to the intracellular nitro-oxidative system, and directly scavenging ROS (Dong et al., 2015). Pd-NPs have demonstrated anti-lipid peroxidation and antioxidant activities (Vijayalakshmi et al., 2019). These observations suggest that both Pd and Pd-NPs stabilize membranes against cardiac and renal injury by reducing oxidative stress, attributable to their antioxidant and anti-peroxidative properties.

Hyperglycemia-induced reactive oxygen species (ROS) is one of the causing oxidative stress and subsequent damage in the heart and kidneys of diabetic rats, leading to inflammation, fibrosis, and apoptosis (Zhou and Wu, 2017; Kaludercic and Di Lisa, 2020). The positive immunoreactivity of ANP and NF-κB observed in diabetic rats in this study suggests that hyperglycemia further amplifies oxidative stress and influences signaling pathways, aligning with previous research findings (Shabab et al., 2021 and Gao et al., 2022). Mitochondrial ROS generation and biochemical assessments were utilized to quantify oxidative damage, followed by examination of inflammatory and apoptotic markers such as ANP and NF-κB (Mittal et al., 2018). Multiple signaling pathways interact to produce these pathological features, with ROS potentially amplifying the NF-κB signaling pathway as an immunological maladaptive modulator that promotes fibrosis and remodeling in the cardiac and renal tissues (Newsholme et al., 2016 and Jia et al., 2018). Lipid accumulation and diastolic dysfunction can result from reduced fatty acid oxidation due to elevated ROS levels (Jia et al., 2016). Our findings indicate that both Pd and Pd-NPs reduce NF-κB and ANP levels, aligning with studies by Mostafa et al. (2021) and Ye et al. (2017). This effect is likely due to the antioxidant and anti-inflammatory properties exhibited by both Pd and Pd-NPs.

Numerous studies have established a direct link between diabetes-related oxidative stress, inflammation, and apoptosis. Hyperglycemia increases ROS levels, leading to the production of pro-inflammatory cytokines (Seddon et al., 2007). Cytokines play a central role in inflammation, regulating innate and adaptive immune responses, tissue injury, defense, remodeling, and repair processes (Medzhitov, 2010).

Our results indicated abnormally elevated levels of pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, IL-18, and IL-23, alongside reduced levels of anti-inflammatory cytokines such as IL-10 and IL-35 in diabetic rats. These findings align with previous studies reporting higher levels of TNF-α, IL-1β, IL-6, IL-18, and IL-23 in diabetic conditions by Moriwaki et al., (2003), Althunibat et al., (2019), and Fatima et al., (2016). Similarly, reduced levels of anti-inflammatory cytokines IL-10 and IL-35 parallel findings from studies by Elsherbiny et al., (2015) and Li et al., (2018).

TNF-α, IL-1β, and IL-6 are pro-inflammatory cytokines that directly contribute to the development of diabetes complications, as well as heart and renal diseases (Khanra et al., 2015 and Zhang et al., 2017). IL-18, another pro-inflammatory cytokine, is produced by various cell types, including those involved in

inflammation and cardiovascular functions (Mallat et al., 2004). Activation of IL-18 can lead to increased generation of free radicals and oxidative damage (Elmarakby and Sullivan, 2012). IL-23 plays a critical role in recruiting inflammatory cells in the pathogenesis of inflammatory diseases (Korn et al., 2009) and is centrally involved in the development of autoimmune inflammatory conditions (Sonderegger et al., 2006).

The current study investigates the role of pro-inflammatory cytokines in the development of diabetes (Fatima et al., 2016). IL-10, an anti-inflammatory cytokine, is crucial in mitigating acute heart and kidney diseases, and low IL-10 levels are associated with these conditions (Lebastchi et al., 2011). Additionally, IL-35 serves as a pivotal anti-inflammatory cytokine, playing a significant role in attenuating inflammatory diseases and autoimmune conditions (Bettini et al., 2012).

These inflammatory mediators play a significant role in diabetic heart and kidney disease, with previous studies establishing a connection between their levels and oxidative stress (Gupta et al., 2015 and Althunibat et al., 2019). Administration of Pd and Pd-NPs appears to provide protective effects against diabetic heart and renal diseases in rats. This protection is likely mediated through the down-regulation of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-18, and IL-23, as well as a decrease in anti-inflammatory cytokines like IL-10 and IL-35 in diabetic rats. Importantly, the inhibition of NF-κB by Pd contributes to the reduction in the expression of these pro-inflammatory cytokines. These findings align with conclusions drawn from previous studies (Ye et al., 2017).

Pd demonstrates both anti-inflammatory and lipid-lowering properties (Zhang et al., 2015). Additionally, Pd-NPs appear to be more effective in ameliorating diabetic cardiomyopathy and nephropathy compared to free Pd in diabetic rats. These findings are consistent with the results reported by Mostafa et al., (2021) and Abdel-Moneim et al., (2020).

5 Conclusions

In conclusion, the present study emphasizes the substantial potential of both Pd and Pd-NPs in alleviating the profound renal and cardiovascular complications associated with diabetes. Administration of Pd and Pd-NPs to diabetic subjects demonstrated evident protective effects, manifesting improvements in various indices of renal and cardiac function. Furthermore, Pd and Pd-NPs exhibited robust antioxidative activities and enhanced levels of critical antioxidants. Significantly, these compounds also demonstrated potent anti-inflammatory effects **(Fig. 7).** This dual antioxidative and anti-inflammatory action holds promise for managing diabetic cardiomyopathy and nephropathy. Particularly noteworthy was the superior efficacy of the novel Pd-NPs formulation compared to Pd alone. Overall, this study contributes valuable insights into the potential roles of Pd and Pd-NPs in safeguarding individuals with diabetes from renal and cardiovascular complications, highlighting their anti-inflammatory and antioxidative properties as promising avenues for further research and clinical applications.

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