Research Article

Jiayue Lin#, Yang Jiang#, Xu Zhou, Ting Zhang, Xu Yan*

# Preparation, characterization, and determination of the therapeutic effects of copper nanoparticles green-formulated by *Pistacia atlantica* in diabetes-induced cardiac dysfunction in rat

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**Abstract:** The development and creation of innovative therapeutic supplements and medications with extraordinary efficacy for addressing severe diabetes are of utmost importance to both developing and developed nations. A bio-inspired method has been documented for producing copper nanoparticles (CuNPs) using *Pistacia atlantica* leaf extract as a natural stabilizing agent. This approach is applicable, easy, and environmentally friendly, as it avoids using any toxic or harmful reagents. The CuNPs that were synthesized through biological processes underwent characterization using sophisticated physicochemical methods such as energy-dispersive X-ray spectroscopy, transmission electron microscopy, field emission-scanning electron microscopy, and Fourier-transformed infrared spectroscopy. It is confirmed that CuNPs exhibit a spherical structure, with an average size ranging from approximately 30 to 70 nm. Diabetes was induced *in vivo* through a fructose-enriched diet combined with streptozotocin. Half the subjects were administered CuNPs (100 µg/kg) via oral gavage. In contrast to the animals that were given regular food, the diabetic animals revealed an increase in serum fasting glucose level and a decrease in glucose tolerance. The administration of CuNPs had a significant impact on reducing glucose intolerance and fasting hyperglycemia. Additionally, it helped alleviate the negative effects of diabetes on cardiac output and work. Furthermore, utilizing CuNPs effectively hindered the rise in cardiac signal transducer and activator of transcription 3-phosphorylation caused by diabetes. The findings from this investigation provide evidence of the therapeutic benefits of CuNPs in mitigating diabetes-induced cardiac dysfunction in rats.

**Keywords:** cardiac dysfunction, diabetes, copper nanoparticles, *Pistacia atlantica* leaf, rat

## 1 Introduction

Nanotechnology offers contemporary solutions for enhancing the effectiveness and safety of disease treatment methods. Despite the clinical approval of various nanomedicines, the potential benefits of nanotechnology for patients are yet to be fully realized in clinical practice [1–6]. Nanoparticles (NPs) possess biological, optical, magnetic, electronic, thermal, and distinctive transport characteristics that remain imperceptible on a molecular or macroscopic level. Nevertheless, these attributes can be harnessed for therapeutic applications. This attribute arises because NPs are approximately the same size as the light wavelength and exhibit a substantial surface-to-volume ratio [2,3]. Because of the relatively large size of NPs compared to biological macromolecule drugs or traditional chemotherapeutic agents, they can be combined with various support components [3–5]. The dissolution elements aid in safeguarding imaging, targeting, degradation, and activation based on stimuli. NPs undergo distinct processing within the body compared to conventional medications. NPs display distinctive biodistribution patterns and hydrodynamic features. It should be emphasized that the interactions taking place at

# Jiayue Lin is the First author and Yang Jiang is the Co-first author.

* **Corresponding author: Xu Yan,** Department of Cardiovascular, Hunan Provincial Hospital of Integrated Traditional Chinese and Western Medicine, No. 58, Lushan Road, Changsha, Hunan, 410006, China, e-mail: 13755069374@163.com, Xu.Yan.13755069374@outlook.com
**Jiayue Lin:** Postgraduate School, Hunan University of Chinese Medicine, No. 300, Bachelor's Road, Yuelu District, Changsha, Hunan, 410208, China
**Yang Jiang**, **Xu Zhou:** Department of Spleen (Stomach and Liver Diseases), Hunan Provincial Hospital of Integrated Traditional Chinese and Western Medicine, No. 58, Lushan Road, Changsha, Hunan, 410006, China
**Ting Zhang:** Department of Cardiovascular, Hunan Provincial Hospital of Integrated Traditional Chinese and Western Medicine, No. 58, Lushan Road, Changsha, Hunan, 410006, China

the nano-bio level have the potential to greatly improve drug delivery. NPs have several applications, including the targeted delivery of drugs to cancerous tumor cells, extracting cancer agents from these cells, and raising the sensitivity of cancer cells for closer imaging and observation [4–8]. NPs show promising potential in the treatment, diagnosis, and prevention of many diseases, including diabetes. Advancements in this field could result in increased revenue and decreased costs. As such, it is crucial to prioritize the formulation of effective policies for the advancement of nanoparticle technology, particularly in the context of diabetes diagnosis and treatment [6–8]. NPs play a crucial role in imaging within the neuroscience field, tracking the behavior of adult stem cells in the nervous system, and treating various nervous system disorders. Lately, researchers have been concentrating on developing novel chemotherapeutic drugs through the utilization of nanoscale structures [7–10]. New studies indicate that biopsy-produced NPs have significant impacts on eradicating and halting cancer cell proliferation. However, there remains a gap in knowledge regarding the mechanisms of oxidative nanoparticle toxicity in human cells. The aforementioned characteristics result in elevated chemical reactivity and increased reactive oxygene species production. Prior investigations have demonstrated that oxidative NPs can elicit apoptotic reactions in pulmonary cells, trigger inflammation, disrupt calcium balance, and induce oxidative stress [1–6]. Furthermore, additional studies have highlighted the advantageous applications of metallic NPs in treating various forms of diabetes [5–10].

Diabetes is a prevalent disease and metabolic disorder that is prevalent worldwide, affecting individuals. It has an average occurrence rate of 0.08 among adults. Apart from severe hyperglycemia, this condition manifests with various symptoms including diabetic ketoacidosis, complications related to both large and small arteries, and, notably, liver disease [11–14]. Besides the assortment of chemical medications utilized for managing diabetes, herbs with minimal and more affordable side effects can serve as substitutes or supplements for chemical drugs. In recent years, a blend of modern and traditional sciences has aimed to develop novel drugs for diabetes control [12–15]. The utilization of nanotechnology in the nanomedicine production derived from medicinal plants has paved the way for advancements in traditional medicine. This innovative approach has caused the development of a highly potent compound that effectively regulates diabetes [16]. The exploration of NPs for diabetes control presents a novel challenge in medicine and pharmacy, as evidenced by recent research findings [17–19]. The toxicity repeated reporting of different NPs in both laboratory animals and human body has been well-documented [18,19]. The presence of NPs in the bloodstream can lead to the manifestation of toxic symptoms. Given the extensive utilization of NPs across various industries, individuals are inevitably exposed to these substances, whether voluntarily or involuntarily. The metal NPs' presence in the food chains and environment is expected to disrupt the well-being of humans and other organisms [17–19]. These particles have the potential to accumulate in different organs of the body, including the muscles, kidneys, liver, lungs, and heart. Upon entering the bloodstream, NPs come into contact with the immune system and the plasma proteins [14–19]. The substances can be absorbed through various pathways, including hemolysis and the complement system. This absorption can lead to several side effects, including an increase in the cells number involved in the immune system, a decrease in the antioxidant activity of cells, oxidative stress stimulation, anti-mitotic effects, and a decrease in blood cell count [13–18].

Copper is a commonly occurring transition metal that is frequently observed in metabolic processes. Copper nanoparticles (CuNPs) possess biocompatibility, indicating their reduced potential for harm compared to other metallic NPs commonly employed in medical environments [20–22]. Researchers have shown a growing interest in biologically produced metallic oxide NPs because of their wide range of therapeutic abilities [20,21]. Researchers have made a significant finding that these NPs exhibit a remarkable inhibitory impact on both α-glucosidase and α-amylase, which are acknowledged as crucial pharmacological targets in treating type 2 diabetes. Sone et al. [22] have reported that CuNPs possess antioxidant, anticancer, and antibacterial properties, which could be beneficial for various biological purposes. Additionally, several research groups have investigated the potential *in vitro* antidiabetic effects of CuNPs [22,23].

It is essential to exercise prudence when employing nanodrugs. On the other hand, the significant advantages of these medications in disease management should not be overlooked. Therefore, it is essential to conduct thorough research to determine the detrimental effects of these nanoparticles at the specified concentrations. As a result, this study encompassed the development and characterization of biopharmaceuticals, as well as the assessment of blood glucose levels in diabetic animals that received treatment with these nanoparticles. Furthermore, an analysis of cardiac tissue was performed to investigate the possible toxic effects of the substances, while concurrently observing the blood glucose levels in diabetic rats. The study thoroughly examined the easy production and analysis of CuNPs using a green method involving *Pistacia atlantica* leaves, as well as its potential therapeutic effects on diabetes-induced cardiac dysfunction in animals.

# 2 Experimental

## 2.1 Materials

The chemicals for the green formulation of CuNPs were acquired from Sigma Aldrich.

## 2.2 Preparation of aqueous extract

The *P. atlantica* leaf was collected and then underwent a drying procedure for 15 days at 25°C. Before the extraction procedure, the dried materials were converted into smaller fragments with dimensions of 1–2 mm. The percolation (soaking) method was employed to conduct the extraction. In this process, 100 g of plant powder were mixed with 800 mL of water. After 48 h at 25°C, the Whatman filter paper was used for filtering the samples. The extraction procedure was repeated four times, and the entire organic solvent was removed by a rotary evaporator. Afterward, the solvent was completely removed through a freeze-drying procedure. The extraction yield was found to be 34%. Previous research suggests that the best method for obtaining bioactive compounds from *P. atlantica* extract, to be utilized in the CuNPs production, is through the preparation of an aqueous extract [24,25].

## 2.3 Green synthesis of CuNPs

After filtering and chilling the aqueous *P. atlantica* leaf extract, 40 mL of the extract was added to 40 mL of $Cu(NO_3)_2{\cdot}3H_2O$ (0.25 M). The mixture was then refluxed for 16 h at 75°C. This process led to the creation of NPs (Cu@*P. atlantica*), which caused the formation of dark brown precipitates. The NPs were subsequently isolated and subjected to four rounds of washing with deionized water. This washing process involved centrifugation at 11,000 rpm for 8 min at specified time points [26,27]. Sophisticated physicochemical methods, including field emission-scanning electron microscopy (FE-SEM), energy-dispersive X-ray spectroscopy (EDX), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FT-IR) analysis, were utilized to examine and analyze the CuNPs synthesized using *P. atlantica* extract [26–29].

## 2.4 Cytotoxicity analysis

Cell viability was clarified through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. This technique relies on the function of succinate dehydrogenase enzyme within the viable cells mitochondria, causing the conversion of yellow MTT solution into purple formazan crystals. The formazan crystals are then dissolved in dimethyl sulfoxide (DMSO) and quantified using an ELISA reader. About $10^4$ human umbilical vein endothelial cells (HUVEC) were plated in a 96-well plate. Subsequently, the cells were treated with NPs, and the well volume was adjusted to 100 µL. The plates were then incubated for 24 and 48 h under the same conditions. Following the specified time, the 96-well plates were removed from the incubator. Subsequently, 10 µL of MTT solution (5 mg/mL) was introduced into each well and incubated for 3 h. Afterward, the MTT incubated wells had 100 µL of DMSO substituted and were left in a dark environment for 30 min. DMSO is used to dissolve the formazan crystals that are produced. Once dissolved in DMSO, these crystals create a purple-colored solution, demonstrating the cells' bioavailability being treated. The plate's optical absorption was then measured at 570 nm [26–28]

$$\text{Cell viability (\%)} = \frac{\text{Total cells} - \text{dead cells}}{\text{Total cells}} \times 100.$$

## 2.5 Antioxidant analysis

The study focused on examining the antioxidant properties of CuNPs in eliminating 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. It is commonly employed to determine the antioxidant capabilities of different substances. In its radical state within ethanol, DPPH exhibits maximum absorption at 517 nm. The antioxidant interacts with this radical, leading to its elimination. Hence, the absorption at 517 nm diminishes, resulting in a transition of the solution's color from purple to yellow. This alteration allows for the examination of the substance's antioxidant capability. In order to create the DPPH radical solution, 2 mg of DPPH was dissolved in 30 mL of ethanol. Various concentrations of NPs were then prepared and combined with the DPPH solution in equal amounts. The mixture was vigorously blended using a vortex for 10 s and stored in a dark environment at 25°C for 30 min. Following this, the mixture absorbance was measured at 517 nm. For this experiment, distilled water and butylated hydroxytoluene were utilized as the negative and positive controls, respectively, in comparison to the effectiveness of copper NPs [29,30]

$$\text{Inhibition ratio (\%)} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100.$$

The absorbance of the blank sample treated with no added extract is denoted as Absorbance 517 of control, while the absorbance in the presence of NPs is represented as Absorbance 517 of the sample.

### 2.6 Diabetes-induced cardiac dysfunction design

The research experiment involved 80 male rats weighing 220 ± 5 g. Initially, diabetes was induced in 75 rats by administering a single dose of streptozotocin at 60 mg/kg. A blood glucose level exceeding 250 mg/dL was used as the criterion for diabetes diagnosis. The rats were subsequently divided into four subgroups. These subgroups consisted of a negative healthy control group that received distilled water, a positive control group that received glibenclamide (20 mg/kg), an untreated negative control group that also received distilled water, and a group that received CuNPs at a concentration of 100 µg/kg. Following a 20-day treatment period, blood samples were extracted from the heart to analyze biochemical parameters.

In the course of the study, the evaluation of function and cardiac performance in isolated Neely working hearts of rats was conducted, as previously explained [31,32]. Heart rate, coronary flow, aortic flow, cardiac output, left ventricular developed pressure (LVDP), and left ventricular end-diastolic pressure (LVEDP) were assessed as cardiac performance indicators. The cardiac work determination involved the multiplication of maximum pressure and cardiac output. The Western blot technique was utilized to identify the phosphorylation of crucial proteins in established cardioprotective signaling pathways, such as extracellular signal-regulated kinase (Erk), protein kinase B (Akt), and signal transducer and activator of transcription 3 (STAT3). Additionally, the apoptosis-related Bcl-XL and Bax proteins activation, as well as the GLUT4 glucose transporter detection, were also observed, following the previously described methodology [33].

### 2.7 Qualitative measurement

The data collected underwent analysis using SPSS-22, including variance one-way analysis and the Duncan *post hoc* test.

## 3 Results and discussion

A method inspired by nature has been recorded for synthesizing CuNPs by utilizing *P. atlantica* leaf extract as a natural stabilizer. This technique is practical, straightforward, and eco-friendly, as it eliminates the need for any hazardous or detrimental chemicals. Different methods, including biological, chemical, and physical techniques, have been devised to produce CuNPs with controlled stability, shape, and size [29–32]. Biological synthesis outperforms physical and chemical methods regarding environmental sustainability, cost, and scalability for large-scale synthesis [29–31]. The biological approach is environmentally friendly, cost-effective, and secure and utilizes advantageous microorganisms, including fungal, bacterial, and plant cell cultures. Plants are the preferred choice over other biological sources for nanoparticle synthesis. This is because they offer a straightforward and efficient procedure that eliminates the need for time-consuming cell culture maintenance and a non-aseptic environment [32–34]. Plants are readily accessible, simple to manage, a plentiful source of various metabolites, and abundant in pharmacological components that serve as reducing and capping agents during NPs production [31–34].

### 3.1 Characterization of the prepared CuNPs

CuNPs morphology, synthesized through biosynthesis, was analyzed through TEM and FE-SEM techniques. Different polymorphic structures of CuNPs were identified, indicating evidence of aggregation. The observed clustering can be associated with the desiccation process performed during the sample preparation for FE-SEM and TEM analysis, as depicted in Figure 1a and b. Additionally, the mean particle size was calculated through the utilization of ImageJ software, yielding a value of approximately 42.86 nm. Gu et al. [35] synthesized the CuNPs with *Calendula officinalis*, revealing a particle size below 100 nm. Liyuan et al. [36] found that the size of CuNPs formulated with *Alhagi maurorum* extract ranged from 10 to 60 nm. Meanwhile, Chinnaiah et al. [37] synthesized CuNPs using *Datura metel* L. instead of CuO and determined that the average crystallite size of the CuNPs was around 19.56 nm. In their study, Kumar et al. [38] utilized leaves from the Andean sacha inchi plant to produce finely dispersed semicrystalline CuNPs via a heating method. However, the size of the CuNPs was found to be around 46 nm. Conversely, Murthy et al. [39] successfully synthesized CuONPs using an extract derived from *Vernonia amygdalina* Del. The monoclinic structure was observed in the synthesized NPs, which had a particle size measuring 19.7 nm. However, synthesizing mixed $Cu_2O$/CuONPs has not been extensively investigated until now. CuNPs were formulated in a small size. It is likely that the reduced size of CuNPs greatly enhances their therapeutic effects. Gu et al. [35] and Liyuan et al. [36] have validated the remarkable therapeutic efficacies of CuNPs with small sizes, specifically ranging from 10 to 50 nm.

The EDX spectrum in Figure 1c displays the CuNPs stabilized by *P. atlantica*, showcasing quantitative results

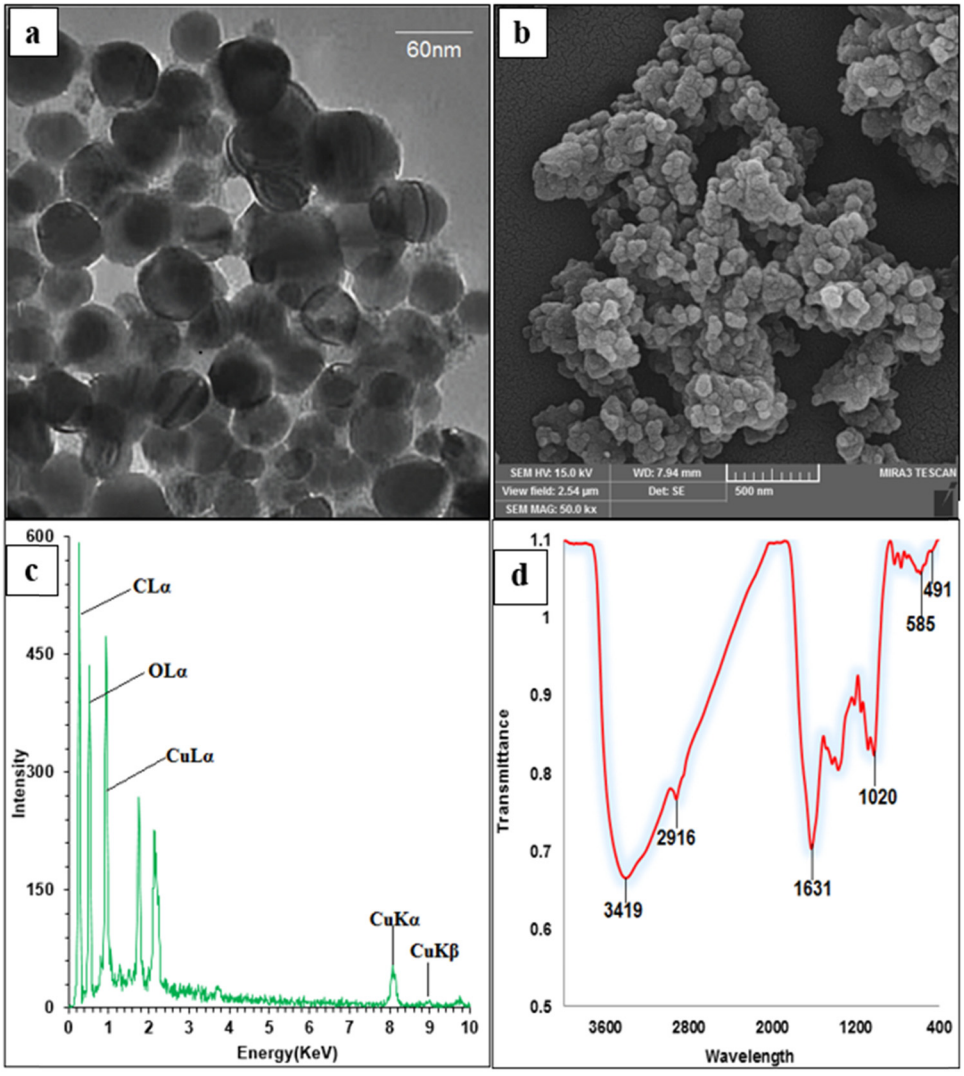


**Figure 1:** Chemical characterization of CuNPs green-mediated by *P. atlantica* leaf extract. (a) TEM image. (b) FE-SEM image. (c) EDX analysis. (d) FT-IR analysis.

and elemental mapping. The signals corresponding to CLα, OLα, CuLα, CuKα, and CuKβ are observed at energy levels of 0.2, 0.5, below 1, around 8, and below 9 keV, respectively. These data are consistent with previous research performed by Gu et al. [35] and Liyuan et al. [36].

An FTIR spectroscopy analysis was conducted to evaluate the active and reducing groups of Cu ions within 400–4,000 $cm^{-1}$ (Figure 1d). The spectrogram illustrates the effectiveness of *P. atlantica* in reducing copper ions. Peaks corresponding to vibrations at 3,419, 2,916, 1,631, 1,020, 585, and 491 wavelengths were observed, indicating the presence of hydroxyl (OH), aliphatic C–H, C═O, carbonyl (CO), alkene, and alkyl groups, respectively.

The copper nitrate salt was combined with plant extract, resulting in a mixture that was incubated for a duration of 180 min. Throughout this period, a noticeable alteration in color was detected within the reaction mixture. The solution's original yellow-orange hue transformed into a deep brown color, confirming the occurrence of the photosynthesis process in the production of CuNPs. The change in color occurred as a direct consequence of the NPs' SPR activity, as indicated by Gu et al. [35] and Liyuan et al. [36]. The intensity of color is determined by the quantity of liberated electrons when converting $NO_3$ to $NO_2$, leading to the reduction of Cu++ ions to metallic ions [36]. The visual change in the color of the solution was confirmed by the findings from Chinnaiah et al. [37], providing support for our research. We conducted an analysis of the UV–Vis spectrum of both the CuNPs and plant extract in order to delve deeper into the color change observed in the reaction solution. An absorption peak at

270 nm was detected in the CuNPs, signifying the stimulation of unbound electrons in the metal as NPs formed, a phenomenon referred to as surface plasmon resonance (Figure 2). A lack of an absorption peak was observed in the plant extract within this particular range; nevertheless, usually, these spectra offer crucial insights into the characteristics and synthesis of colloidal CuNPs. The presence of an absorption peak between 250 and 300 nm [36,38] is a notable feature of CuNPs.

## 3.2 Cytotoxicity and antioxidant data analysis of the prepared CuNPs

During our study, we examined the CuNPs impact on treated cell lines using the widely recognized MTT test for 72 h to assess cytotoxicity levels on HUVEC (Figure 3). The results indicated that copper NPs did not exhibit any toxicity toward HUVEC cells.

Throughout history, plants have consistently held a significant role as vital sources of food and medicine in human existence. Over the past century, herbal medicine, a subset of traditional medicine, has been approved to be instrumental in treating various ailments. Various medications derived from natural sources are commonly utilized worldwide to treat several illnesses, including fungal, viral, and bacterial infections, as well as various metabolic disorders and cancer [40,41]. Given the drawbacks and adverse reactions associated with chemical preservatives, it is possible to substitute them with natural compounds for preserving different types of food. Nevertheless, despite the widespread use and proven efficacy of natural compounds, there remains a preference for pharmaceuticals and chemical compounds in certain cases. It is worth noting

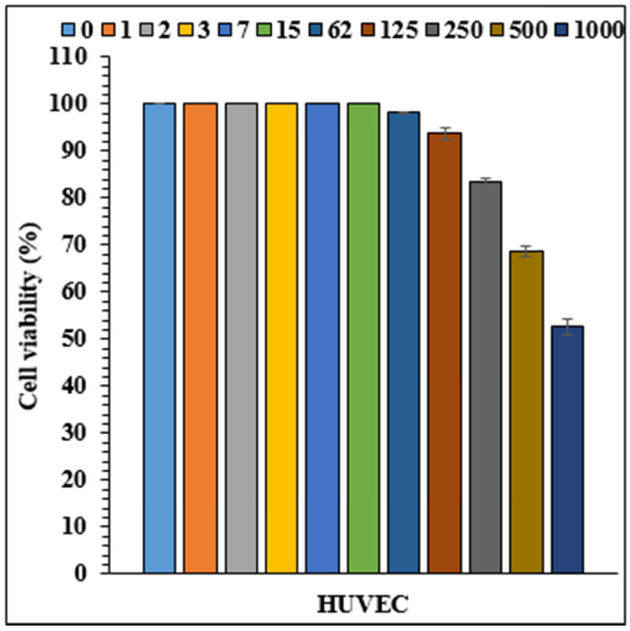


**Figure 3:** The cytotoxicity activities of CuNPs against HUVEC (normal) cells.

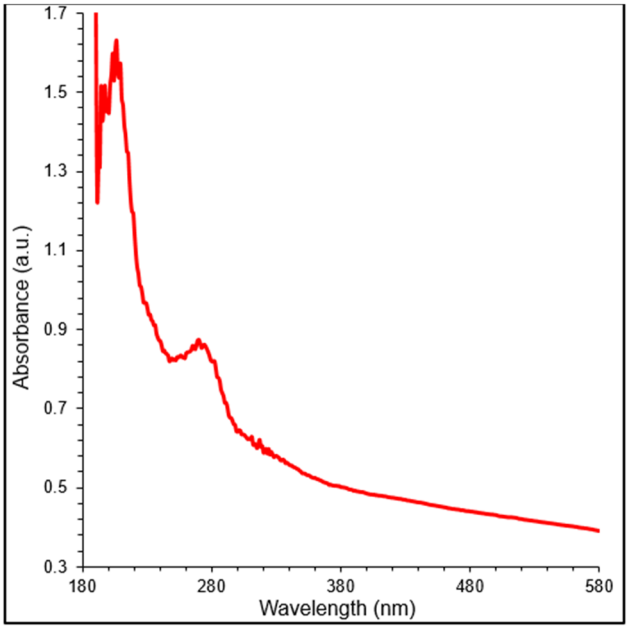


**Figure 2:** UV–Vis analysis of CuNPs green-mediated by *P. atlantica* leaf extract.

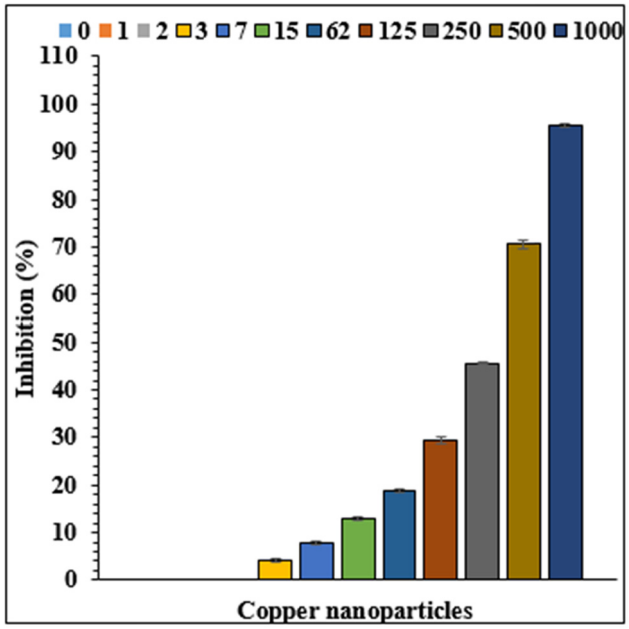


**Figure 4:** The antioxidant properties of CuNPs against DPPH.

that no publicly announced alternative has been identified thus far that can surpass their effectiveness [40,42]. There exist natural compounds that have not yet been thoroughly researched or have received limited attention. As a result, ongoing studies by researchers are focused on exploring the functional properties of these natural compounds and metabolites [42–45]. Genuine antioxidants not only enhance the effectiveness of plasma antioxidants but also lower the risk of cardiovascular disease and stroke, while also preventing the development of cancer that results from DNA damage. Extensive evidence exists to demonstrate the harmful effects of artificial antioxidants like *tert*-beta-hydroxyquinone and butyl hydroxy anisole when added to food products [40–45]. Therefore, the demand for natural antioxidants that are more potent and less harmful is an unavoidable requirement. Typically, consuming natural antioxidants leads to improved therapy and reduced adverse reactions [40–44]. Bio-NPs containing antioxidant compounds can alleviate oxidative stress caused by free radicals [40–42]. Inflammation is a beneficial response that occurs when the body is damaged by chemical or mechanical factors. Various inflammatory conditions exhibit elevated free radical levels, hyperactive phagocytes, protein denaturation, alterations in membrane permeability, and altered membrane functions. Therefore, using antioxidants and anti-inflammatory agents is crucial in combating oxidative and inflammatory stress [42–46].

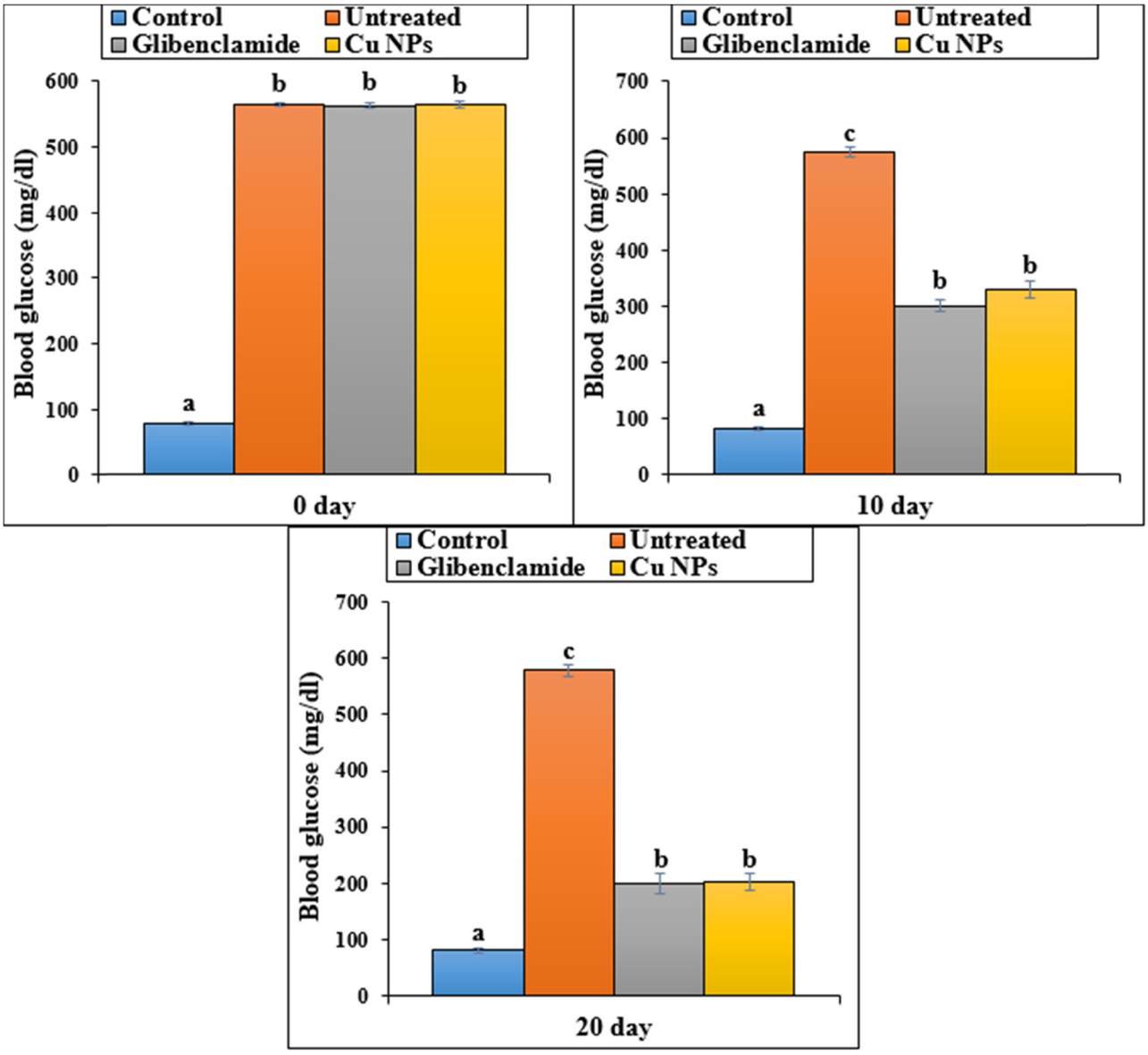


**Figure 5:** The effect of CuNPs on blood glucose levels on several days.

In the latest research, Figure 4 illustrates the scavenging capacity of copper NPs at various concentrations, presented as percentage inhibition. The $IC_{50}$ value for copper NPs in the antioxidant test on DPPH was 294 µg/mL.

## 3.3 Antidiabetic potential of CuNPs

Diabetes is a prevalent condition that impacts a significant number of individuals, leading to various concerns. This disease can affect multiple areas of the body, including the arteries, heart, and kidneys. It is important to acknowledge that diabetes undeniably contributes to the narrowing of arteries, which in turn can pose a potential risk to the heart [47–49]. If diabetes reaches an advanced stage, it can impact the blockage and clogging of arteries in the heart due to its numerous arteries. Consequently, individuals with diabetes are prone to developing various heart diseases [48–52]. Among these heart diseases, coronary artery disease is particularly prevalent in diabetic patients as it hampers the blood flow to the heart [49–52]. Plaque accumulation within the blood vessel walls and the arteries supplying the heart is responsible for the progression of this ailment, resulting in the stiffening of these blood vessels [48–51]. Consequently, this arterial rigidity can

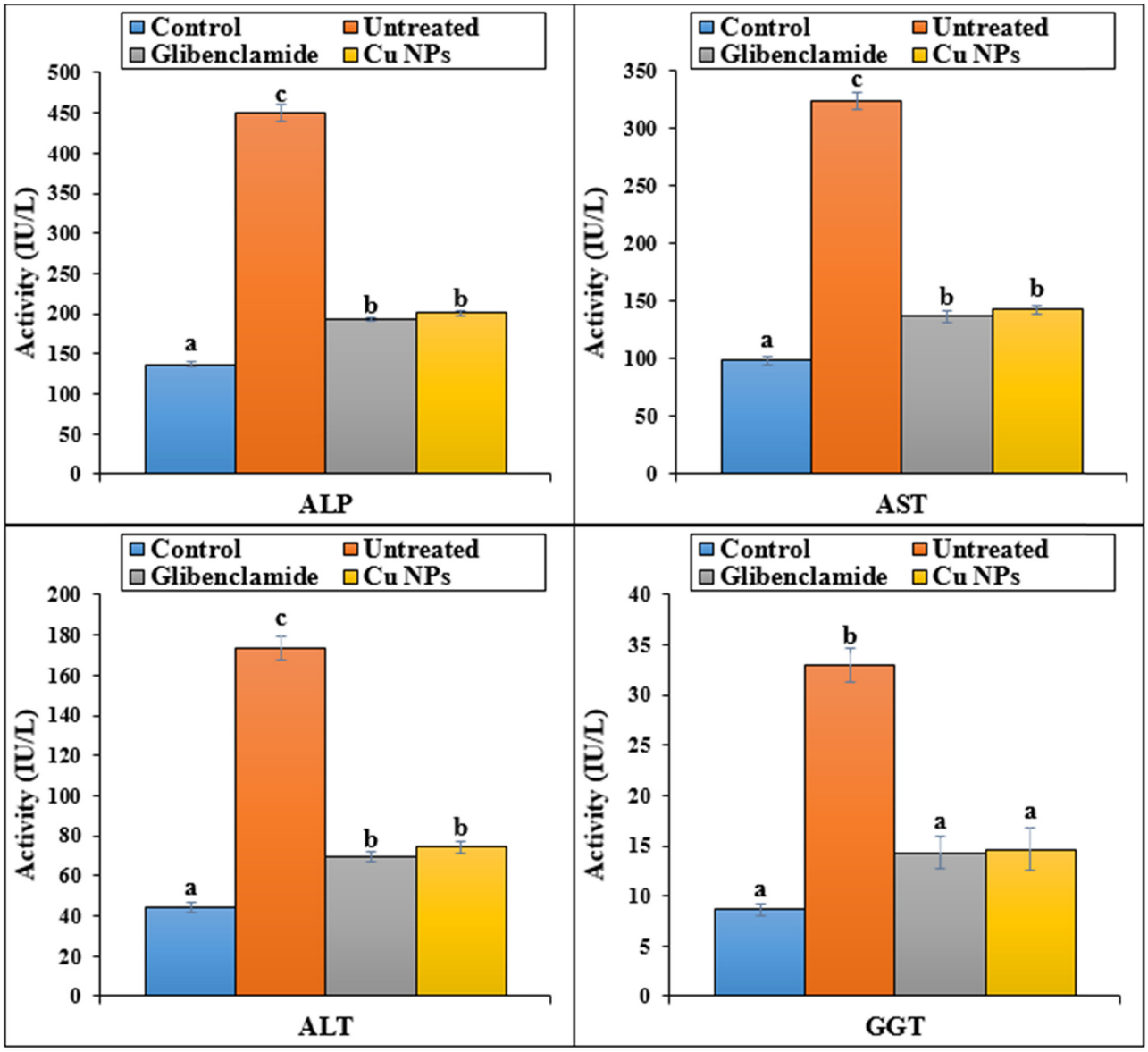


**Figure 6:** The effect of CuNPs on biochemical parameters.

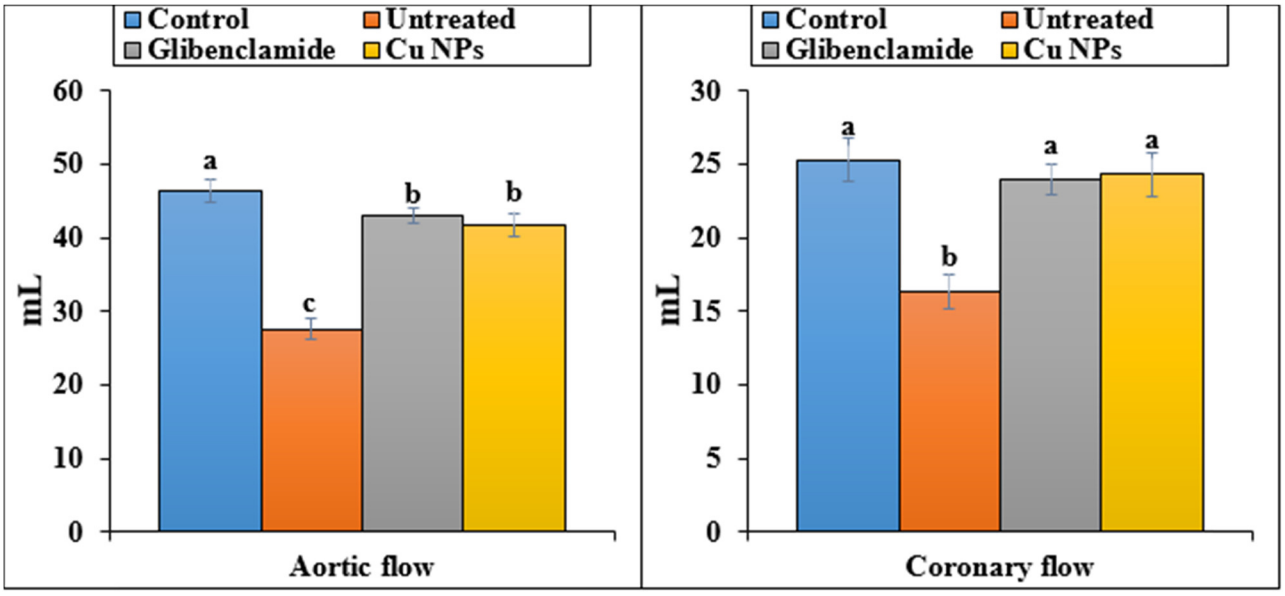


**Figure 7:** The effect of CuNPs on coronary flow and aortic flow levels.

potentially trigger heart attacks or strokes in affected individuals. Peripheral arterial disease, also known as PAD [47–49], can manifest as the hardening of blood vessels in various regions of the body. This condition serves as an initial indication of heart-related issues in individuals with diabetes. Diabetic patients often encounter numerous challenges associated with elevated blood glucose levels, all of which contribute to cardiovascular disease development [50–55]. High blood pressure can lead to heart disease as it increases the strain on the heart. When combined with diabetes, the developing risk of these conditions is significantly higher. Individuals with diabetes often have poor blood cholesterol levels, which can in turn lead to issues with the heart's arteries [48–54].

Figure 5 illustrates the demonstrated impact of the CuNPs on the fasting blood glucose in diabetic rats. The untreated diabetic rat's blood glucose levels raised by approximately 660% ($p \leq 0.05$) compared to the untreated diabetic animals. Throughout the study, there was no significant alteration observed in the normal control rat's

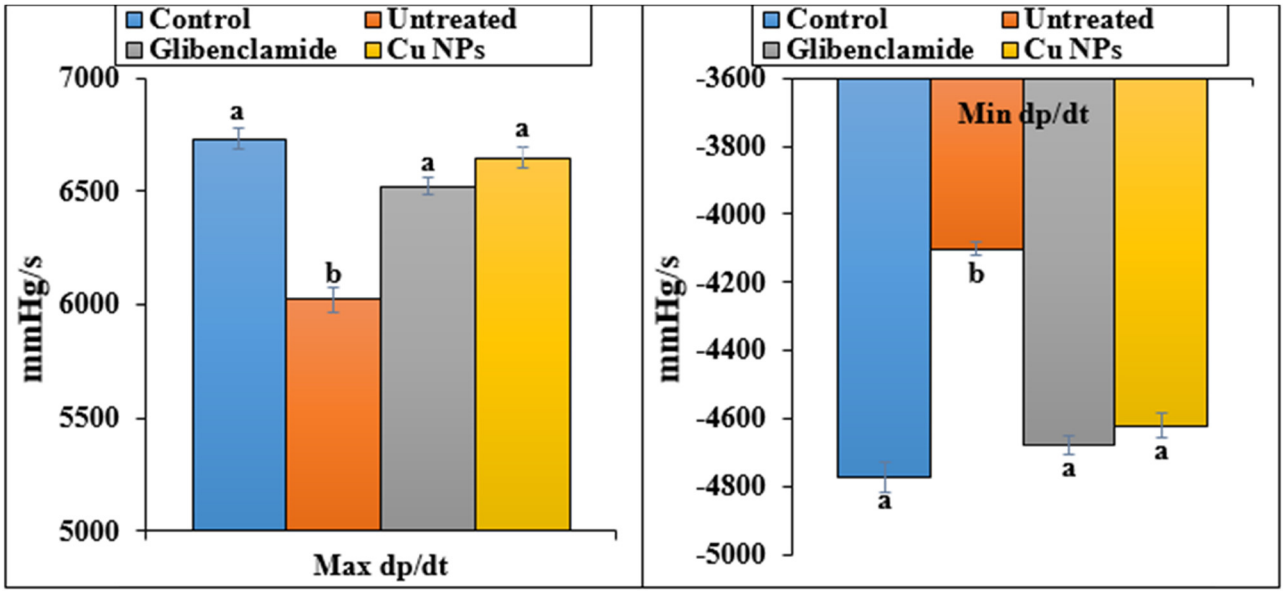


**Figure 8:** The effect of CuNPs on Min d*p*/d*t* and Max d*p*/d*t* levels.

blood glucose level. The administration of CuNPs to streptozotocin (STZ)-diabetic rats resulted in a notable decrease in blood glucose levels ($p \leq 0.05$) comparable to those treated with glibenclamide after the study. Furthermore, the CuNPs exhibited the greatest impact on day 20. There was no notable variance ($p \leq 0.05$) observed between the effects of CuNPs and glibenclamide on fasting blood glucose levels on days 10 and 20.

The liver parameter values are provided in Figure 6. STZ-induced toxicity cause a notable rise in alkaline phosphatases (ALP), aspartate aminotransferase (AST), alanine aminoteransferase (ALT), and gamma-glutamyl transpeptidase (GGT) levels compared to the control group. Treatment with CuNPs resulted in a notable reduction in the elevated levels of ALP, AST, ALT, and GGT compared to the untreated group. There was no notable change in GGT levels between the CuNPs, glibenclamide, and control groups.

The Diabetes group exhibited significantly impaired cardiac work, cardiac output, and aortic flow, which are indicative of systolic heart function when compared to the control group (Figures 7–9). These findings suggest that diabetes has adverse effects on the heart. The CuNPs administration led to a notable enhancement in cardiac output and cardiac work, indicating potential positive impacts on the heart under diabetic conditions. Additionally, there was a tendency for an increase in LVEDP in the Diabetes group.

The Diabetes group exhibited a notable increase in STAT3 phosphorylation, a response lessened by the

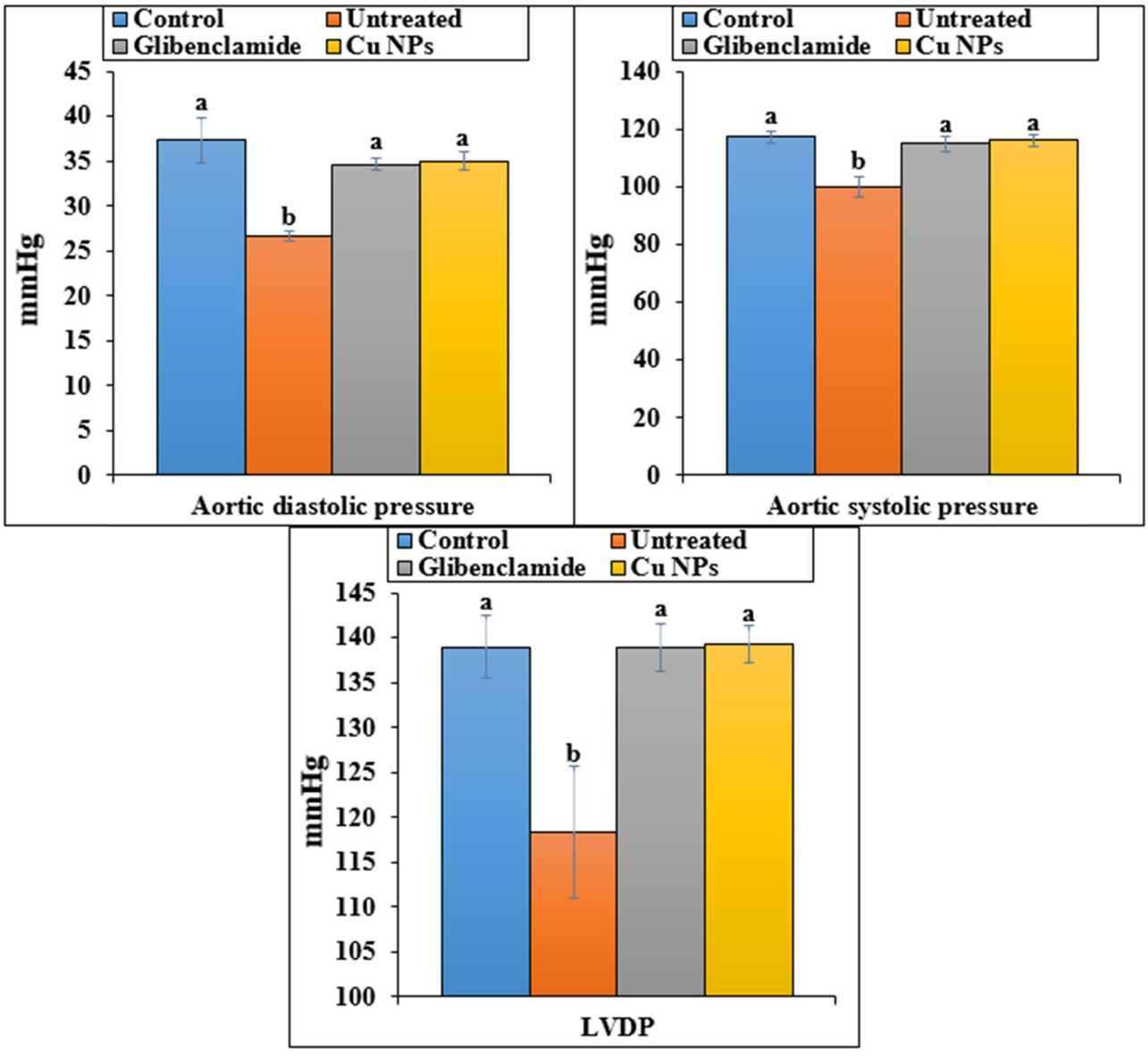


**Figure 9:** The effect of CuNPs on LVDP levels, aortic systolic pressure, and aortic diastolic pressure.

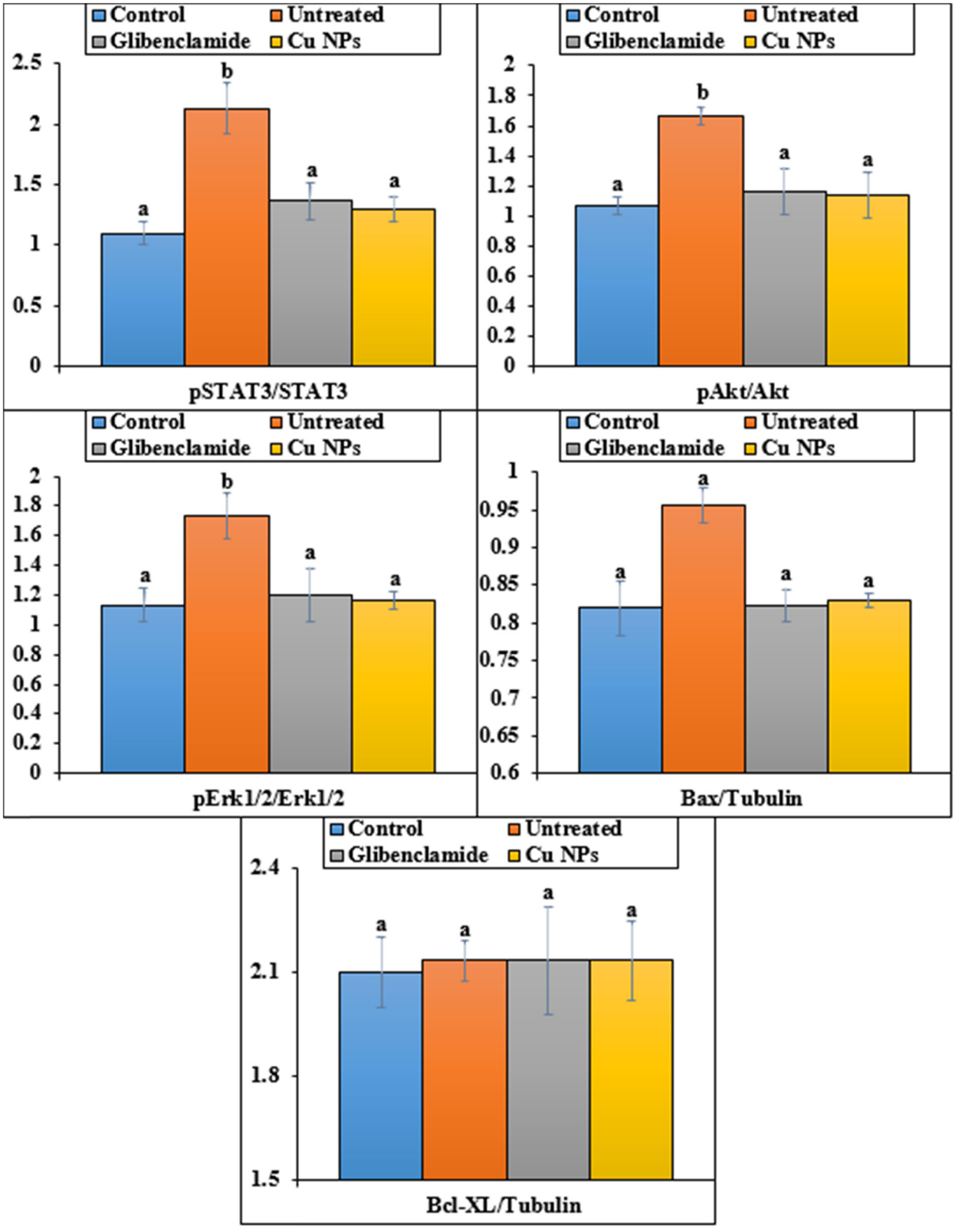


**Figure 10:** The effect of CuNPs on pSTAT3/STAT3, pAkt/Akt, pErk1/2/Erk1/2, Bax/Tubulin, and Bcl-XL/Tubulin levels.

administration of CuNPs (Figure 10). This implies a correlation with the positive impact of CuNPs on cardiac function. The levels of antiapoptotic Bcl-XL and proapoptotic Bax proteins remained unaffected by both Diabetes and CuNPs treatment.

Elevated fasting blood glucose levels were observed in both untreated diabetic rats and treated diabetic rats when compared to non-diabetic rats. These results indicate that STZ induces diabetes by causing damage to the β-cells in the pancreas. The decrease in synthesizing natural insulin caused by this harm results in a decline in the uptake of glucose by tissues. This rise can be ascribed to the detrimental impact of STZ, which selectively attacks and eradicates the beta cells in the pancreas. As a result, the damage leads to a notable rise in blood sugar levels, potentially due to the substantial decrease in insulin production triggered by STZ. The normal group exhibited no significant changes in blood glucose levels, whereas the positive control group of diabetes displayed a noticeable increase in blood glucose levels on days 10 and 20 following STZ induction. Our results are consistent with the research conducted by Ghasemi and Jeddi [56], indicating that STZ has been an effective method for inducing hyperglycemia in Wistar rats within 24 h following injection over the past 10 years. In the study of Hussein et al. 2023, diabetic rats treated with CuNPs at doses of 0.5 and 5 mg/kg exhibited a notable decrease in glucose levels compared to untreated diabetic rats. Nevertheless, the optimal dosage for antidiabetic management is 5 mg/kg when administering CuONPs. These NPs are acknowledged for their potential in managing type 2 diabetes [57]. Hemmati and colleagues (2018) demonstrated that numerous trace metals exhibit potential therapeutic properties for lowering blood glucose levels. Additionally, the insulin-mimetic characteristics of these substances contribute to their hypoglycemic efficacies [58]. This finding aligns with previous research carried out by Martín Giménez et al. [1], who determined that CuNPs possess beneficial therapeutic characteristics for treating type 2 diabetes. Furthermore, the findings are consistent with the research conducted by Umar et al. [59], which demonstrated that CuONP led to a significant decrease in blood glucose levels in rats, as well as other relevant factors. The results indicate that CuONP hold promise as a potential candidate for remedial advancements in the management of diabetes at two distinct dosages. Meanwhile, the Safety Data Sheet, by Regulation (EC), Sigma-Aldrich, 2020, and Assy et al. [60], has documented the safety profile of CuNPs administration at a daily oral gavage dose of 125 mg/kg for 28 days.

# 4 Conclusion

The research involved the successful incorporation of highly stable CuNPs into *P. atlantica* leaf extract through an environmentally friendly and straightforward method that eliminates the need for any harmful chemicals. Subsequently, the structural analysis of CuNPs was conducted by FT-IR, TEM, SEM, and EDX techniques. It has been discovered that *P. atlantica* leaf extract is uniformly dispersed with CuNPs, with an average particle diameter ranging from 30 to 70 nm. In rats, CuNPs appear to protect diabetes-induced cardiac dysfunction, although this efficacy does not seem to be influenced by the modulation of glucose tolerance or fasting hyperglycemia. CuNPs have been found to inhibit STAT3 phosphorylation in the heart, potentially contributing to the positive impact on cardiac health. Additional research, such as clinical trials, is essential to comprehensively grasp the action mechanism of the medication and its benefits for diabetic patients. If the clinical trial studies in humans can confirm the antidiabetic and cardioprotective efficacies of the recently produced NPs using the biological method, it could potentially bring about a significant revolution in the pharmaceutical industry. This revolution would aim to treat diabetes with minimal side effects and at the lowest cost possible.

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