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## Therapeutic effects of *Citrullus colocynthis* nanoparticles on Streptozotocin-induced diabetic rats: Improvement of pancreatic histology

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### **Abstract**

#### **Objective**

Diabetes mellitus is a chronic metabolic disorder. This disorder occurs when insulin secretion is insufficient or insulin function is impaired, and blood sugar levels increase. Continuously elevated blood sugar levels cause oxidative stress and structural damage in pancreatic tissue. This damage is most commonly seen in beta cells of the islets of Langerhans. One of the medicinal plants with antidiabetic properties is colocynthis (*Citrullus colocynthis*). Therefore, our aim was to study the therapeutic effects of colocynthis nanoparticles (CCNPs) on pancreatic histology, insulin levels, oxidative stress markers, and blood glucose levels in streptozotocin (STZ)-induced diabetic rats.

#### **Materials and methods**

Four groups of male Wistar rats were randomly selected. These four groups included the control group, untreated diabetic rats, diabetic rats treated with colocynthis (*Citrullus colocynthis*) extract, and diabetic rats treated with CCNPs. We used an intraperitoneal injection of streptozotocin (STZ) to induce diabetes. The treatment period was 28 days. We used standard biochemical methods to measure blood glucose and serum insulin levels. We measured the antioxidant effect of the treatments using oxidative stress parameters. We examined pancreatic tissues histologically to examine structural changes in the islets of Langerhans.

#### **Results**

Treatment with CCNPs significantly reduced blood glucose levels. In the other groups, namely the untreated diabetic and extract-treated groups, insulin levels increased significantly. Oxidative stress markers improved in the CCNP group mice. In these mice, beta cell density increased, pancreatic islet architecture was restored, and cellular destruction was reduced. On the other hand, in untreated diabetic animals, pancreatic islets were severely shrunk and beta cells were damaged.

Therefore, it can be said that the therapeutic efficacy of the nanoparticle formulation is greater than that of the crude plant extract.

### Conclusion

Our experimental results confirmed the protective and restorative role of *Citrullus colocynthis* nanoparticles on pancreatic tissue in diabetic mice. CCNPs improved blood sugar control. In addition, their use increased insulin secretion and reduced oxidative stress. Therefore, it can be concluded that the use of nanoparticle-based formulations from medicinal plants may potentially provide a promising and innovative approach for diabetes management.

**Keywords:** antidiabetic, citrullus colocynthis nanoparticles, diabetes mellitus, oxidative stress, pancreatic histology

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### Introduction

Diabetes mellitus (DM) is a chronic and multifactorial metabolic disorder that affects millions of people worldwide and is associated with high morbidity and mortality. This disease causes persistent hyperglycemia, and this persistent hyperglycemia is either due to impaired insulin secretion or impaired insulin action, or sometimes both. In experimental research, streptozotocin (STZ) is widely used to induce diabetes in animal models because it selectively destroys pancreatic  $\beta$ -cells and mimics type 1 diabetes (Lenzen, 2008). This model is used to study the effects of new therapeutic agents, as it may lead to better management of diabetes. Synthetic drugs are very expensive and have undesirable side effects (Salehi et al., 2019). Much research has been done to reduce the limitations and find herbal alternatives that are both safer and cheaper (Mortazavi et al., 2005). Natural products have attracted strong scientific interest because of their safety profile and biological effectiveness (Kooti et al., 2016). The therapeutic role of many medicinal plants has been investigated over the past years. The results have shown

that these plants play important roles in the treatment of diabetes and atherosclerosis (Mohammadabadi & Mozafari, 2018), cardiovascular diseases (Maritim et al., 2003), neurological disorders (Heidarpour et al., 2011), and cancer (Sabu & Kuttan, 2002). It has been found that there is a significant association between these conditions and oxidative stress and disturbances in redox balance (Ghorbani, 2005). Thus, to reduce oxidative damage and improve overall health, it is better to use medicinal plants that have antioxidant activity (Zarrabi et al., 2020). In the past, when chemical anti-diabetic drugs did not exist and insulin was not yet available, patients used medicinal plants and traditional medicine to treat themselves (Ostovar et al., 2020; Mohammadabadi et al., 2009). Plants lower blood glucose by acting on several mechanisms. They may stimulate insulin production or they may activate enzymes that produce insulin (Ostovan et al., 2017). Some other hormones act by preventing the insulin produced from being destroyed or by causing the pancreatic beta cells to regenerate (Souto et al., 2019). In addition, if plant-based dietary fibers are consumed, the rate of absorption of carbohydrates decreases. This causes blood sugar levels to drop (Sarkhel et al., 2024). *Citrullus colocynthis* (bitter apple) is a medicinal plant that has antioxidant properties and lowers blood sugar. It is rich in bioactive compounds such as flavonoids and alkaloids. The antidiabetic activity of this plant has been investigated and confirmed by various methods, including traditional methods and modern experimental methods (Matough et al., 2012). This plant is also used in traditional medicine to treat hyperlipidemia and obesity. Recent findings indicate that fruit extracts of *C. colocynthis* can reduce lipid levels without negatively affecting liver biochemical parameters (Dhir et al., 2024). Moreover, its extracts may prevent fat accumulation around vital organs such as the liver, kidneys, and pancreas. In hyperlipidemic rats, the plant increased high-density lipoprotein cholesterol (HDL-c) and decreased triglycerides, total cholesterol, and low-density lipoprotein cholesterol (LDL-c). In addition, seed extracts improved laying performance and immune responses in commercial layer hens (Alsalihi et al., 2025). Nanotechnology has opened new opportunities to improve the therapeutic efficiency of plant-derived compounds. Nanoparticles can enhance drug delivery, increase cellular uptake, and improve bioavailability. Bioactive molecules can attach to the surface of nanoparticles. So, they can penetrate biological membranes more effectively, which increases their therapeutic action. Therefore, to enhance the biological activity of medicinal plant extracts, they can be formulated as nanoparticles. Therefore, our aim was to study the therapeutic effects of *Citrullus colocynthis* nanoparticles (CCNPs) on pancreatic histology, insulin levels, oxidative stress markers, and blood glucose levels in streptozotocin (STZ)-induced diabetic rats.

## Materials and method

First, we collected and washed the fresh roots of *Citrullus colocynthis*. After drying in the shade, we converted them into fine powder. We defatted the powdered material using petroleum ether (60-80°C). Then, we performed extraction with 90% ethanol using a Soxhlet apparatus by hot percolation method and with distilled water by soaking method, respectively. To obtain maximum yield, we repeated the extraction process three times. We filtered all the extracts. Then, we concentrated them under reduced pressure using a rotary evaporator (Bucci, USA). The final yield of the dry extract we obtained was approximately 15% (w/w) of the original plant material. The required streptozotocin (STZ) (Sigma-Aldrich, USA) was also purchased.

A green synthesis method based on plant extract reduction was used to *Citrullus colocynthis* nanoparticles (CCNPs). This method is based on the method presented by Karunakaran and Hari (2022). In this method, we can use the plant extract as a reducing agent. It also takes advantage of the stabilizing properties of the extract. To produce stable particles, the synthesized nanoparticles were first dried. Then, they were calcined at 700 °C. We used scanning electron microscopy (SEM) to examine the surface morphology. We used transmission electron microscopy (TEM) (JEM, Japan) to determine the size and shape of the particles. Particle measurements showed that their average size was approximately 50 nm. To understand whether nanoparticles were formed or not, we used UV-Visible spectroscopy (UV-Biotechnology Medical Service, China). We used X-ray diffraction (XRD) in the  $2\theta$  range from 20 to 70° at 40 kV using a JEOL diffractometer with drop casting technique to analyze the structural properties. The morphology of CCNPs observed under SEM and TEM is shown in Figure 1. Figure 2 shows the UV-Vis absorption spectrum and XRD pattern of the synthesized nanoparticles.

Twenty-four male Wistar rats (180–220 g) were used in this study. We obtained these animals from the Cancer Research Center and Medical Genetics, Iraq, Baghdad. We obtained the necessary permits for all experiments (Animal Ethics Committee, Baghdad College of Health and Medical Technology). We maintained the animals under standard laboratory conditions (12-h light/dark cycle, controlled temperature, and free access to food and water). An acclimation period was provided for all animals before the start of the experiment. We used an intraperitoneal injection of STZ (60 mg/kg) dissolved in citrate buffer (pH 4.5) (Lenzen, 2008) to induce diabetes. After 72 h, we measured fasting blood glucose levels. Any animal with a blood glucose level higher than 250 mg/dL was considered diabetic. Four groups of male Wistar rats were randomly selected. These four groups included the control group (Group I), untreated diabetic rats (Group II), diabetic rats treated with colocynthia (*Citrullus colocynthis*) extract (200 mg/kg/day, orally= Group III), and diabetic rats treated with CCNPs (100 mg/kg/day, orally= Group IV). The treatment period lasted 28 days.

We used a glucometer to measure blood glucose levels weekly. We also used an ELISA kit to determine serum insulin levels. We used spectrophotometric methods to measure oxidative stress markers. These methods measure malondialdehyde (MDA) and superoxide dismutase (SOD).

At the end of the experimental period, we euthanized the mice. We collected the pancreatic tissues. Then we fixed them in 10% formalin. After fixation, we embedded them in paraffin and sectioned them. We stained the sections with hematoxylin and eosin (H&E). We planned the experiments in such a way that we could perform microscopic examinations. With these examinations, we measured and reported the morphology of beta cells, the architecture of the islets of Langerhans, the size of the islets of Langerhans, and the infiltration of inflammatory cells.

We reported the data as mean  $\pm$  standard deviation (SD). We performed statistical analysis using one-way analysis of variance (ANOVA). We then used Tukey's post hoc test. We considered a p value of less than 0.05 to be statistically significant.

## Results and discussion

The SEM analysis results in this study show that the synthesized nanoparticles tend to aggregate into larger clusters. It is believed that the drying process during sample preparation could be one of the main reasons for this aggregation. However, despite all this, their overall spherical shape was maintained. The particle surfaces were rough and irregular. This could be due to the phytochemical compounds of *Citrullus colocynthis*. Because these compounds act as coating and stabilizing agents (Rasool et al., 2020). We also observed various other shapes. These shapes included spherical, cubic, triangular, and rod-shaped structures. This diversity in our results indicates the successful green synthesis with natural biomolecules that influence particle formation.

The UV–Vis absorption spectrum (Figure 2) showed a strong absorption peak at approximately 430 nm. This peak confirms that nanoparticles have formed. It also confirms that surface plasmon resonance (SPR), which is typical for metal nanoparticles, has been generated. The sharp and symmetrical peak indicated that the particle size had a relatively uniform distribution. In this study, after seven days, we did not observe any major changes in the position or intensity of the peak. This indicated that the nanoparticles were well stable over time and had minimal aggregation (Mubeen et al., 2022).

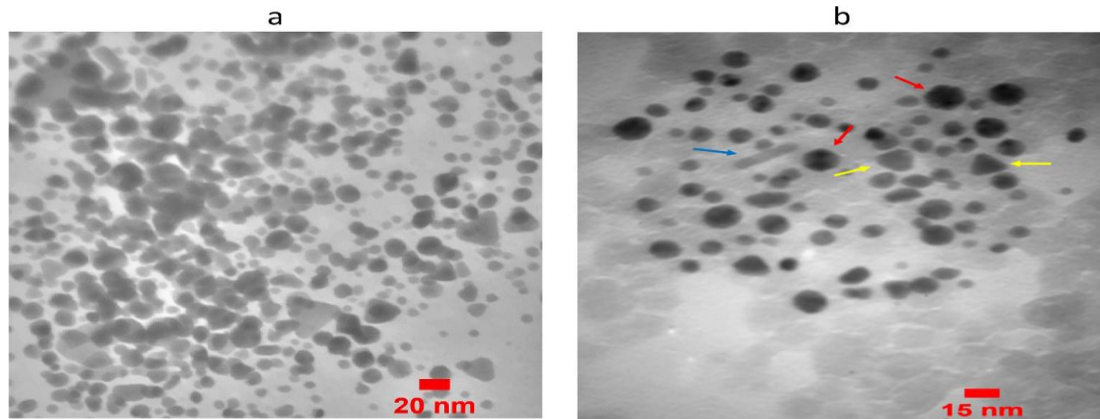


Figure 1. SEM image and particle size distribution of CCNPs. Arrows show various nanoparticle morphologies (spherical, triangular, and rod-shaped forms)

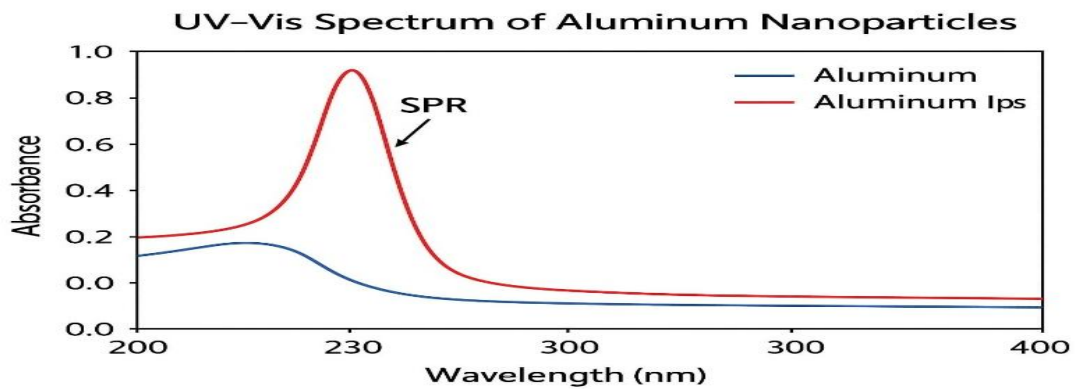


Figure 2. UV-Vis absorption spectra of *Citrullus colocynthis* extract and CCNPs. The blue line is for *Citrullus colocynthis* extract and the red line is for CCNPs

Our TEM experimental results showed that most of the nanoparticles were spherical in shape and their size ranged from 10 to 40 nm (Figure 3). The dispersion of the particles was very good. However, we also observed some small clusters. Our TEM images were of high resolution and their lattice fringes were clear. These results confirmed the crystalline nature of the nanoparticles. The particle sizes we observed were consistent across all TEM, SEM, and XRD calculations.

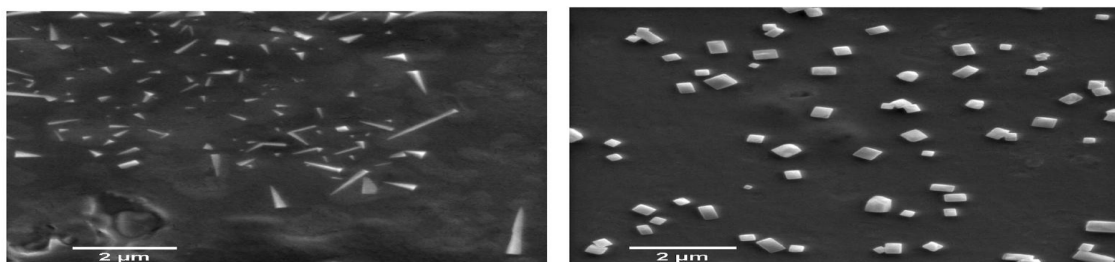
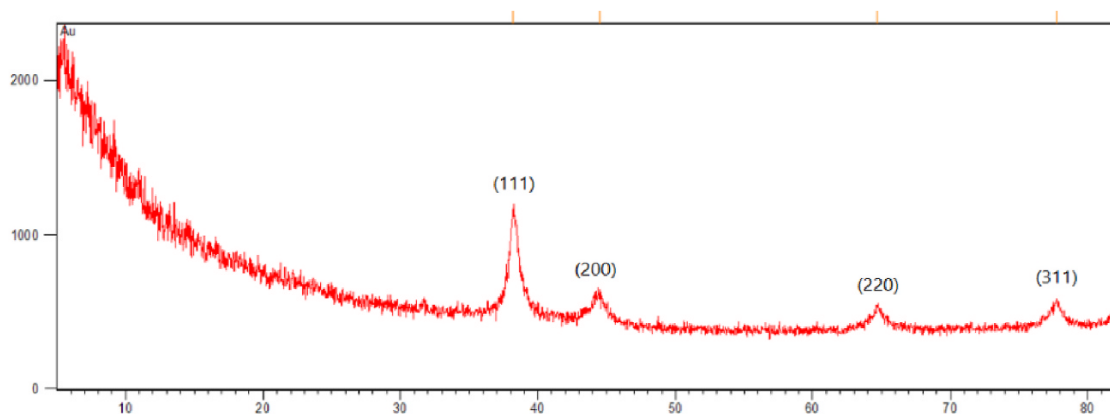


Figure 3. Morphology TEM image of CCNPs. Morphologies are spherical and nanoscale

XRD analysis confirmed the crystalline structure of the nanoparticles synthesized in our experiment (Figure 4). We observed distinct diffraction peaks at  $2\theta$  values corresponding to the (111), (200), (220) and (311) planes of a face-centered cubic (fcc) structure (JCPDS No. 04-0783). The sharpness and intensity of the peaks in our experimental results indicated a high degree of crystallinity. Using the Debye-Scherrer equation, the average crystallite size in our experiment was calculated to be approximately 20 nm. These results were consistent with TEM observations (Hafeez et al., 2023).



**Figure 4. Reported XRD pattern of CCNPs. Peaks indicate crystalline nanoparticles**

Table 1 shows blood glucose and serum insulin levels at baseline and after 28 days of treatment. At Day 0, there were no significant differences among diabetic groups. After 28 days, untreated diabetic rats maintained high blood glucose levels ( $295 \pm 18$  mg/dL) and low insulin levels ( $3.1 \pm 0.5$   $\mu$ IU/mL). Treatment with *C. colocynthis* extract significantly reduced blood glucose to  $190 \pm 15$  mg/dL ( $p < 0.05$ ). However, CCNP treatment resulted in a greater reduction to  $120 \pm 12$  mg/dL ( $p < 0.01$ ). Serum insulin levels were also significantly increased in the CCNP group compared with the untreated diabetic group. These results suggest that nanoparticle formulation enhanced the antidiabetic activity of the plant extract.

**Table 1. Effect of treatments on blood glucose and serum insulin levels after 28 days**

Group	Day 0 Glucose (mg/dL)	Day 28 Glucose (mg/dL)	Serum Insulin ( $\mu$ IU/mL)
Control	$85 \pm 5$	$88 \pm 6$	$12.5 \pm 1.2$
Diabetic untreated	$310 \pm 20$	$295 \pm 18$	$3.1 \pm 0.5$
Diabetic + Extract	$312 \pm 22$	$190 \pm 15^*$	$7.8 \pm 0.9^*$
Diabetic + CCNPs	$305 \pm 19$	$120 \pm 12^{**}$	$10.6 \pm 1.1^{**}$

\*Significant vs. diabetic untreated,  $p < 0.05$ ; \*\* $p < 0.01$

As shown in Table 2, STZ significantly increased malondialdehyde (MDA) levels and reduced superoxide dismutase (SOD) activity compared with the control group. Treatment with plant extract partially reduced oxidative stress. However, CCNP treatment resulted in a more significant decrease in MDA and a significant increase in SOD activity ( $p < 0.01$ ). These findings indicated that CCNPs improved antioxidant defense mechanisms. They also reduced lipid peroxidation. Various studies have shown that oxidative stress plays an important role in the progression of diabetes and that reducing oxidative damage is important for protecting the pancreas (Liu et al., 2025).

**Table 2. Effect of treatments on oxidative stress markers**

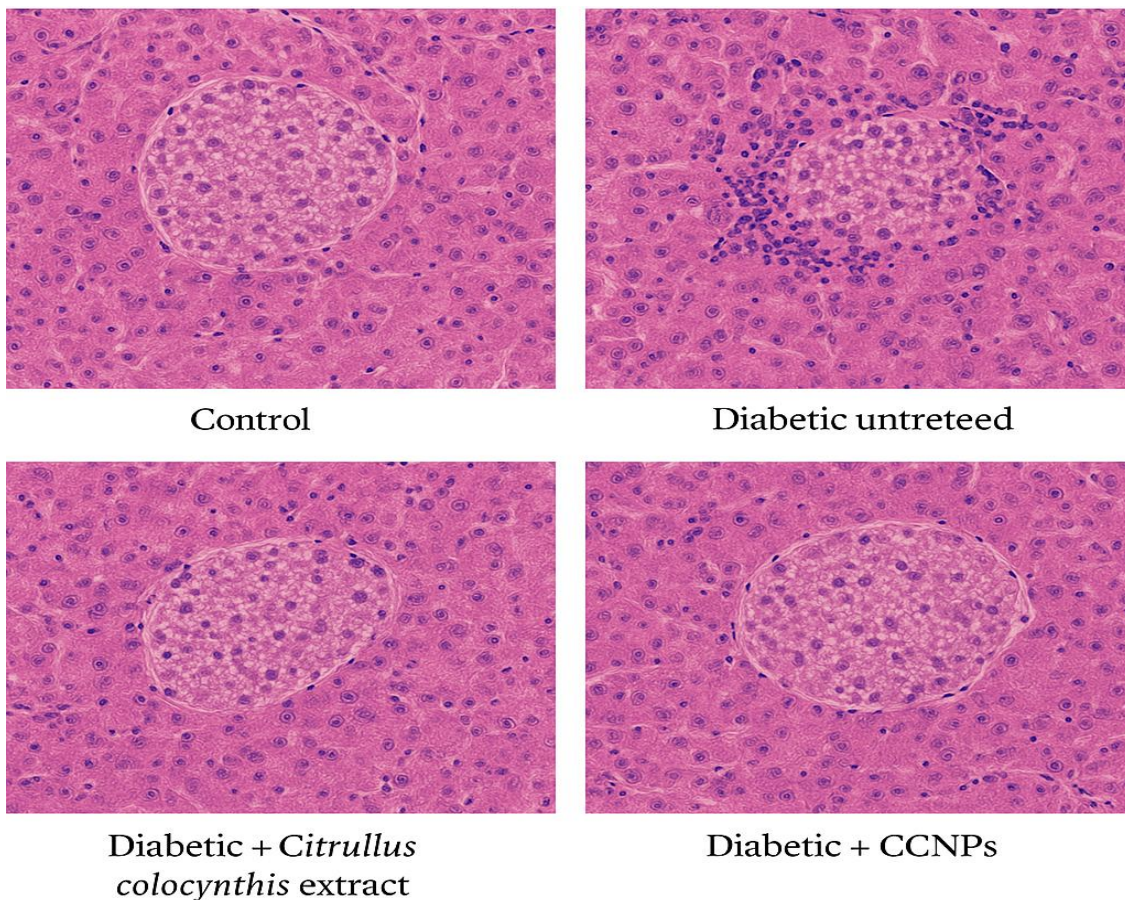
Group	MDA (nmol/mg protein)	SOD (U/mg protein)
Control	1.5 ± 0.2	12.1 ± 1.3
Diabetic untreated	4.5 ± 0.5	5.2 ± 0.8
Diabetic + Extract	3.0 ± 0.4*	8.7 ± 1.0*
Diabetic + CCNPs	2.1 ± 0.3**	10.9 ± 1.2**

\*Significant vs. diabetic untreated,  $p < 0.05$ ; \*\* $p < 0.01$

The histological analysis of pancreatic tissue is shown in Figure 5. Normal islets of Langerhans with intact beta cells were reported by the control group. On the other hand, severe degeneration of beta cells, reduction in islet size and inflammatory cell infiltration were reported by the untreated diabetic group. Partial improvement in islet structure and mild improvement of beta cells was reported by the mice treated with *C. colocynthis* extract. Significant restoration of islet architecture, increased beta cell density and reduced inflammatory infiltration were also reported by the CCNP-treated group. Overall, the size of the islets was close to normal compared to the control group.

The death of all the  $\beta$ -cells is the cause of STZ induced hyperglycemia. The administering of CCNPs resulted in a significant reduction in blood glucose levels of the mice, as well as an increase in insulin levels. The effect was more than that of the extract on its own. Perhaps due to the improved bioavailability and antioxidant activity of nanoparticles. Oxidative stress plays a major role in the development of diabetes. CCNPs decreased lipid peroxidation and improved oxidative stress markers (decreased MDA, increased SOD) through enhanced enzymatic defense (Liu et al., 2025). Biochemical data show that CCNPs prevented injury of pancreatic islets and  $\beta$ -cells from STZ histologically (Kanwugu et al., 2022). However, our results suggest that nanoparticle formulation enhances these therapeutic effects. Overall, our results suggest that the use of greenly synthesized CCNPs can provide better antidiabetic and antioxidant effects than the

crude extract alone. Therefore, nanotechnology may be able to improve the therapeutic potential of medicinal plants in diabetes management.



**Figure 5.** Histopathological slices of pancreatic tissue (H&E stain, 400x). (A) Control group has normal islets; (B) Untreated diabetic group has  $\beta$ -cell degeneration and inflammation; (C) Diabetic + extract group with partial recovery; (D) Diabetic + CCNP group has significant restoration of islet structure

**Conclusion:** The nanoparticles made from *Citrullus colocynthis* have the potential to be tested for antidiabetic and antioxidant activities against STZ-induced diabetic rats which improve pancreatic histology and biochemical markers. Diabetes treatment can be improved through the use of CCNPs.

#### Author contributions

All activities were performed based on standard procedures by author.

#### Data availability

The author can provide data supporting this study upon reasonable request.

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### Ethical consideration

This study approved by Ethical Committee at Middle Technical University College of Health and Medical Technique (Ethical code 7/27/345.Medicol.Mtu).

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### Conflict of Interest

The authors declare no conflict of interest.

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## اثرات درمانی نانوذرات هندوانه ابوجهل (*Citrullus colocynthis*) بر رت‌های دیابتی

### القاشده با استرپتوزوتوسین: بهبود بافت‌شناسی پانکراس

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#### چکیده

**هدف:** دیابت ملیتوس یک اختلال متابولیک مزمن است که با هیپرگلیسمی ناشی از کاهش ترشح انسولین یا اختلال در عملکرد آن مشخص می‌شود. هیپرگلیسمی پایدار موجب استرس اکسیداتیو و آسیب ساختاری در بافت پانکراس می‌گردد؛ این آسیب به‌ویژه در سلول‌های بتای جزایر لانگرهانس مشهود است. گیاه *Citrullus colocynthis* (هندوانه ابوجهل) در مطالعات پیشین دارای خواص ضد دیابتی گزارش شده است. بنابراین، هدف این پژوهش ارزیابی اثرات درمانی نانوذرات هندوانه ابوجهل (CCNPs) بر بافت‌شناسی پانکراس، سطح انسولین، شاخص‌های استرس اکسیداتیو و سطح گلوکز خون در رت‌های دیابتی القاشده با استرپتوزوتوسین (STZ) بود.

**مواد و روش‌ها:** رت‌های نر ویستار به‌صورت تصادفی به چهار گروه تقسیم شدند: کنترل، دیابتی بدون درمان، دیابتی تیمار شده با عصاره هندوانه ابوجهل، و دیابتی تیمار شده با نانوذرات هندوانه ابوجهل. دیابت با یک تزریق داخل صفاقی استرپتوزوتوسین (STZ) القا شد. دوره درمان ۲۸ روز به طول انجامید. سطح گلوکز خون و انسولین سرم با روش‌های بیوشیمیایی استاندارد اندازه‌گیری شد. برای ارزیابی اثر آنتی‌اکسیدانی تیمارها، شاخص‌های استرس اکسیداتیو بررسی شدند. در پایان آزمایش، بافت پانکراس جمع‌آوری و از نظر بافت‌شناسی برای ارزیابی تغییرات ساختاری در جزایر لانگرهانس مورد بررسی قرار گرفت.

**نتایج:** نتایج نشان داد که درمان با CCNPs در مقایسه با گروه دیابتی بدون درمان، کاهش معنی‌داری در سطح گلوکز خون ایجاد کرد. همچنین در مقایسه با گروه‌های دیابتی بدون درمان و تیمار شده با عصاره، رت‌های دریافت‌کننده CCNPs افزایش معنی‌داری در سطح انسولین نشان دادند. شاخص‌های استرس اکسیداتیو در گروه CCNP به‌طور قابل توجهی بهبود یافت. بررسی بافت‌شناسی نشان داد که در رت‌های تیمار شده با CCNPs تراکم سلول‌های بتا افزایش یافته، ساختار جزایر پانکراسی به‌طور واضح بازسازی

شده و دژتراسیون سلولی کاهش یافته است. در مقابل، گروه دیابتی بدون درمان کاهش شدید اندازه جزایر و آسیب سلول‌های بتا را نشان داد. اثربخشی درمانی فرمولاسیون نانوذره‌ای بیشتر از عصاره خام گیاه بود.

**نتیجه‌گیری:** نتایج این مطالعه نشان داد که نانوذرات هندوانه ابوجهل دارای اثرات محافظتی و ترمیمی بر بافت پانکراس در رت‌های دیابتی هستند CCNPs. کنترل گلیسمی را بهبود بخشیده، ترشح انسولین را افزایش داده و استرس اکسیداتیو را کاهش دادند. بنابراین، استفاده از فرمولاسیون‌های نانوپایه مشتق از گیاهان دارویی می‌تواند رویکردی نوآورانه و امیدبخش در مدیریت دیابت محسوب شود.

**کلمات کلیدی:** استرس اکسیداتیو، بافت‌شناسی پانکراس، دیابت ملیتوس، ضد دیابت، نانوذرات هندوانه ابوجهل

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