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The Green Synthesis of Co_3O_4 Nanoparticles Using *Artemisia herba alba* and their Potential Application as Anti-Hyperglycemia Agents

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Abstract | The extract of *Artemisia herba alba* and cobalt nitrate, $Co(NO_3)_2$, were used as a precursor in the green synthesis process to create Co_3O_4-NPs. Post-calcination, the nanoparticles were characterized using Fourier Transform Infrared Spectroscopy, scanning electron microscopy, and X-ray diffraction. A post-calcination procedure can be used to enhance crystallinity and purity. The pure form of Co_3O_4-NPs was analyzed using FTIR, SEM, EDX, XRD, AAS, and UV-VIS spectroscopy. The XRD analysis confirmed the monoclinic crystalline structure of Co_3O_4-NPs, with an average size of 73 nm. UV-Vis spectroscopy showed a distinctive absorption peak in the 200–800 nm. At 232 nm, a noticeable absorption peak was seen. The carbonyl (C=O), hydroxy (O-H), alkane (C-H), alkene (C=C), and (Co-O) groups were identified by the FTIR analysis with a noticeable peak at 570 cm−1. Cobalt is the most common element, making up 6.2% of the elemental composition, according to the EDX analysis. The spectra show that carbon and oxygen, which make up 36.9%, 19.5% of the composition, respectively, are present in addition to cobalt. The biosynthesized CoO-NPs' main XRD 2θ value peaks at 24.95◦, 30.00◦, and 37.65◦. Co_3O_4-NPs show a sharp peak at 2θ = 29.00 with the diffraction of the 111 planes, suggesting that the synthesized Co_3O_4-NPs are monoclinic and crystalline. Treated animals with alloxan substance led to an increase in glucose (483.66mg/dl) as compared with the control group (136.16mg/dl), while after being treated with CoNPs, there was an improvement in glucose and insulin levels (118.50 mg/dl) and (2.32ng/ml) as compared with the alloxan group. This study outlines the generation of Co_3O_4 nanoparticles and use of animal as model to study the exploitation of these nanoparticles.

Keywords | Artemisia herba alba, Co_3O_4-NPs, Diabetic mellitus, Histopathology. SEM

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INTRODUCTION

Artemisia herba alba, also known as white wormwood, is a perennial plant found in Mediterranean dry steppes, characterized by its white, woolly stems and foliage (Mohammed *et al*., 2021). White wormwood, or Artemisia herba-alba, is well-known for a wide variety of bioactive substances that support its therapeutic qualities. *Artemisia herba alba* therapy was linked to higher blood levels of albumin and plasma proteins in addition to decreased liver enzyme levels. This is significant because the liver produces albumin, a crucial protein whose levels reflect both overall health and liver function (Belinskaia *et al*., 2024). Research on *Artemisia herba alba*'s potential impacts on kidney health has shown that it exhibits both potentially nephrotoxic and nephroprotective qualities in

certain situations (Sekiou *et al*., 2021). The antioxidant qualities of A. herba alba shield the tissue of the renal from oxidative stress, minimizing damage to the kidneys in particular that results from diabetes and elevated levels of blood glucose (Darenskaya *et al*., 2023). Nanotechnology was used to clarify in every technology that can be used to manipulate matter at the level of molecules and make matters, devices, and structures having a dimension of (1-100 nm) at least in one direction. The drugs were delivered directly to specific parts of the body using nanoparticle engineering, increasing treatment effectiveness and reducing adverse effects. For instance, medical drugs for neurological disorders can be delivered using nanoparticles, which can cross the blood-brain barrier (Elumalai *et al*., 2024). Formulations based on nanotechnologies, such as solid lipid nanoparticles, polymeric nanoparticles, and nanoemulsions, improve hydrophobic drug solubility by encapsulating medications, shielding them from deterioration, and promoting improved absorption (Sultana *et al*., 2022). Especially for low-permeability medications, nanoparticles have enhanced drug transport into systemic circulation by efficiently penetrating biological membranes and using paracellular or transcellular pathways to bypass the epithelial barriers of the gut (Zheng *et al*., 2024). The research focuses on developing glucose-responsive nanoparticles that improve the release of insulin in response to blood glucose levels, potentially enhancing glycaemic management compared to traditional insulin therapy (Rege *et al*., 2017).

The management of glycemia could be improved by using nanotechnology to produce glucose-responsive nanoparticles that release insulin in response to an increase in blood sugar levels (Andreadi *et al*., 2024). Cobalt nanoparticles possess unparalleled physicochemical properties and nanoscale dimensions that enable their use toward a wide range of advanced applications, including catalysis, energy storage, imaging, medicine, microelectronics, and drug delivery systems. The fundamental magnetic characteristics enable cobalt nanoparticles to serve as a targeted transporter for drug delivery, offering an opportunity for site-specific therapeutic interventions. Moreover, their special surface properties and small size make cobalt nanoparticles effective sensors for detecting several substances, supporting their incorporation into biomedical, analytical, and monitoring technologies (Vodyashkin *et al*., 2022).

This study aims evaluation and effectiveness of the new synthesized cobalt nanoparticles coated with Artemisia herba alba, *Artemisia herba alba* nanoparticles, and the extract of Artemisia herba alba on hyperglycemia induced in laboratory male rats.

MATERIALS AND METHODS

Collection and preparation of Artemisia herba alba

In Basrah, Iraq, *Artemisia herba alba* was gathered from the neighbourhood market. Sultana *et al*. (2009) state that in independent studies, 100 grammes of air-dried powdered plant material were extracted using 500 milliliters of an aqueous methanol solvent (methanol: water, 80% v/v) for eight hours under Soxhlet conditions on a water bath. The plant extract was liberated, and its solvent was concentrated using a rotary evaporator set at 45 °C. To determine the yield, the dried crude concentrated extracts of Artemisia herba alba were weighed and kept in a refrigerator at 4 °C until they were utilized.

Hydrothermal method of CoNPs biosynthesis

According to Ansari *et al*. (2017), 10 ml of the Artemisia herba alba extract (200 mg/ ml) concentration was added to cobalt nitrate solution (6 g) to synthesize cobalt oxide (Co_3O_4) nanoparticles, and then a KOH solution was added, followed by 15 min stirring, after adding the hydrazine monohydrate and oleic acid solutions, the mixture's volume reached two-thirds of the autoclave's total volume. It was then stirred for two hours to completely dissolve the solid reagents before being transferred into a Teflon-coated autoclave and placed in an electric oven, where it was kept at 160 °C for twenty-four hours. The autoclave has been allowed to naturally cool to ambient temperature after twenty-four hours. Ultimately, the solution's final preparation was centrifuged, the liquid phase was decanted, and the resulting black precipitate was dried in an oven at 70 °C to separate the produced Cobalt NPs from the liquid phase (Ansari *et al*., 2017).

Experimental animals

Mature domestic 30 male rats in good health, weighing between 300 and 400 grams. Before the research, the animals were given a week to acclimate to the lab environment under strict clean, hygienic and standard managing conditions at temperature (20-25°C), controlled room on a 12 hour light and 12 h dark (Shehab *et al*., 2024). The animals were divided into five groups, each containing eight male rats, and were given the following care:

- Group 1: A control group, giving normal saline 0.5ml (I.P)
- Group 2: Each rat was injected with alloxan (150 mg/kg) (Nagappa *et al*., 2003)
- Group 3: Each rat was daily treated with cobalt nanoparticles coated with *A. herba alba* (104.5µg\ml)
- Group 4: Each rat was daily treated with *Artemisia herba alba* nanoparticles (146µg\ml)
- Group 5: Each rat was daily treated with *Artemisia herba alba* extract (500 mg/kg) (Awad *et al*., 2012)

Collection of blood sample and organs

At the end of the trail, blood samples have been collected from the hearts of the rats for biochemical analysis (glucose and insulin). Four ml of the blood was placed in an anticoagulant-free tube and centrifuged for 15 minutes at 4000 rpm. The serum was used for glucose and insulin analysis (Hafth *et al*., 2025). The pancreas was isolated and weighed using an electronic balance. The organ has been fixed by using formalin 10% for the histological examinations, and it was kept in a test cup until the examination.

RESULTS

The Co_3O_4-NPs solution exhibited characteristic absorption peaks in the 200–800 nm wavelength range. The absorbance values of biogenic cobalt nanoparticles (Co_3O_4 NPs) in (Figure 1) show several peaks between 214, 319, and 232 nm. The reduction of cobalt ions is shown by the distinct peak that was seen at 232 nm. The absorption spectrum of *Artemisia herba alba* extract is distinguished by prominent bands with several peaks occurring at wavelengths of 250, 261, 292, and 660 nm. The first three peaks are likely due to the presence of flavonoids, phenolic acids, and terpenoids in the leaves and seeds extract. Lastly, the peak at 660 nm is indicative of the presence of chlorophyll (Figure 2). A shift in the Ar-Co NPs absorption peak following conjugation onto cobalt oxide nanoparticles suggests that artemisia herba alba and the CoO surface are interacting. enhanced or altered absorption peak intensity, indicating that the nanocomposite was successfully formed. Thus, the successful integration of *Artemisia* into CoO as well as the creation of nanoparticles are confirmed by the UV-Vis spectrum. Fourier-transform infrared spectroscopy (FT-IR) has been used to identify the functional groups on the surfaces of the Co_3O_4-NPs as well as the functional groups of the plant metabolites present in the aqueous extractof *Artemisia herba alba*. The FT-IR spectra showed strong peaks at wavenumbers such as 3554, 3415, and 3284 cm^{-1} that were generated by O-H and N-H stretch vibrations (Figure 3). The C=C and C=O vibration modes are prominent in 1734 and 1600, while the peaks at 2924 and 2854 cm^{-1} are associated with the s vibration of C-H groups. The band originating from the Co-O stretching vibration showed a prominent peak at 570 cm^{-1}, with comparable peaks at 430 cm^{-1}. According to the FT-IR results, acids, proteins, polyphenols, alkaloids, and carboxylic acid functional groups could be found in the Co3O4-NPs (Figure 4) (Abdulkareem *et al*., 2025). Scanning electron microscopy (SEM) has been employed to examine the surface morphology of the biosynthesized cobalt oxide nanoparticles (CoO-NPs). The appearance of small, smooth, spherical nanoparticles, individually and clustered, and the reason for this clustering may be due to the rapid evaporation of the sample for examination. The SEM image shows several NP sizes, including 56.85 nm, 80.72 nm, and 83.63 nm (Figure 5).

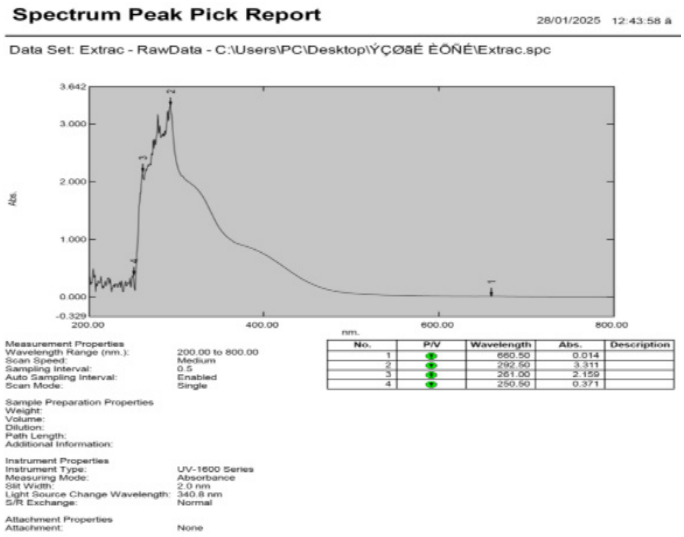

Figure 1: UV-Vis spectrum of biosynthesized Co_3O_4-NPs.

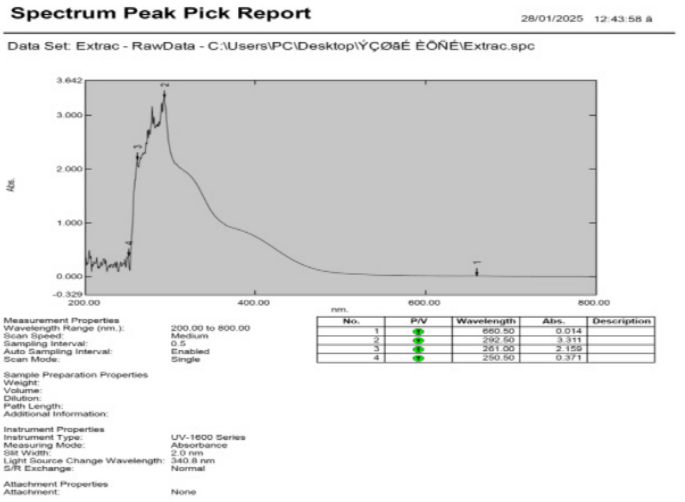

Figure 2: UV-Vis spectrum of *Artemisia herba alba* extract.

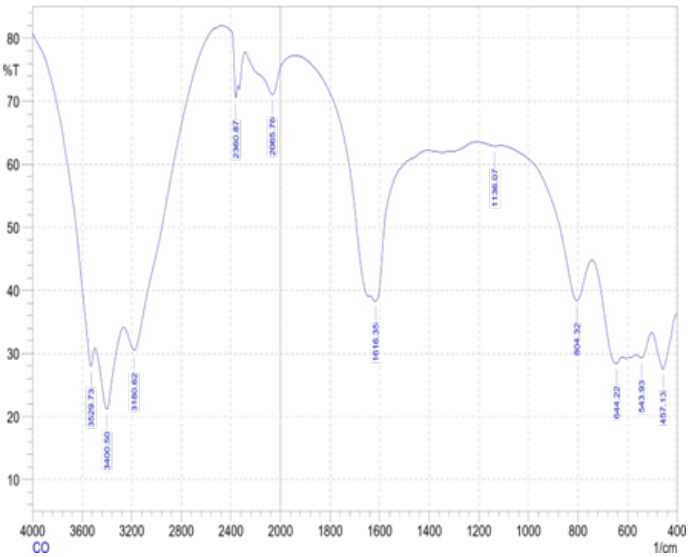

Figure 3: FT-IR Spectrum of Biosynthesized Co_3O_4-NPs

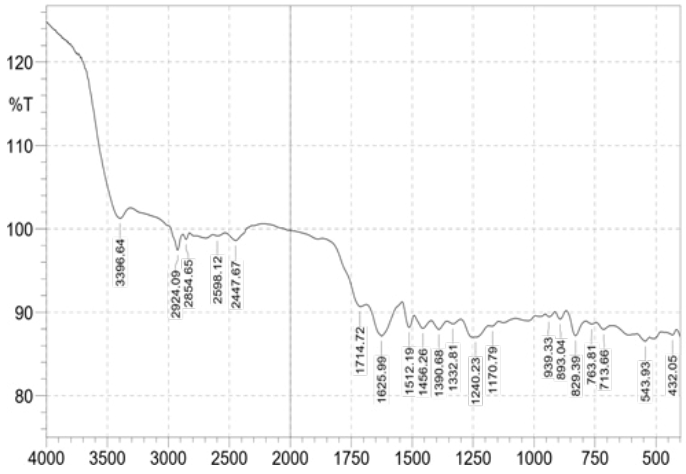

Figure 4: FT-IR Spectrum of *Artemisia herba alba* extract.

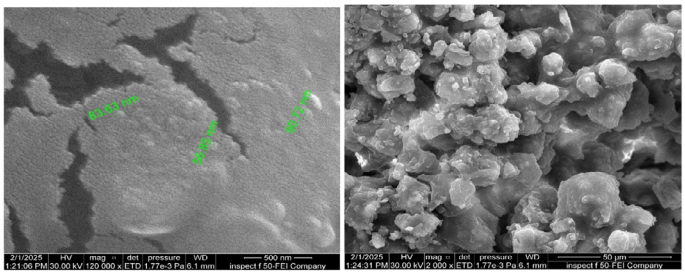

Figure 5: Artemisia -Co_3O_4 nanoparticle size and morphology by are displayed by field emission scanning electron microscopy (FeSEM).

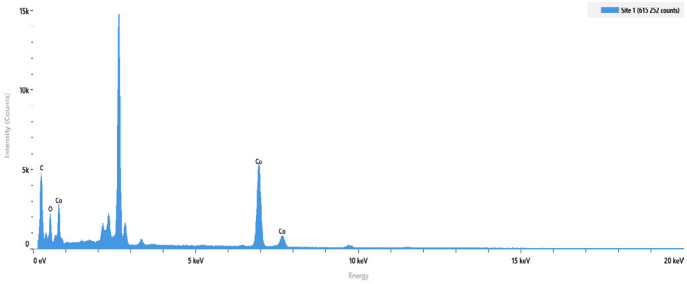

Figure 6: EDX images of biosynthesized Co_3O_4-NPs.

The images reveal a mixture of cuboidal and spherical morphologies, though some degree of agglomeration is observed. Such agglomeration is common in the synthesis of nanoparticles and can be attributed to factors such as van der Waals forces and high surface energy between particles. The EDX spectrum displays a significant signal in the Cobalt area, confirming CoNPs production. Because of plasmon surface resonance, metallic Cobalt nanocrystals often display an optical absorption peak at 1.5 KeV (Figure 6). Data from energy dispersive X-ray (EDX) reveal highly significant Cobalt. The presence of a dense Cobalt peak verified the reduction of Co(NO3)3 to nanoparticles. According to Figure 6, the EDX spectrum result showed that the elements C, O, Cl, and Co had the highest percentage of weight (63.9%, 19.5%, 10.0%, and 6.2%, respectively), while the elements K and Mo had the lowest percentages (0.3%) and 0.1%, respectively. The presence of carbon and oxygen indicates that the surface of the nanoparticles is accompanied by organic molecules, most likely phytochemicals from the green synthesis process. These phytochemicals are thought to play a crucial part in the production and stabilisation of the Co3O4-NPs by acting as both capping and reducing agents during synthesis. The synthesis of the Co3O4 NPs has been confirmed via X-ray diffraction (XRD) analysis. The Bragg's reflections at (100), (101), and (201), respectively, were represented by the diffraction characteristics pertaining to 2θ at 24.95◦, 30.00◦, and 37.65◦ (Figure 7).

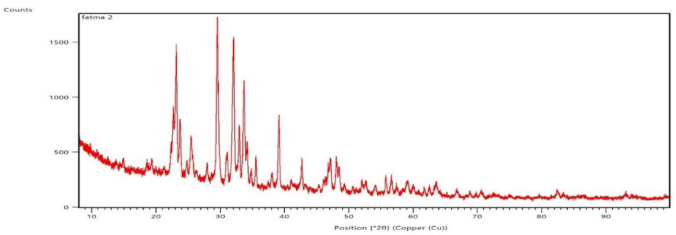

Figure 7: XRD analysis of biosynthesized Co_3O_4-NPs.

The nanoparticles crystal size was 109 nm. Debye Scherrer's equation was used to determine the average crystalline structure size of the biosynthesised Co_3O_4-NPs. In this equation, k stands for the shape constant of the geometric factor (0.9), for wavelength, for line broadening at half-maximum intensity, for Bragg angle, and D for average crystalline size of the nanoparticles. According to Figure 7, the diffraction of the (111) plane has a sharp peak at 2θ = 29.00 that corresponds to Co_3O_4-NPs. This suggests that the synthesised Co_3O_4-NPs are monoclinic and crystalline. During synthesis, photomolecules were deposited on the surfaces of the Co_3O_4-NPs, causing these unknown peaks to form (Figure 7).

$$D = k\lambda / \beta \frac{1}{2} \cos\theta$$

As shown in Table 1, the glucose and HbA1c have been significantly increased at (p≤0.05) after administrated of alloxan, but insulin has been significantly decreased at (p≤0.05) compared with the control group. Treated with cobalt nanoparticles causes a significant decrease in glucose and HbA1c levels but a significant increase in insulin levels at (p≤0.05) compared with the control group. Artemisia herba alba nanoparticles cause a significant decrease in glucose and HbA1c, and a significant increase in the levels of insulin (p≤0.05) compared with the untreated group. Additionally, the table shows a significant decrease in glucose and HbA1c, and a significant increase in insulin level (p ≤ 0.05) compared with the alloxan group. Figure 8 show the levels of glucose, HbA1c, and insulin in all groups. The histological section of the pancreas from the control group reveals the normal architecture of pancreatic parenchyma, comprising normal Langerhans islets and

beta cells (Figure 9A). The pancreas of the alloxan group exhibits a disrupted architecture of pancreatic parenchyma, characterized by atrophied Langerhans islets, degenerative changes in beta cells, and cystic dilation of the pancreatic parenchyma (Figure 9B). CoNPs coated with *A. herba alba* pancreas show regenerated architecture of pancreatic parenchyma, consisting of normal Langerhans islets, normal beta cells (Figure 9C).

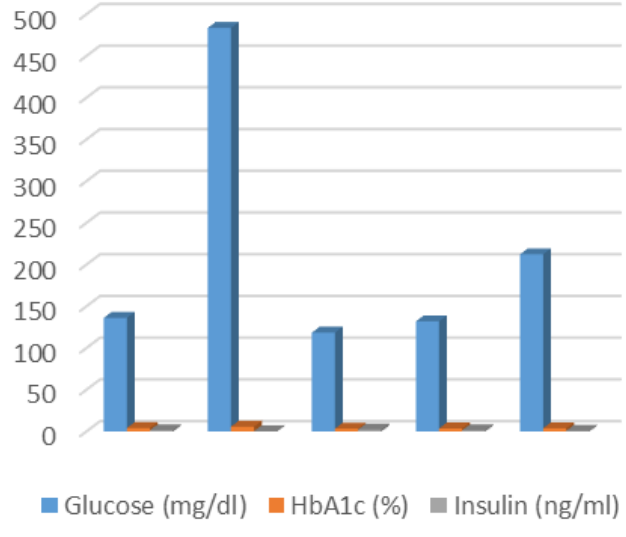

Figure 8: Levels of glucose, HbA1c, and insulin litters refer to significant differences among groups (P≤ 0.05).

Table 1: Glucose, HbA1c, and insulin values in male rats.

Parameters/ Groups	Glucose (mg/dl)	HbA1c (%)	Insulin (ng/ml)
Control (0.9% N.S)	c 136.16 ±15.36	b 4.17 ±0.10	b 1.61 ±0.52
Alloxan (150mg/kg)	a 483.66 ±15.50	a 5.72 ±0.68	e 0.40 ±0.19
CoNPs coated with AHA(104.5µg\ml)	e 118.50 ±8.43	e 3.40 ±0.18	a 2.32 ±0.59
Artemisia herba alba NPs(146µg\ml)	d 132.00 ±12.99	d 3.65 ±0.40	c 1.42 ±0.31
Artemisia herba alba (500mg/kg)	b 212.33 ±37.90	c 3.83 ±0.55	d 0.84 ±0.34
LSD	4.16	0.17	0.19

DISCUSSION

This study showed that treating diabetic animals with *Artemisia herba alba* aqueous extract at a dose of 500 mg/kg once daily for one month caused a significant decrease

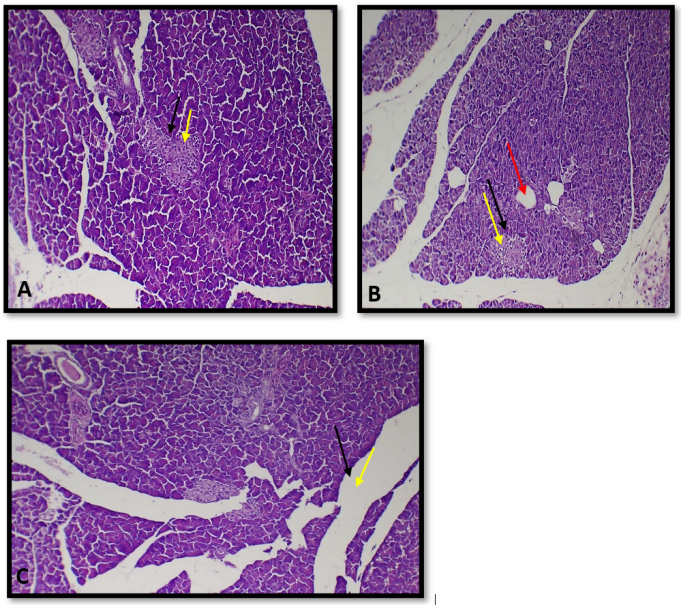

Figure 9: (A) Histological micrograph of the pancreas of the G1 control the normal architecture of pancreatic parenchyma, consisting of normal Langerhans islets (black arrow), normal beta cells (yellow arrow) H&E stain 100X. (B) Histological micrograph of the pancreas of the G2 (alloxan group) shows the disrupted architecture of pancreatic parenchyma, of atrophied Langerhans islets (black arrow), degenerative changes of beta cells (yellow arrow), cystic dilation of pancreatic parenchyma (red arrow). H & E stain 100X. (C) Histological micrograph of the pancreas of the G3(CoNPs coated with *AHA*) treatment group shows architecture of pancreatic parenchyma, consisting of normal Langerhans islets (black arrow) normal beta cells (yellow arrow). H & E stain 100X).

in the levels of glucose. This result was consistent with (Afolayan and Sunmonu, 2013; Mansi and Lahham, 2008). *Artemisia herba alba* contains glycosides and saponins, which reduce the glucose levels (Abdel-Hassan *et al.*, 2002). It also contains flavonoids, which lower cholesterol and triglycerides, increase the secretion of insulin, and stimulate the beta cells of the pancreas to secrete more insulin by increasing the enzyme hepatic glucokinase (Al-Ishaq *et al.*, 2019). The blood glucose-lowering effect could be due to causes beyond the pancreas, such as stimulating its uptake by peripheral tissues (Naik *et al.*, 1991; Obatomi *et al.*, 1994), increasing the activity of glycogenolytic enzymes (Naik *et al.*, 1991), or *Artemisia herba alba* may decrease the secretion of opposing regulatory hormones such as cortisone, growth hormone, and glucagon (Roman-Ramos *et al.*, 1995), or reduce the absorption of glucose from the digestive tract (Akhtar, 1991; Sharma *et al.*, 1996). The study showed that treating diabetic animals with *A. herba alba* aqueous extract at a dose of 500 mg/kg once a day for one month leads to a significant increase in insulin levels in the blood serum. The result was consistent with (Mansi and Lahham, 2008). This is attributed to the fact that

Artemisia herba alba extract contains active substances with an insulin-like effect. This stimulates pancreatic beta cells to secrete more insulin into the bloodstream (Wadood *et al*., 1992), which leads to increased glycogen deposition in the liver, leading to a reduction in glucose levels or an increase in insulin receptors (Kouzi *et al*., 1994).

The saponins found in wormwood also stimulate the secretion of insulin from undamaged pancreatic beta cells (Afolayan and Sunmonu, 2011). The presence of flavonoids in *Artemisia herba alba*, which lowers cholesterol and triglycerides, increases the secretion of insulin, and stimulates the beta cells of the pancreas to secrete more insulin by increasing the enzyme hepatic glucokinase (Al-Ishaq *et al*., 2019) or by increasing the activity of glycogenolytic enzymes (Naik *et al*., 1991), Through increased cell viability, decreased apoptosis, and improved insulin signaling pathways, an ethanolic extract of *AHA* was shown to reduce hyperglycemia (Ansari et al., 2022). *AHA*'s strong antioxidant properties help reduce oxidative stress caused by diabetes. By improving the function of the mitochondrial and reducing reactive oxygen species (ROS) levels, *AHA* protects pancreatic cells and enhances their insulin secretion efficiency (Caturano *et al*., 2023) Due to the properties of the plant and the characteristics of nanotechnology that makes the drugs could be delivered directly to specific parts of the body using nanoparticle engineering, increasing the effectiveness of the treatment and reducing adverse effects (Elumalai *et al*., 2024), and they corset the encapsulated nanoparticles from early metabolic collapse by gut wall or liver enzymes, guaranteeing that the active medication reaches its intended location. By using other absorption pathways to circumvent initial hepatic metabolism, they also eliminate the first-pass impact (Zheng *et al*., 2024).

We reported that *Artemisia herba alba* nanoparticles show an improvement in the decrease in blood glucose levels more than when treated with the extract of Artemisia herba alba. Furthermore, we revealed for the first time that using CoNPs coated with *A. herba alba* to treat diabetes improves glucose, HbA1c, and insulin levels more than using *Artemisia herba alba* extract due to the improvement of the pancreatic beta cells. The study's findings demonstrated that, compared to the negative control group, male rabbits with diabetes had a higher incidence of pancreatic alterations. This finding aligns with research conducted on rats (Rasheed *et al*., 2021). Degenerative alterations in the Endocrine glands secreting from the pancreas, including islet atrophy, islet cell rupture, vascular congestion, and the presence of inflammatory cells, were observed in the diabetic control group. Oxidative stress may link these alterations, as high blood sugar lowers antioxidant levels and increases oxygen radicals (Nugent *et al*., 2008). Moreover, diabetes is frequently linked to deteriorating issues that impact the macrovascular and microvascular systems (Hanssen, 1997), which can lead to congestion. These results are in line with those of Jones *et al*. (2010), who noted that the islets of Langerhans displayed significant necrotic alterations and elevated connective tissue congestion, resulting in a relative reduction in the islets' size. Additionally, diabetic pancreatic tissue sections treated with 500 mg/kg of the aqueous extract showed improvement. This outcome was in line with a study (Ghazanfar *et al*., 2014) that demonstrated that beta cells in pancreatic tissue were repaired and protected by alcoholic wormwood extract at 500 and 250 mg/kg.

The current study provides the first report demonstrating the therapeutic potential of cobalt nanoparticles coated with *Artemisia herba alba* in reduced the hyperglycemia and enhancing the function of pancreatic in alloxan-induced diabetic rats. The treatments show significantly decreased in the levels of glucose and HbA1c while the insulin concentration is increase, indicating enhanced glycemic control. This study revealed markedly pancreatic regeneration in the histological analysis, with restored Langerhans islets and normal beta cells, predominately in the cobalt nanoparticle-treated group. Notably, compared with the others treatment the cobalt nanoparticles exhibited superior efficacy, suggesting their promising role as potent antidiabetic and pancreatic restorative agents in experimental diabetes.

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NOVELTY STATEMENT

This study utilized cobalt oxide nanoparticles to treated diabetes mellitus. To our knowledge, it represents the first investigation to coated cobalt oxide nanoparticles with *Artemisia herba alba* as a drug delivery. While prior research has been explored the effect of Artemisia herba alba extract on diabetic rats. Using cobalt oxide nanoparticles coated with Artemisia herba alba is a practical and effective approach on treating diabetes.

AUTHOR'S CONTRIBUTION

The work that has been provided here was a collaborative effort by all authors. Furthermore, to writing the final publication, each of the authors' influence on the idea, design, the data collection, analysis and interpretation of

the study.

Ethical approval

The study was approved by the Research Ethics Committee of the College of Veterinary Medicine, University of Basrah, according to Protocol No. 94/37/2025, dated September 1, 2024.

Generative AI and AI-assisted technology statement

The authors declare that no genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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