



## Physiological Effects of Cobalt Nanoparticles Coated with *Artemisia herba-alba* Extract on Alloxan-Induced Diabetic Rats

### Article Info.

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#### Abstract

The present study examines the effects of cobalt nanoparticles(CoNPs) coated with *Artemisia herba alba*, *Artemisia herba alba* nanoparticles, and alcoholic extract of *Artemisia herba alba* on diabetic rats on body weight, organs' weight, and selective hematological parameters compared with the untreated group. 30 male laboratory rats were divided into five groups. The control group was injected (I.P) with normal saline (0.9%), and the second group was injected with alloxan (150 mg/kg). For 30 days of treatment, the third group was treated by CoNPs coated with *Artemisia herba alba* (104.5 $\mu$ g/ml), whereas the fourth and fifth groups were treated with *Artemisia herba alba* nanoparticles (146 $\mu$ g/ml) and *Artemisia herba alba* extract (500 mg/kg), respectively. The results show that 30 days of treatment with CoNPs coated with *A. herba alba*, *Artemisia herba alba* nanoparticles and the extract of *Artemisia herba alba*, there is a significant increase in RBC (6.61 $\pm$ 0.24, 6.75 $\pm$ 0.19, 7.04 $\pm$ 0.38) respectively, compared with the untreated group (4.22 $\pm$ 0.48), also there is a significant increase in HB and PCV (10.66  $\pm$  0.38, 32.03  $\pm$  1.06) whereas there is a significant decrease in WBC (8.75  $\pm$  0.75) when treated with CoNPs coated with *Artemisia herba alba* (8.75 $\pm$ 0.75). The results of the current study indicate that treating with cobalt nanoparticles coated with *Artemisia herba alba* extract has a significantly greater therapeutic effect on diabetic rats compared with other interventions tested.

**Keywords:** *Artemisia herba alba*, Cobalt nanoparticles, Diabetes mellitus, Erythropoiesis, Hemoglobin

## Introduction

Nanotechnology is a rapidly advancing field that focuses on the nanoscale manipulation of matter, typically ranging from 1 to 100 nanometers (1). The nanotechnology applications in the medical field are known as "nanomedicine," and this technology addresses the diagnosis, treatment, monitoring, and prevention of diseases (2). Because of their usual characteristics, which include nanoscale size, wide surface chemistry, and customized drug kinetics, nanoparticles are consequently excellent prospects for controlled drug delivery systems. Additionally, nanomaterials have a special surface-to-volume ratio that makes it easy for them to enter tissues deeply and pass through cell membranes (3). Using nanoparticle engineering, medications may be administered specifically to particular bodily areas, improving therapy efficacy and minimizing side effects (4). By promoting drug delivery to particular organs or tissues, nanoparticles reduce toxicity in non-target tissues and systemic exposure. Nanoparticles can avoid the reticuloendothelial system with surface modulation like PEGylation, which lengthens circulation durations and increases accumulation at the target site (5). They protect the encapsulated nanoparticles from early metabolic collapse by gut wall or liver enzymes, guaranteeing that the active medication reaches its intended location. Cobalt nanoparticles (CoNPs) exhibit promising anti-cancer activities in recent years (6). Cobalt is a non-accumulating element in the human body (7), indicating that it does not cause metal toxicity due to cobalt accumulation. Cytotoxicity studies show that cobalt nanoparticles exhibit moderate antiproliferative properties against cancer cells and safe properties toward normal cells. It has also been shown that cobalt nanoparticles are compatible with human red blood cells and have no mischievous effects in the human bloodstream (8). Moreover, cobalt nanomaterials have other uses in medicine, such as antidiabetic and anticholinergic activities (6). Cobalt nanomaterials are considered safe towards normal cells and do not pose any detrimental effects in the circulation of blood, which permits their abundant biological and medical applications. White wormwood plant, also called *Artemisia herba-alba*, is a perennial plant in Mediterranean dry steppes, distinguished by its white, woolly stems and foliage (9). The *Artemisia herba-alba* plant is widely distributed across several geographic areas, including the Iberian Peninsula. It is notably prevalent in eastern, southeastern, and southern Spain, where it forms extensive populations (10). Throughout long-term treatment, *Artemisia herba-alba* may improve metabolic health biochemical indicators and guard against diabetes complications, including peripheral neuropathy (11). Particularly when it comes to managing diabetes, *Artemisia herba-alba* (AHA) has several modes of action that contribute to its hypoglycemic effects (12). By improving the peripheral tissues' ability to absorb glucose, *Artemisia herba alba* increases insulin sensitivity and lowers blood sugar levels (13). The plant may cause the secretion of the insulin hormone from the beta cells in the pancreas. Increased insulin production and release into the bloodstream may result from either the stimulation of precursor cells in the pancreatic duct or the regeneration of beta-cell bulk (14). Significant antioxidant qualities that have been found in *Artemisia herba alba* aid in reducing the oxidative stress linked to diabetes. *Artemisia herba alba*

can preserve and enhance the function of pancreatic beta cells by reducing oxidative damage, which will boost insulin secretion and general metabolic health (15).

## Materials and Methods

### Collection of the *Artemisia herba alba*:

*Artemisia herba alba* has been collected from the Basrah local market and stored in tightly capped glass bottles away from light, heat, and moisture until ready for use. The Agriculture College and the College of Science at the University of Basrah performed the plant's identification (16).

### Preparation of plant extract

To prepare the extract of the plant, 100 g of air-dried ground plant material was extracted using an aqueous methanol solvent (methanol: water, 80% v/v) (500 ml) for eight hours under Soxhlet extraction on a water bath in independent tests. A rotary evaporator was used to concentrate and remove the solvent from the extracts at 45°C while the pressure was lowered. Before being employed, the dried crude concentrated extracts were kept in a refrigerator at 4°C after being weighed to determine the yield (17).

### Hydrothermal Method of CoNPs Biosynthesis

With certain adjustments, Ansari *et al.* (18) produced cobalt nanoparticles using the hydrothermal technique. To create cobalt oxide (Co<sub>3</sub>O<sub>4</sub>) nanoparticles, 10 millilitres of *Artemisia herba alba* extract at a concentration of 200 mg/ml was first added to 6 grams of cobalt nitrate solution. A KOH solution was then added, and the mixture was stirred for fifteen minutes. After adding hydrazine monohydrate and oleic acid solutions until the mixture's volume equalled two-thirds of the autoclave's total volume, the mixture was stirred for two hours to completely dissolve the solid reagents. And after that, the mixture was put into an electric oven with a Teflon coating and kept at 160 °C for 24 hours to allow the reaction to set. The autoclave was left to cool to ambient temperature after twenty-four hours spontaneously. In order to separate the produced CoNPs from the liquid phase, the final solution was centrifuged, the liquid phase was decanted, and the resulting black precipitate was dried in an oven set at 70 °C (18).

### Experimental Animals

Test animals, 30 mature domestic 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. The room temperature was 21-25°C, and the light period was 12h using 2 fluorescent lamps (19). The animals were randomly divided into five groups, each containing six male rats, and were given the following care for a month:

Group 1: a control group, giving normal saline 0.5ml (I.P)  
 Group 2: Each rat was injected with alloxan (150 mg/kg) (20).  
 Group 3: Each rat was daily treated with cobalt nanoparticles coated with *A. herba alba* (104.5 $\mu$ g/ml) (I.P)  
 Group 4: Each rat was daily treated with *Artemisia herba alba* nanoparticles (I.P) (146 $\mu$ g/ml)  
 Group 5: Each rat was orally treated daily with *Artemisia herba-alba* extract (500 mg/kg) (20).

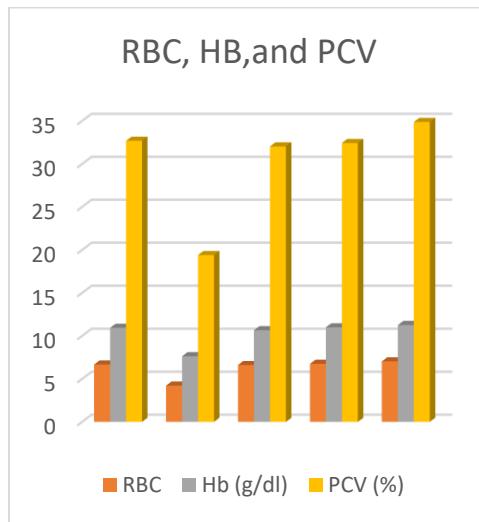
## Results

In the table (1), injection of alloxan leads to a decrease in (RBC, Hb, PCV) compared to the control at ( $P \leq 0.05$ ). At the same time, the treated cobalt nanoparticles coated with AHA show no significant difference in RBC, Hb, and PCV compared with the control group ( $P \leq 0.05$ ); the values are close to those of the control. In the treated group of *Artemisia herba alba* nanoparticles, there was a significant increase in RBC and Hb, and no significant difference in PCV concentration ( $P \leq 0.05$ ) compared to the control group. In the same table, the data show a significant increase ( $P \leq 0.05$ ) in RBC, Hb, and PCV concentration compared with the healthy group.

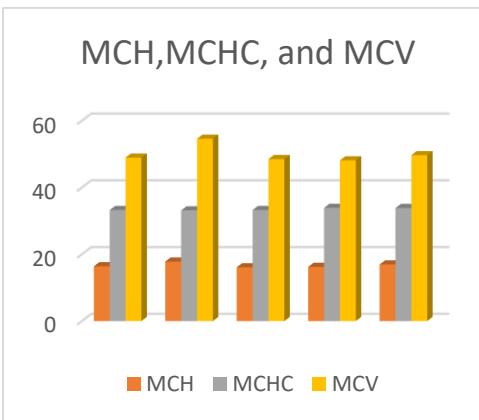
**Table (1): Effect of CoNPs, *Artemisia herba alba* nanoparticles and *A. herba alba* extract on RBCs, Hb, PCV**

Parameters	RBC (10 <sup>12</sup> /L)	Hb (g/dl)	PCV (%)
<b>Groups</b>			
<b>Control (0.9% N.S)</b>	c 6.67 ±0.46	c 10.94 ±0.56	b 32.69 ±2.07
<b>Alloxan (150mg/kg)</b>	e 4.22 ±0.48	e 7.63 ±0.34	e 19.40 ±9.53
<b>Cobalt nanoparticles coated with AHA(104.5<math>\mu</math>g/ml)</b>	d 6.61 ±0.24	d 10.66 ±0.38	d 32.03 ±1.06
<b>Artemisia herba alba nanoparticles (146<math>\mu</math>g/ml)</b>	b 6.75 ±0.19	b 11.00 ±0.41	c 32.43 ±1.02
<b>Artemisia herba alba (500mg/kg)</b>	a 7.04 ±0.38	a 11.27 ±0.72	a 34.88 ±2.98
<b>LSD</b>	0.06	0.05	0.26

Different litters refer to significant differences among groups ( $P \leq 0.05$ )

**Figure 1: RBC, HB, and PCV)**

The table(2) shows a significant increase ( $p \leq 0.05$ ) in MCH and MCV in the diabetes group and no significant difference in MCHC. While the treated with cobalt nanoparticles show no significant difference compared with the control, the value is close to the control group at ( $p \leq 0.05$ ). At the same time, Artemisia herba alba nanoparticles treated show no significant difference in MCH, a significant increase in MCHC, and a significant decrease in MCV at ( $p \leq 0.05$ ). Treating with Artemisia herba alba causes a significant increase in MCH and MCHC at ( $p \leq 0.05$ ), and there is no significant difference in MCV.

**Figure 2 (MCH, MCHC, and MCV)**

**Table (2): Effect of CoNPs, *Artemisia herba alba* nanoparticles and *A. herba alba* extract on MCH, MCHC, and MCV.**

Groups	parameters	MCH (pg)	MCHC (g/dl)	MCV (FL)
<b>Control (0.9% N.S)</b>	c 16.40 ±0.49	a 33.26 ±0.26	c 48.96 ±0.60	
<b>Alloxan (150mg/kg)</b>	a 17.80 ±0.87	a 33.15 ±0.16	a 54.60 ±2.20	
<b>Cobalt nanoparticles coated with AHA(104.5µg/ml)</b>	e 16.10 ±0.17	a 33.26 ±0.17	d 48.50 ±0.54	
<b>Artemisia herba alba nano(146µg/ml)</b>	d 16.23 ±0.20	a 33.90 ±1.47	e 48.13 ±1.68	
<b>Artemisia herba alba (500mg/kg)</b>	b 16.95 ±1.23	a 33.86 ±1.60	b 49.68 ±2.77	
<b>LSD</b>	0.13	0.00	0.36	

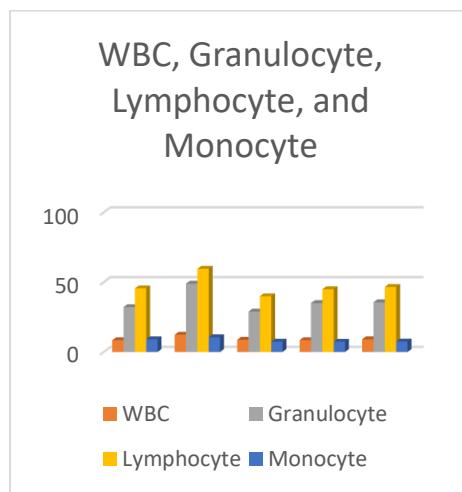
Different litters refer to significant differences among groups (  $P \leq 0.05$ ), the same litters refer to no significant differences among groups (  $P \leq 0.05$  )

Table (3) shows a significant increase in WBC, granulocyte, lymphocyte, and monocyte at ( $p \leq 0.05$ ) compared with the control group when injected with alloxan. Treatment with cobalt nanoparticles results in a significant decrease in WBC, lymphocyte, granulocyte, and monocyte counts ( $p \leq 0.05$ ) compared with the alloxan group. While treated with *Artemisia herba alba* nanoparticles, there is also a significant decrease in granulocyte monocyte, WBC, and lymphocyte at ( $p \leq 0.05$ ), compared to the alloxan group. *Artemisia herba alba* treatment results in a significant decrease in WBC, granulocytes, lymphocytes, and monocytes ( $p \leq 0.05$ ) compared with the alloxan group.

**Table (3): Effect of CoNPs, *Artemisia herba alba* nanoparticles and *A. herba alba* extract on WBC, granulocyte, lymphocyte, and monocyte concentration**

Groups	Parameters	WBC (10 <sup>9</sup> /L)	Granulocyte (%)	Lymphocyte (%)	Monocyte (%)
<b>Control (0.9% N.S)</b>	e	8.44 ±1.50	32.13 ±4.70	45.60 ±3.11	9.15 ±1.07
<b>Alloxan (150mg/kg)</b>	a	12.41 ±1.02	a 48.95 ±5.37	a 59.63 ±2.24	a 10.56 ±0.78
<b>Cobalt nanoparticles coated with AHA(104.5µg/ml)</b>	c	8.75 ±0.75	e 28.96 ±1.26	e 39.95 ±1.64	d 7.35 ±0.88
<b>Artemisia herba alba nano(146µg/ml)</b>	d	8.46 ±0.99	c 35.06 ±3.31	d 44.98 ±2.49	e 7.33 ±1.07
<b>Artemisia herba alba (500mg/kg)</b>	b	9.11 ±1.09	b 35.61 ±2.74	b 46.61 ±2.40	c 7.45 ±0.94
<b>LSD</b>	0.02	0.55	0.61	0.01	

Different litters refer to significant differences among groups (  $P \leq 0.05$  )

**Figure 3 (WBC, Granulocyte, Lymphocyte ,Monocyte)**

## Discussion

The study results showed that the induction of diabetes with alloxan caused a significant decrease in the number of RBCs in the affected animals compared to the healthy control (Table 3). This is consistent with the results of the study conducted on rats. (21) This may be due to the development of diabetes in rats, which results in a reduction in the activity of the Na-K-ATPase enzyme in the membranes of red blood cells, resulting in hemodynamic dysfunction due to the change in microvascular blood flow, and rheological abnormalities are hastened by decreased RBC deformability and increased fluidity. This leads to a disturbance in capillary circulation, which can cause the decomposition of some red blood cells and the occurrence of anemia (22). According to (21), hyperglycemia is associated with modifications in the lipid membrane's constituent components, which alter the flexibility of red blood cells and ultimately lead to their breakdown. Red blood cell counts rise after treatment with the *Artemisia herba alba* aqueous extract. This outcome aligns with research (23) that demonstrates administering the aqueous extract of the plant causes a notable increase in red blood cells and returns them to normal levels. Because *Artemisia herba alba* plants have potent antioxidants that can save red blood cells from harm, this illustrates the plant's hematoprotective (or disease-preventing) properties. resulting from a rise in free radicals, which lowers the amount of lipid peroxide in RBC membranes, reducing the cells' capacity to clot (24). The results showed that alloxan-induced diabetes leads to a significant decrease in blood hemoglobin levels (HB) in male albino rats compared to healthy rats. The study was consistent with the results of (21), which were conducted on rats, and with the findings of (25, 26), who indicated in their study on diabetic patients that hemoglobin levels were lower, and the decrease increased when accompanied by an increase in creatinine concentration. The increase in antibodies may demonstrate the decrease in hemoglobin levels, the release of which is enhanced when diabetes occurs, resulting from the destruction of beta cells in the islets of Langerhans as a result of the effect of autoimmunity, which results in a reduction in B12 absorption, and damage to the intestinal mucosa (27). It is believed that the cause is due to the reduction of insulin, which leads to increased lysosomal activity, resulting in the breakdown of RBCs (28). Increased blood glucose levels cause hemoglobin to react with glucose to create HbA1c, which lowers hemoglobin levels in the blood (29). The significant increase in hemoglobin in the blood cells is caused by the aqueous extract of *A. herba alba*'s ability to regulate blood parameters. This result is similar to a study of (30) that found that when treated with *Artemisia herba alba* aqueous extract had higher hemoglobin because of a decrease in blood glucose levels, improving and bringing their hemoglobin levels closer to normal. This makes the connection between low hemoglobin levels and high blood glucose strong. According to this study's findings, male albino rats' blood levels of PCV drop when alloxan is employed to cause diabetes (31). This agrees with the study conducted by (23) on male albino rats that were administered diabetes. They noticed that the amounts of hemoglobin in the blood were closely connected with the volume of packed cells. Additionally, they found that factors influencing the number of red blood cells also influenced the volume of packed cells and hemoglobin concentration (20).

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The study showed that administering an aqueous extract of *A. herba alba* once daily for a month at a concentration of 500 mg/kg enhanced the volume of packed cells in mice with oxytocin-induced hyperglycemia. This result is consistent with a study (23) that showed the volume of packed cells was enhanced by an aqueous extract of *Artemisia herba alba*. The results show a significant increase in RBC, Hb, and PCV when treated with CoNP coated with AHA because it is a component of vitamin B12, which is beneficial for the treatment of anemia since it increases the formation of RBCs (6). Red blood cells, hemoglobin, and PCV all improve when *Artemisia herba alba* nanoparticles are used. Apart from the glycosylation of proteins, hyperglycemia also affects red blood cells through increased clumping, osmotic fragility, and changed mechanical and intrinsic viscosity, all of which affect hemodynamic characteristics and red blood cell structure. These changes may be reflected in one or all of the red blood cell parameters, such as red blood cell count, hemoglobin, HCT, MCV, MCH, and MCHC (32, 33). In Table 2, Treated with CoNP coated with AHA and *A. herba alba* nanoparticles, there is an improvement in (MCH, MCHC, and MCV). In Table 3, the results of the study have shown that alloxan-induced diabetes leads to an increase in the number of white blood cells in the blood of male albino rats. This is consistent with the result of (34) on rats with alloxan-induced diabetes. The number of white blood cells increases due to an increase in the rate of neutrophil production. The study, which was conducted on the effect of alloxan on the number of WBCs in rats with diabetes, indicated that the reason for the increase in neutrophils was that the cells themselves suffer from Discouragement in migration in the diabetic patients (35).

The inhibition of neutrophil migration in individuals with insulin-dependent diabetes due to pancreatic antigens causes them to accumulate in the bloodstream and subsequently increase in number (36). High blood glucose levels affect chemotaxis and obstruct neutrophil migration, or glucose may interact directly with receptors on the surface of neutrophils (36) or lead to the glycation of some blood proteins. The glycated proteins then bind to receptors on the surface of neutrophils, obstructing chemotaxis and inhibiting the migration of these cells to various tissues in the body (37). Given that neutrophils represent a large proportion of the body's population. When treated with the aqueous extract of wormwood, it may cause a decrease in the number of white blood cells, and this is consistent with (30, 23). *This is due to the presence of the main component of the herb artemisinin, which is used effectively against fungal and Parasitic infections, especially the malaria parasite* (38). The result of CoNP coated with AHA and *A. herba alba* nanoparticles shows a significant decrease in WBC level, more than when treated with *A. herba alba* extract. For the first time in our study, we found that diabetic rats treated with cobalt nanoparticles coated with *Artemisia herba alba* (AHA) had notable improvements in haematological indicators. Specifically, after delivering cobalt nanoparticles coated with AHA, the number of red blood cells (RBCs), hemoglobin concentration (Hb), and packed cell volume (PCV) all greatly rose. In contrast, the number of white blood cells (WBCs) dramatically dropped.

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Furthermore, after being treated with *Artemisia herba alba* nanoparticles (AHA-NPs), rats with experimentally induced diabetes had notable alterations in blood parameters, including increased erythropoiesis and reduced leukocyte counts. These findings show that AHA-NPs and AHA-coated cobalt nanoparticles have the potential to improve haematological profiles in diabetic animal models. For the first time, our study showed that treating diabetes in diabetic rats with cobalt nanoparticles coated with *Artemisia herba alba* (AHA) led to a significant improvement in haematological markers. Specifically, the injection of cobalt nanoparticles coated with AHA led to a notable rise in hemoglobin (Hb) level, packed cell volume (PCV), and red blood cell (RBC) count and a substantial drop in white blood cells (WBCs). Furthermore, following treatment with *Artemisia herba alba* nanoparticles (AHA-NPs), rats with experimentally induced diabetes showed comparable tendencies of enhanced erythropoiesis and reduced leukocyte levels, leading to notable changes in their hematological investigations. These results suggest that AHA-NPs and AHA-coated cobalt nanoparticles may be utilised in medicine to alter blood parameters in diabetic rats.

## Conclusion

This study concludes by showing that diabetic laboratory rats' haematological parameters are significantly altered by treatment with cobalt nanoparticles coated with *Artemisia herba alba* (AHA), *A. herba alba* nanoparticles, and the ethanolic extract of AHA. In particular, all techniques led to a significant decrease in the number of white blood cells (WBCs) and a significant rise in hemoglobin (Hb), packed cell volume (PCV), and red blood cells (RBC). Although the decrease in WBCs necessitates additional research to evaluate the immunological implications and safety profile of these interventions, the comparable trends seen across the various treatments indicate that both the composite nanoparticles and the plant extract of *Artemisia herba alba* play significant roles in improving erythropoietic and oxygen-carrying capacities in diabetic rats.

## Conflicts of interest

The authors declare that there is no conflict of interest.

## 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.

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### التأثيرات الفسيولوجية لجسيمات الكوبالت النانوية المغلفة بمستخلص عشبة الشيح على الجرذان المصابة بمرض السكري المستحدث بالألوكسان

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### الخلاصة

هدفت هذه الدراسة إلى تحديد تأثير جسيمات الكوبالت النانوية المحمل عليها الشيح, جسيمات الشيح النانوي, والمستخلص الكحولي لنبات الشيح على بعض المعايير الفسيولوجية في ذكور الفئران المصابة بمرض السكري باستخدام مادة الألوكسان. تم استخدام 30 جرذ مختبري محلي, وقسمت إلى خمس مجموعات تضم (6) حيوانات لكل مجموعة. المجموعة الأولى هي مجموعة الضبط وقد تم حقنها بمحلول فسيولوجي ملحي لمدة شهر وعند مجموعة السيطرة السالبة. أما المجموعة الثانية والثالثة والرابعة والخامسة فقد استحدثت بها مرض السكري عن طريق حقنها بمادة الألوكسان (150 ملغم/كغم) من وزن الجسم تحت الصفاق, وعند المجموعة الثانية مجموعة السيطرة الإيجابية. أما المجموعة الثالثة تم علاجها بجسيمات الكوبالت النانوية المحمل بها الشيح بجرعة 104.5 مائكروغرام/مل. المجموعة الرابعة تم علاجها بجسيمات الشيح النانوي وبجرعة 146 مائكروغرام/مل. أما المجموعة الخامسة فقد تم علاجها بمستخلص الشيح الكحولي بجرعة 500 ملغم/كغم, ولمدة شهر. جمعت عينات الدم بعد شهر من العلاج وتم فحص تعداد الدم الكامل. أظهرت نتائج الدراسة أن العلاج لمدة ثلاثين يوماً لذكور الجرذان المستحدث فيها مرض السكري بجسيمات الكوبالت النانوية المحملة بالشيح أدى إلى تحسن واضح في مؤشرات الدم الحيوية، حيث لوحظ ارتفاع معنوي في عدد كريات الدم الحمراء والهيموغلوبين وحجم كريات الدم المكدة مقارنةً بالمجموعة غير المعالجة مع انخفاض ملحوظ في الخلايا البيضاء. كما لوحظ أن المجموعة الرابعة والخامسة أظهرت تأثيرات علاجية مشابهة لكن أقل فاعلية. سجلت الدراسة، ولأول مرة، قدرة جسيمات الكوبالت النانوية المحملة بالشيح على تعزيز وتحسين تكون الكريات الحمراء والقدرة على تقليل خلايا الدم البيضاء، مما يدل على دورها الفعال في تحسين الحالة الدموية والوظائف المناعية لدى الجرذان المصابة بالسكري.

**الكلمات المفتاحية:** نبات الشيح الأبيض, جسيمات الكوبالت النانوية, داء السكري, تكون الخلايا الدم الحمراء والهيموغلوبين.