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Investigating the Therapeutic Role of *Withania somnifera* in Metabolic Regulation in Diabetic Rat Models

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Abstract Withania somnifera (Ashwagandha) is a traditional plant with medicinal properties used in the treatment of various diseases. It possesses potential in the management of diabetes; however, its efficacy relative to standard therapies and the advantages of nano-formulation remain inadequately investigated. This study aims to clarify the relative effects of Withania somnifera extracts (WSE) and nanoparticles (WNAE) compared to metformin on alloxan-induced diabetic rats. Focusing on the moderation of metabolic regulatory indices, such as glucose level, insulin sensitivity, and lipid metabolism, very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), the research assesses the mechanisms underlying how WS preparations influence metabolic pathways, as well as their effectiveness compared to the existing standard of care. The experimental groups consisted of 32 Wistar male rats, which were divided into the following groups: non-diabetic control (G1), diabetic untreated (G2), diabetic AE-treated (G3), diabetic NAE-treated (G4), and diabetic metformin-treated (G5). Metabolic parameters were monitored over a 30-day period. The findings revealed reduced blood glucose levels and normalized insulin levels in rats treated with both AE and NAE. Markedly improved lipid profiles, with NAE attaining the most significant reductions in LDL and VLDL and the highest increases in HDL, surpassing the effects of metformin. Weight loss was minimal in the metformin and NAE groups compared to the diabetic control group. The Nano-formulation enhances the bioavailability and efficacy of ashwagandha in regulating glucose, insulin, and lipid levels, exhibiting effects comparable to or superior to those of metformin. These results suggest that NAE represents a promising adjunctive therapy for diabetes management, enhancing the bioavailability and efficacy of ashwagandha in regulating glucose, insulin, and lipid levels, with effects comparable to or superior to those of metformin. Thus, NAE represents a promising adjunctive therapy for diabetes management.

Key words: *Withania somnifera*, *Ashwagandha* nanoparticles, antidiabetic, alloxan, metformin.

Introduction Diabetes mellitus is a global public health crisis that affects many people worldwide with type 2 diabetes (T2D), making up most (90-95%) of the cases [1]. This metabolic disorder is manifested in chronic and persistent hyperglycemia due to the functional abnormalities in the production of insulin and/or vasodilation, and subsequently the development of serious complications associated with cardiovascular disease, neuropathy, nephropathy, and retinopathy [2]. The pathophysiology of type 2 diabetes (T2D) is complex and depends on the interaction between genetic predisposition and environmental factors. The key processes are insulin resistance and β-cell dysfunction of the pancreas[3]. This is because with the current increase in the prevalence of diabetes throughout the

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world, treatment plans that not only address and control the diabetes but also prevent the development and complications of the associated metabolic derangement are urgently needed.

The current pharmacological treatment, particularly metformin, has been proven effective in treating individuals with T2D through various mechanisms, including the inhibition of hepatic glucose production and the enhancement of insulin sensitivity [4]. However, the traditional interventions have some significant drawbacks, including gastrointestinal side effects, risks of vitamin B12 deficiency as the treatment progresses, and incomplete recovery of metabolic homeostasis [5]. Moreover, even with proper dosing, a substantial proportion of patients still do not respond well to glycemic control, further underscoring the need to use alternative or complementary therapeutic approaches [6]. Thus, there is a growing search for new drugs that are safer and more effective, resulting in scholars focusing on natural products with anti-diabetic properties.

WS, also known as ashwagandha, is a highly popular medicinal plant that is recommended for purchase with or without a prescription [7]. Modern scientific studies have confirmed most of the traditional indications and have developed a wide pharmacological profile, of antioxidant, anti-inflammatory, immunomodulatory, and, in particular, anti-diabetic effects [8]. Much of the therapeutic effect of WS is attributed to its phytochemical homogeneity, primarily the steroidal lactones (withanolides), which have been observed to have significant biological effects in various disease models [9].

Comprehensive scientific research has demonstrated the anti-diabetic properties of WS through a multifaceted mode of action of these compounds which enhance insulin sensitivity and glucose homeostasis, particularly by protecting pancreatic beta cells against oxidative stress and inducing glucose uptake in peripheral tissues [10,11]. Additionally, the antioxidant effects of WS are crucial in mitigating oxidative stress in pancreatic islets, which is a key factor in the pathogenesis of diabetes[12][13].

Overall, promising results were published in the preclinical studies; however, conventional WS extracts have their therapeutic power limited by poor aqueous solubility, low bioavailability, and high systemic clearance [14]. Recent advances in nanotechnology offer a promising solution to these shortcomings: nanoparticle formulations of WS have demonstrated enhanced dissolution rates, greater protection of bioactive constituents, and increased cellular uptake [15]. These formulations can allow for reducing the doses used in therapy while maintaining the same or even increasing the pharmacological effect [16].

Although the actions of both WS and metformin on type 2 diabetes management have been independently proven separately, were there is a lack of comparative analysis of their relative efficacies [17].

Material and methods

Ethical Considerations

The Committee for Animal Care and Use at the Faculty of Veterinary Medicine, University of Al Qadisiyah, reviewed and approved all procedures for this study (Approval Number: 3726, dated 8/9/2025).

Ashwagandha extraction

Ashwagandha root was extracted with distilled water using a Soxhlet method over four days, then dried and reconstituted for testing at a concentration of 2 mL per animal. Initially, roots were finely ground; later, an ethanol-water mixture was used for extraction, and the solution was evaporated to remove solvents [18].

Ashwagandha nanoparticle extraction

The dry residue from the previous extraction step was re-dissolved in a mixture of ethanol and distilled water for Nano-formulation. It was homogenized with a sonicator using 375 mL and 500 mL of distilled water to optimize dispersion [19]. After homogenization, the extract was re-evaporated, collected, and stored in a refrigerator. About 2 grams of the dried extract were dissolved in 10 mL of distilled water with PBS at pH 7.2, then 2 mL was administered to each rat daily [20].

Experimental animals

Thirty-two adult Wister male rats were included in this experiment; 24 rats received 1 mL of alloxan (0.43 g/kg) via injection (diabetes developed within 48 hr), while the last eight received distilled water only as a negative control. Rats were randomly divided into five groups (8 each).

- Negative group (G1): Eight rats received distilled water (not diabetic).
- Positive group (G2): Eight rats induced diabetes without treatment [21].
- Ashwagandha group (G3): Eight diabetic rats were treated with ashwagandha extract [22].

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- Nanoparticle group (G4): Eight diabetic rats were treated with nanoparticles of ashwagandha [23].
- Metformin group (G5): Eight diabetic rats received metformin (200 mg/kg/day) [24].

Body weight

Experimental animals were weighed on day zero and at the end of the experiment. Rats were gently placed in an empty container, and their weights were quickly recorded to minimize errors and detect treatment-related weight differences.

Blood Collection and Biochemical Analysis

Blood Collection

At the end of the experiment, about 5 mL of blood was drawn directly from the heart via cardiac puncture after inhalation anesthesia with chloroform (Cristalco, France) using a 5 mL disposable syringe. The blood samples were transferred into plain gel tubes, allowed to clot at room temperature for 10 to 20 minutes, and then centrifuged at 3500 rpm for 5 minutes. The serum was subsequently aliquoted into labeled Eppendorf tubes (Sigma, England) and stored at -20°C until analysis of insulin and lipid profiles [25].

Glucose Measurement

Blood samples were collected from tail vein after disinfected with 70% alcohol and glucose levels were measured using a portable meter (Glucometer, Hansa Medical) to enhance accuracy and minimize invasiveness [26].

Insulin Measurement

Insulin concentrations were quantified in serum samples according to the established blood collection protocol. Fasting insulin levels were ascertained utilizing a radioimmunoassay kit, after an overnight fast of eight hours [27].

Lipid Profile Measurement

Serum concentrations of VLDL, LDL, and HDL were measured using radioimmunoassay (RIA) [28].

Histopathological studies

After sacrificing the anesthetized animal with chloroform, tissues from the liver and kidney were fixed in 10% formalin for 2-3 days. Then samples were fixed in a sectioned to of 0.5 µm thickness and placed in plastic cassettes [29]. The Italian tissue processor Histo-Line Laboratories ATP1000 automatically dehydrates and clears the tissue. Dehydrated tissues were embedded in paraffin wax using the Histo-Line Laboratories HESTION TEC2900 embedding system, controlled by a TEC2900 Thermal Console (Histo-Line Laboratories, Italy). Tissue blocks were sectioned at 4-5 µm with a Histo-Line Laboratories MRS3500 rotary microtome. Sections were floated in a water bath at 37°C and on a hot plate, both of which were regulated by the TEC2900 Thermal Console, before being mounted on slides. Sections were stained with Hematoxylin and Eosin (H&E, Dakocytomation, Denmark) and examined under a light microscope (Olympus, Japan) at 40× and 10× magnifications for histological assessment [30,31].

Data Analysis

The findings of the present study were analyzed utilizing the SPSS software, version 27, and are expressed as mean ± SEM. Quantitative variables were examined using a one-way analysis of variance (ANOVA), with the Least Significant Difference (LSD) test employed as a post-hoc analysis to compare the means across different rat groups. Results with a P-value less than 0.05 were deemed to be statistically significant [32].

Result

Effect of treatment on body weight

Table present the mean body weights (± standard error) of five groups of rats before and after treatment, along with the corresponding weight changes. The G1 group exhibited a slight but non-significant increase in body weight (248.37 ± 7.02 to 251.37 ± 7.05 g), with a mean weight gain of 3 ± 0.46 g. In contrast, the G2 exhibited a significant weight loss of -6.75 ± 2.12 g (from 258.62 ± 9.72 to 246.87 ± 9.35 g), indicating the adverse effects of diabetes. Furthermore, G3 also experienced weight loss (250.12 ± 8.70 to 246.62 ± 7.29 g), with a mean reduction of -3.50 ± 1.47 g, while the group G4 showed a similar trend, with a weight loss of -2.62 ± 0.38 g (from 255.50 ± 8.60 to 252.87 ± 8.74 g). Meanwhile, the G5 demonstrated a comparatively lower weight loss of -1.75 ± 0.72 g (from 261.75 ± 3.57 to 260 ± 3.28 g). Statistical analysis revealed significant differences in weight change among groups, with different superscript letters indicating significance at $P < 0.05$ according to the LSD test.

Table 1. The table represents the weights (Mean ± SE) of five groups of rates.

Groups	Day zero	End the experiment	Weight change
G1	248.37±7.02a	251.37±7.05a	3±0.46a

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G2	258.62±9.72a	246.87±9.35a	-6.75±2.12c
G3	250.12±8.70a	246.62±7.29a	-3.50±1.47bc
G4	255.50±8.60a	252.87±8.74a	-2.62±0.38bc
G5	261.75±3.57a	260±3.28a	-1.75±0.72ab
LSD(P<0.05)	22.51	21.40	15.07

1- G1 (negative Control): No person with diabetes treated.
2- G2 (positive control): Treated with alloxan to induce diabetes.
3- G3 (*Ashwagandha* extract): Diabetic with alloxan, and treated with *ashwagandha* extract to study its effect on treating diabetes.
4- G4 (Nano *Ashwagandha*): Diabetic with alloxan, and treated with banana *ashwagandha* to study its impact on treating diabetes.
5- G5 (Metformin): Diabetic with alloxan, and treated with metformin to study its effect on treating diabetes.
Different letters between any two means, vertically denoted, indicate significant differences at P < 0.05.

Effect of Ashwagandha Treatment on the Insulin and Glucose Levels

The insulin concentrations (Mean ± SE) measured in the different experimental groups revealed significant variations following treatment, Table 2. G1 maintained near-normal insulin levels at 14.43 ± 0.20 µIU/mL. In contrast, the diabetic rats in (G2), showed a substantial and statistically significant reduction in insulin secretion, with levels dropping to 8.69 ± 0.62 µIU/mL,. Notably, rats treated with ashwagandha extract exhibited the highest insulin levels at 16.33 ± 1.06 µIU/mL, indicating a potential stimulatory effect on insulin secretion. The rats in G4, also showed improved insulin levels (13.86 ± 0.89 µIU/mL), which were significantly higher than those in G1. Similarly, metformin treatment presented an increase in insulin concentrations of 15.17 ± 0.92 µIU/mL, which closely aligns with the values observed in the ashwagandha-treated groups. These results demonstrate the potential of both ashwagandha and metformin to restore insulin secretion in diabetic conditions.

Glucose measurements also revealed significant differences among the treatment groups, Table 2. The G1 maintained normal blood glucose levels at 99.25 ± 2.54 mg/dL. As expected, the G2 showed a dramatic and statistically significant elevation in glucose concentration, reaching 459.5 ± 30.4 mg/dL, indicative of severe hyperglycemia. Treatment with ashwagandha extract showed a significant reduction in glucose levels to 128.25 ± 12.3 mg/dL. Likewise, Nano-ashwagandha lowered glucose to 117.88 ± 12.19 mg/dL, demonstrating slightly greater efficacy than the conventional extract. Metformin-treated rats also experienced notable improvement, with glucose levels at 127 ± 15.1 mg/dL, comparable to those in the ashwagandha groups. All treatment groups (G3–G5) showed statistically significant reductions in glucose levels compared to the diabetic control, supporting their therapeutic value in glycemic regulation.

Table 2. Insulin and glucose levels in diabetic rats treated with *ashwagandha* (Mean ± SE)

Groups	Insulin	Glucose
G1	14.43±0.20ab	99.25±2.54b
G2	8.69±0.62c	459.5±30.4a
G3	16.33±1.06a	128.25±12.3b
G4	13.86±0.89b	117.875±12.19b
G5	15.17±0.92ab	127±15.1b
LSD(P<0.05)	2.32	49.16

1- G1 (negative Control): No person with diabetes treated.
2- G2 (positive control): Treated with alloxan to induce diabetes.
3- G3 (*Ashwagandha* extract): Diabetic with alloxan, and treated with *ashwagandha* extract to study its effect on treating diabetes.
4- G4 (Nano *Ashwagandha*): Diabetic with alloxan, and treated with banana *ashwagandha* to study its impact on treating diabetes.
5- G5 (Metformin): Diabetic with alloxan, and treated with metformin to study its effects in treating diabetes.
Different letters between any two means, vertically denoted, indicate significant differences at P < 0.05.

Effect of Treatment with Ashwagandha on Lipid Profile Concentrations

Table (3), illustrated the mean values (± SE) of lipid profile parameters (VLDL, HDL, and LDL) in five groups

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of rats following treatment. The G2, exhibited significant dyslipidemia, as indicated by elevated VLDL (22.75 ± 1.27 mg/dL) and LDL (156.94 ± 3.21 mg/dL) levels, alongside a marked reduction in HDL (37.75 ± 0.95 mg/dL). In contrast, the G1 maintained normal lipid levels, with VLDL at 19.19 ± 0.37 mg/dL, HDL at 48.38 ± 1.67 mg/dL, and LDL at 56.25 ± 2.34 mg/dL. Treated rats in G3 significantly improved lipid parameters, resulting in a reduction in LDL (55.13 ± 4.74 mg/dL), an increase in HDL (58.13 ± 1.73 mg/dL), and a slight increase in VLDL level to 20.25 ± 0.49 mg/dL.

Similarly, G4 group demonstrated strong hypolipidemic effects, with VLDL levels at 19.23 ± 0.70 mg/dL, an increase in HDL levels at 56.75 ± 1.03 mg/dL, and an increase in LDL levels at 52.61 ± 2.28 mg/dL. Metformin-treated rats also showed improvement over the diabetic control, with moderately regulated lipid levels: VLDL at 21.31 ± 0.52 mg/dL, HDL at 51.50 ± 1.67 mg/dL, and LDL at 60.08 ± 3.48 mg/dL. Statistical analysis revealed significant differences among groups ($P < 0.05$), indicating the beneficial impact of ashwagandha and metformin in restoring lipid balance in diabetic conditions.

Table 3. Lipid profile levels in rats treated with ashwagandha (Mean ± SE)

Groups	VLDL	HDL	LDL
G1	19.19±0.37 b	48.38±1.67 b	56.25±2.34 b
G2	22.75±1.27 a	37.75±0.95 c	156.94±3.21a
G3	20.25±0.49 b	58.13±1.73 a	55.13±4.74 b
G4	19.23±0.70 b	56.75±1.03 a	52.61±2.28 b
G5	21.31±0.52 ab	51.50±1.67 b	60.08±3.48 b
LSD(P<0.05)	2.14	4.18	9.59

1- G1 (negative Control): No person with diabetes treated with any substance.

2- G2 (positive control): Treated with alloxan to induce diabetes.

3- G3 (*Ashwagandha* extract): Diabetic with alloxan, and treated with *ashwagandha* extract to study its effect on treating diabetes.

4- G4 (Nano *Ashwagandha*): Diabetic with alloxan, and treated with banana *ashwagandha* to study its impact on treating diabetes.

5- G5 (Metformin): Diabetic with alloxan, and treated with metformin to study its effect on treating diabetes.

Different letters between any two means, vertically denoted, indicate significant differences at $P < 0.05$.

Histopathological Findings

Liver

Histological sections of the rat's liver in G1 revealed a standard structure, characterized by regular hepatic plates and normal central nuclei, with no signs of fatty degeneration or inflammatory infiltration, Figure (1A). The liver appears healthy, without any signs of degeneration or necrosis. In contrast, clear pathological changes were observed, including varying degrees of fatty degeneration, hepatocyte hyperplasia, and periportal inflammatory infiltration, as well as marked congestion of the central blood vessels and blood retention within the liver sinusoids in G2, Figure (1B).

The liver sections in G3 rats showed a significant improvement in histological structure compared to the G2 and control groups, with reduced fatty degeneration and hypertrophic cells, and only mild inflammatory infiltration, indicating a relative protective effect of the plant extract, Figure (1C). Likewise, diabetic rats treated with nanoparticles showed improvements that were more pronounced compared to group 3, with the liver structure becoming more closely aligned with normal, exhibiting a relative absence of fatty degeneration and a significant reduction in inflammatory infiltration, indicating a greater hepatoprotective effect of the plant-derived nanoparticles, figure (1D). On the other hand, the liver sections of G5 rats showed moderate improvement in liver structure compared to the untreated diabetic group, with a reduction in fatty degeneration and congestion; however, moderate perivascular inflammatory infiltration remained, figure (1E).

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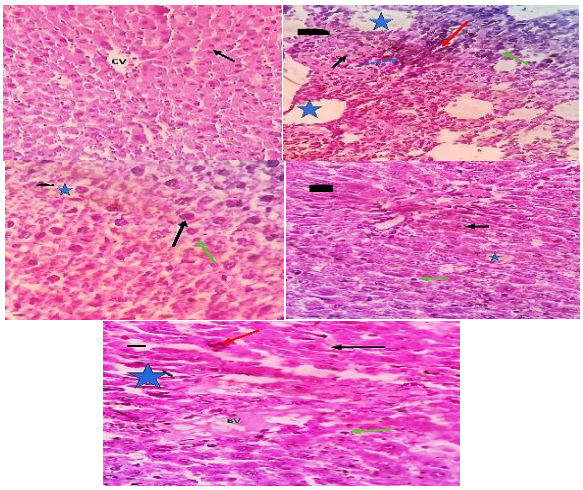

Figure 1: Histological sections of the rat liver. A: showed a normal structure, with regular hepatic plaques and normal central nuclei (black arrow) and normal central vein (CV). H&E stain; X400. B: Histopathological image of the liver of mice was observed, including varying degrees of fatty degeneration (blue stars), hepatocyte hyperplasia (black arrow), and periportal inflammatory infiltration (blue arrow), marked congestion of the blood vessels (red arrow), and blood retention within the liver sinusoids (green arrow). H&E stain; X400. C: from diabetic rats treated with ashwagandha extract was reduced fatty degeneration and hypertrophic cells (black arrow), and only mild inflammatory infiltration (green arrow), and lower degrees of fatty degeneration (blue stars). H&E stain; X400. D: diabetic rats treated with ashwagandha nanoparticles showed improvement more pronounced compared to group 3, with liver structure becoming more closely aligned with normal, with a reduction of fatty degeneration (blue star) and a significant reduction in inflammatory infiltration (green arrow), reduced hypertrophic cells (black arrow), H&E stain; X400. E: showed a moderate improvement in liver structure compared to the untreated diabetic group, with a reduction in fatty degeneration (Blue star) and congestion (red arrow). Still, a moderate perivascular inflammatory infiltration remained (green arrow), and reduced hypertrophic cells (black arrow). H&E stain; X400

Kidney

The kidneys of rats in G1 exhibited normal glomerular and tubular structures, with no significant pathological changes, figure (2A). However, the kidney section of G2 rats exhibited degenerative changes in the renal tubules, including narrowing of the tubular lumen, dilation of the interglomerular spaces, and increased thickness of the basement membrane, accompanied by inflammatory cell infiltration, figure (2B). In contrast, kidney sections of diabetic rats treated with ashwagandha extract exhibited an improved histological structure, characterized by a reduced degree of tubular damage and a marked improvement in glomerular structure. However, mild infiltration persisted, figure (2C). The rat in group 4 exhibited the best preservation of kidney structure in the kidney section, closely approximating the normal shape, with a relative absence of infiltration and damage, indicating the high efficacy of nanoparticles in mitigating diabetes-induced damage, figure (2D). Similarly, the results presented moderate improvement, with a relative decrease in degenerative changes. However, some samples showed slight tubular dilatation and moderate inflammatory infiltration in rats G5, figure (2 E).

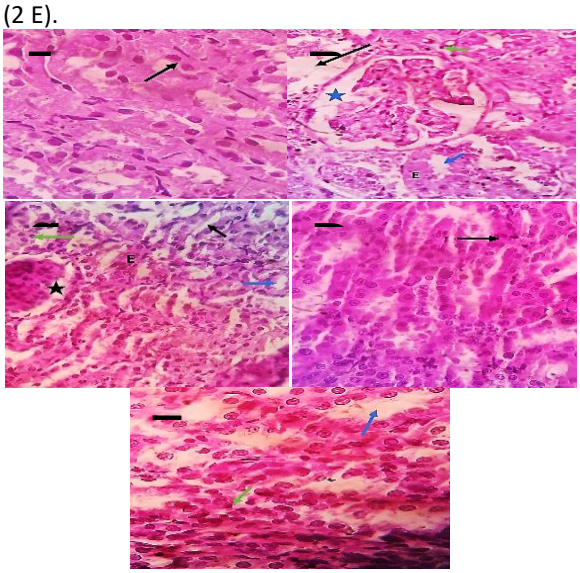

Figure 2. Histopathological image of the rat kidney. A: The negative control group showed normal glomeruli and tubule structure (black arrow), with no significant pathological changes. H&E stain; X400. B: positive control group showed degenerative changes were observed in the renal tubules (black arrow), narrowing of the tubular lumen (blue arrow), dilation of the interglomerular spaces (black arrow), and increased basement membrane thickness (E), with inflammatory cell infiltration (green arrow), H&E stain; X400. C: G3 showed reduced degenerative changes in the renal tubules (black arrow), less narrowing of the tubular lumen (blue arrow), less dilation of the interglomerular spaces (black arrow), and decreased basement membrane thickness (E),

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along with mild inflammatory cell infiltration (green arrow), H&E stain; X400. D: Rats treated with ashwagandha nanoparticles exhibited the best preservation of kidney structure (black arrow), closely resembling normal shape, with minimal infiltration and damage, indicating the high efficacy of nanoparticles in reducing diabetes-induced damage. H&E stain; X4000. E: revealed a moderate improvement, characterized by a relative decrease in degenerative changes. However, some samples showed slight tubular dilatation (blue arrow) and moderate inflammatory infiltration (green arrow). H&E stain; X400.

Discussion

Effect of Ashwagandha treatment on body weight

The study investigated the impact of ashwagandha treatments on body weight in rats with alloxan-induced diabetes. The body weights of rats in G1 showed a slight but statistically insignificant increase over time. This suggests that, under normal physiological conditions, the rats managed to maintain a healthy body weight without noticeable fluctuations. In contrast, the body weight of rats in G2 decreased significantly, which can be attributed to diabetes-related pathophysiology resulting from inadequate blood sugar control. Excess sugar in the blood causes an increased urination, leading to fluid loss and, consequently, weight loss. This weight change is typical of the classic catabolic state, characterized by the destruction of β-cells, insulin deficiency, hyperglycemia, glucose insensitivity, and increased breakdown of proteins and fats [33,34].

The research also showed that the Nano-particles of ashwagandha experienced a similar weight reduction compared to the non-treated ashwagandha extract group. This suggests that Nano-particles can also be effective in addressing weight loss associated with diabetes. Nanoscale delivery systems are well known for enhancing the bioavailability and effectiveness of herbal extracts, thereby improving the success of clinical management [35][36]. Although the extensive cellular protection and antioxidant effects of WS formulations are well-established, the group treated with metformin highlights the drug's unique ability to reduce catabolic processes associated with diabetes.

In the current study, the group of respondents treated with metformin experienced a statistically significant reduction in body weight compared to other treatment groups. This finding supports previous data showing metformin's effectiveness in managing diabetes by increasing insulin sensitivity and decreasing hepatic glucose production [37]. A key feature of metformin is the activation of the highly effective AMPK-driven metabolic pathway, which limits gluconeogenesis in the liver, enhances glucose uptake and utilization in the periphery, and directly improves insulin sensitivity by addressing main metabolic imbalances [38]. Although the extensive cellular protection and antioxidant effects of WS formulations are well-established, the group treated with metformin highlights the drug's unique ability to reduce catabolic processes associated with diabetes.

Effect of Ashwagandha Treatment on the Insulin and Glucose Levels

The findings of the present study indicate notable improvements in insulin and glucose levels in all treatment cohorts relative to diabetic controls lacking intervention, thereby demonstrating the therapeutic potential of these modalities for experimental diabetes.

Individuals in the diabetic control group, exposed only to alloxan, failed to produce insulin in significant quantities and exhibited increased blood glucose concentrations, unequivocally demonstrating that alloxan has cytotoxic effects on pancreatic β-cells [39]. Such results are summaries of other works, as reported, which indicate that alloxan selectively targets β-cells by producing reactive oxygen species (ROS), leading to insulinopenia and hyperglycemia [33].

Based on this study, these results are in agreement with other publications, which support the theory that ashwagandha is a compound that increases insulin secretion by shielding beta-cells against oxidative damage and stimulating cell regeneration [40,41]. The insulin-sensitizing effect of withanolides may be another factor contributing to the anti-hyperglycemic effect of WS [42].

Nano-particles exhibited a slightly better glucose-lowering effect, confirming the idea that the Nano-formulation can be more bioavailable and penetrate cells to increase cellular compound activity [43]. This observation aligns with an increasing number of studies suggesting that delivery systems incorporating nanotechnologies can improve the pharmacokinetic profile of herbal extractions, thereby enhancing their therapeutic value [44]. The nanoparticles from WS exhibit anti-inflammatory, anticancer, antimicrobial, antioxidant, and anti-aging properties, facilitating targeted drug delivery. The plant-based nanoparticles

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exhibit superior characteristics, including enhanced bioavailability, improved solubility, and extended shelf life. The nanoparticles of WS play a crucial role in mitigating damage caused by oxidative stress and neutralizing free radicals, and reducing oxidative damage, while increasing the enzyme antioxidant activity [45] [46][47]

Similarly, metformin yields a significant increase in insulin and glucose levels; these results contribute to the previously established ability of metformin to enhance insulin sensitivity and suppress gluconeogenesis in the liver [48]. It is also interesting that ashwagandha can be used as a suitable supplement or alternative to traditional antidiabetic medications [17].

Collectively, these data highlight the strong therapeutic potential of WS as a multi-targeted agent in managing diabetes, particularly in restoring β-cell function, enhancing insulin secretion, and improving glycemic control.

Effect of Ashwagandha Treatment on Lipid Profile Concentrations

The findings of this investigation demonstrated that alloxan-induced diabetes profoundly disrupts lipid homeostasis, as evidenced by significantly elevated levels of VLDL and LDL, accompanied by a marked reduction in HDL in diabetic rats. These observations are consistent with prior research indicating that hyperglycemia promotes lipolysis within adipose tissue, resulting in augmented hepatic synthesis of VLDL and LDL, while concurrently diminishing reverse cholesterol transport owing to decreased HDL concentrations [49].

Treatment with ashwagandha extract effectively reversed the diabetic-related lipid imbalance, resulting in a significant decrease in LDL levels and a slight decrease in VLDL levels, while increasing HDL levels. These results support previous research that links the lipid-lowering effects of WS to its bioactive compounds, including withanolides and flavonoids, which influence liver lipid metabolism and enhance antioxidant defenses[22][50]. Additionally, the rise in HDL levels may indicate improved reverse cholesterol transport, a key process in protecting against atherosclerosis.

On the other hand, the Nano-Ashwagandha formulation demonstrated even greater efficacy, with (VLDL) levels comparable to those observed in the control group. This formulation resulted in the recording of LDL. This enhanced activity is likely attributable to improved bioavailability and targeted delivery facilitated by nanotechnology, which enhances the absorption and cellular uptake of active phytochemicals [51]. Furthermore, HDL levels were significantly elevated, underscoring its potential as a superior therapeutic option for managing diabetic dyslipidemia.

Similarly, metformin also enhanced lipid profiles, demonstrating moderate reductions in LDL and increases in HDL, consistent with its established effects on lipid metabolism, which include the inhibition of hepatic gluconeogenesis and reduction of lipotoxicity, according to [52]. Nonetheless, the superior capacity of ashwagandha formulations to optimize the HDL/LDL ratio indicates potential complementary or synergistic mechanisms beyond the primary glucose-lowering effect of metformin.

Histopathological Findings

Liver

The results of G1 indicate that the liver retained its normal structure; this reflects the liver's ability to maintain metabolic balance and regulate the metabolism of carbohydrates, lipids, and proteins. These results confirm what [53] reported about the microarchitecture of the healthy liver, as well as their findings. Another study demonstrated that normal livers in rodents are free of pathological changes unless exposed to stress [54]. The absence of degeneration and inflammatory infiltration indicates that the experimental conditions did not affect the liver. Therefore, this group serves as a primary reference for comparing changes in other groups.

Histological findings from the second group showed pathological changes in the liver. These changes reflect metabolic and functional disturbances caused by metabolic or inflammatory stress. Hepatic steatosis is an early sign of lipid metabolism disorders and may predispose to nonalcoholic steatohepatitis (NAS) [55], while hyperplasia represents a compensatory mechanism to maintain functional mass [56]. Inflammatory infiltration also suggests an immune response to lipid accumulation or toxic agents [57], and congestion reflects impaired hepatic flow and increased vascular resistance [58]. These features combine to form a pathological picture indicating the onset of complex fatty liver disease, consistent with previous studies in rodent models.

The results of the third group showed a significant improvement in liver histological structure compared to the second and control groups. This improvement

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reflects the protective effect of ashwagandha extract, which has been demonstrated in previous studies to reduce oxidative stress and hepatic inflammation associated with hyperlipidemia, as well as to stimulate tissue regeneration mechanisms [59]. These results support the potential role of active plant compounds in protecting the liver against metabolic and inflammatory damage caused by excess sugars or fats. Similarly, the results of the fourth group showed a greater improvement in liver histological structure compared to the third group, with the liver tissue approaching its normal shape. This improvement reflects the potent protective effect of the plant nanoparticles, which enhance the absorption of ashwagandha's active compounds and increase their biological efficacy in reducing oxidative stress and hepatic inflammation [60]. These results support the promising role of nanotechnology in improving the pharmacological efficacy of plant extracts to protect the liver from metabolic and inflammatory damage.

Results from the fifth group showed moderate improvement in liver histological structure compared to the untreated diabetic group, with reduced steatosis and congestion. This improvement reflects metformin's ability to enhance glucose and lipid metabolism, as well as mitigate hepatic oxidative stress; however, it may not eliminate the inflammatory response induced by high glucose levels [61]. These results confirm the partial protective role of metformin in protecting the liver from metabolic damage associated with hyperglycemia, underscoring the need for an integrated treatment approach that incorporates other strategies to reduce chronic inflammation.

Kidney Samples

The results of G1 showed that the kidneys maintained a normal structure of the glomeruli and tubules without any pathological changes, reflecting the integrity of renal tissue under normal experimental conditions [53]. In contrast, the second group exhibited marked degenerative changes in the renal tubules. These changes are consistent with the effects of chronic hyperglycemia on renal tissue, which leads to early diabetic nephropathy through inflammatory stimulation, increased oxidative stress, and hypertrophy of the tubular and glomerular basement membranes [62,63].

While the third group exhibited a significant improvement in kidney histological structure, characterized by reduced tubular damage and enhanced glomeruli, indicating a partial protective effect of ashwagandha extract against diabetes-associated kidney damage [59]. Similarly, the fourth group exhibited the best preservation of kidney structure, approaching normal, with a relative absence of infiltration and damage, demonstrating the effectiveness of nanoparticles in enhancing the bioavailability of active compounds and reducing oxidative stress and renal inflammation [60]. While the fifth group showed moderate improvement in histological structure, with a decrease in some degenerative changes, some samples exhibited slight dilatation of the renal tubules and moderate inflammatory infiltration, reflecting the partial protective effect of metformin against diabetic damage [61].

Conclusion

This study showed that Nano-formulated *Withania somnifera* extract (NAE) exhibits superior antidiabetic effects compared to conventional extract (AE) and metformin in alloxan-induced diabetic rats. NAE significantly reduces glucose levels, restores insulin, and normalizes lipids (LDL and HDL), making ashwagandha, especially in Nano form, a promising supportive therapy for diabetes. These results suggest that nanotechnology can optimize herbal medicine delivery and need further clinical study.

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CONFLICT OF INTERESTS

The author declared that there is no commercial or financial conflict of interest.

AUTHOR CONTRIBUTION

The authors made equal contributions**.**

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References

1.American Diabetes Association. American Diabetes Association diabetes guidelines: summary recommendations from NDEI 2016. 2016.

2.Haider R, Mehdi A. The history of diabetes mellitus. Int J Sci Multidiscip Res. 2023; 1:1259-80. https://doi.org/10.55927/ijsmr.v1i10.6494

3.Bhatti JS, Sehrawat A, Mishra J, Sidhu IS, Navik U, Khullar N, et al. Oxidative stress in the

pathophysiology of type 2 diabetes and related complications: current therapeutic strategies and future perspectives. Free Radic Biol Med. 2022; 184:114-34. https://doi.org/10.1016/j.freeradbiomed.2022.03.019

4.Bailey CJ. Metformin: therapeutic profile in the treatment of type 2 diabetes. Diabetes Obes Metab. 2024;26:3-19. https://doi.org/10.1111/dom.15663

5.Chen S, Gan D, Lin S, Zhong Y, Chen M, Zou X, et al. Metformin in aging and aging-related diseases: clinical applications and relevant mechanisms. Theranostics. 2022; 12:2722-42. https://doi.org/10.7150/thno.71360

6.Hinault C, Caroli-Bosc P, Bost F, Chevalier N. Critical overview on endocrine disruptors in diabetes mellitus. Int J Mol Sci. 2023;24:4537. https://doi.org/10.3390/ijms24054537

7.Balkrishna A, Kumar A, Rohela A, Arya V, Gautam AK, Sharma H, et al. Traditional uses, hepatoprotective potential, and phytopharmacology of Tinospora cordifolia: a narrative review. J Pharm Pharmacol. 2024;76:183-200. https://doi.org/10.1093/jpp/rgae013

8.Nargis KM, Gaur R, Singh A, Kanta C. Exploring the antioxidant, antidiabetic and anticancer potential of Withania somnifera and Withania coagulans: a comparative study. J Environ Bio-sci. 2024;38. https://doi.org/10.59467/JEBS.2024.38.377

9.Gupta A, Vasundhara M. Withanolides as prospective drug candidates: production and therapeutic applications – a review. Planta Med. 2025. https://doi.org/10.1055/a-2659-7263

10.Chary JS, Sharma A. Withania somnifera: A Rasayana Herb for Sustainable Management of Human Health and Overall Well-being. In: Advanced green technology for environmental sustainability and circular economy. Boca Raton: CRC Press; 2025. p. 33-47. https://doi.org/10.1201/9781003517108-3

11.Ishtar WR, Mohammed WA. Bioactivity effects of phlorizin extract on diabetic mice compared to metformin. Al-Qadisiyah J Vet Med Sci. 2025;24(1):31-40. https://doi:10.29079/qjvms..2024.152671.1033.

12.Al-Eisa RA. A review on selected pharmacological and phytochemical activities of ashwagandha (Withania somnifera). Egypt J Vet Sci. 2025:1-23. https://doi.org/10.21608/ejvs.2025.311925.2328

13.Alhasani RH, Alsharif I, Albalawi AE, Albalawi FE, Mohammedsaleh ZM, Saleh FM, et al. Anti-inflammatory and anti-diabetic role of ashwagandha (Withania somnifera) in a type 2 diabetes mellitus mouse model: a study using histological, molecular and pathological parameters. Protoplasma. 2025:1-11. https://doi.org/10.1007/s00709-025-02096-4

14.Brahma S. Initiation to formulate, standardize and evaluate an anti-diabetic herbal formulation [dissertation]. 2024.

15.Lelli SM, de Viale LCSM, Mazzetti MB. Response of glucose-metabolism enzymes in an acute porphyria model: role of reactive oxygen species. Toxicology. 2005; 216:49-58. https://doi.org/10.1016/j.tox.2005.07.016

16Tuteja J, Vyas A, Sand A. Utility of nanomedicine and nanocarriers for non-infectious disease treatment. In: Nanotechnology for drug delivery and pharmaceuticals. Amsterdam: Elsevier; 2023. p. 207-23. https://doi.org/10.1016/B978-0-323-95325-2.00014-6

17.Ram H, Singh AK. Improvements in insulin resistance and β-cell dysfunction by DPP-4 inhibition potential of Withania somnifera (L.) Dunal root extract in type 2 diabetic rats. Biointerface Res Appl Chem. 2020;11:8141-55. https://doi.org/10.33263/BRIAC111.81418155

18.Niawanti H, Lewar YS, Octavia NN. Effect of extraction time on Averrhoa bilimbi leaf ethanolic extracts using the Soxhlet apparatus. IOP Conf Ser Mater Sci Eng. 2019; 543:012018. https://doi.org/10.1088/1757-899X/543/1/012018

19.Jorgensen JH, Turnidge JD. Susceptibility test methods: dilution and disk diffusion methods. In: Manual of clinical microbiology. 11th ed. Washington: ASM Press; 2015. p. 1253-73. https://doi.org/10.1128/9781555817381.ch71

20.Mahire SP, Patel SN. Extraction of phytochemicals and study of its antimicrobial and antioxidant activity of Helicteres isora L. Clin Phytoscience. 2020;6:40. https://doi.org/10.1186/s40816-020-00156-1.

21.Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina (B Aires) 2017; 53:365–74.

22.Ali MH. Ashwagandha (Withania somnifera) and their effects on the reproductive hormones of male rats. J Hum Endocrinol. 2021; 37:1-22. https://doi.org/10.21608/jhe.2021.169285.

23.Elgar K. Ashwagandha: a review of clinical use and efficacy. Nutr Med J. 2021; 1:68-78.

24.Al-Za'abi M, Ali BH, Al Suleimani Y, Adham SA, Ali H, Manoj P, et al. The effect of metformin in diabetic and non-diabetic rats with experimentally-induced chronic kidney disease. Biomolecules. 2021;11:814. https://doi.org/10.3390/biom11060814

25.Abbasa MI, Jawad ZJM. Hepatoprotective effect of alcoholic extract of Ficus carica leaves against cypermethrin-induced liver toxicity in male albino rats. Iraqi J Vet Med. 2023; 47:64-72. https://doi.org/10.30539/ijvm.v47i2.1601

26.Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. J Cereb Blood Flow Metab. 2020; 40:1769-77. https://doi.org/10.1177/0271678X20943823

27.Xie Y, Xiao L, Li S. Effects of metformin on reproductive, endocrine and metabolic characteristics of female offspring in a rat model of letrozole-induced polycystic ovarian syndrome with insulin resistance. Front Endocrinol. 2021;12:701590. https://doi.org/10.3389/fendo.2021.701590

28.Tiwari DD, Thorat VM, Pakale PV, Patil S, Chavan D, Patil SJ. Evaluation of lipid-profile modulation by Berberis asiatica, Withania somnifera and their synergy in type 2 diabetic Wistar rats. Cureus. 2024;16:e67974. https://doi.org/10.7759/cureus.67974

29.Jarad AS, Al-Kaisei BI. Effect of L-arginine on some biochemical and pathological parameters in diabetes mellitus induced by alloxan monohydrate in rats. Anbar J Vet Sci. 2013; 6:89-100.

30.Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. 6th ed. Edinburgh: Churchill Livingstone/Elsevier; 2008.

31.Spitalnik PF, Witkin JW. Histology laboratory manual. New York: Columbia University College of Physicians and Surgeons; 2016.

32.Daniel WW, Cross CL. Biostatistics: a foundation for analysis in the health sciences. 11th ed. Hoboken: John Wiley & Sons; 2018.

33.Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50:537-46. https://doi.org/10.33549/physiolres.930111

34.Rohilla A, Ali S. Alloxan-induced diabetes: mechanisms and effects. Int J Res Pharm Biomed Sci. 2012;3:819-23.

35.Sharma Y, Bashir S, Irshad M, Nag TC, Dogra TD. Dimethoate-induced effects on the antioxidant status of the liver and brain of rats following subchronic exposure. Toxicology. 2005; 215:173-81. https://doi.org/10.1016/j.tox.2005.06.029

36.Antonelli-Ushirobira TM, Blainski A, Fernandes HG, Moura-Costa GF, Costa MA, Campos-Shimada LB, et al. Acute toxicity and long-term safety evaluation of the crude extract from rhizomes of Limonium brasiliense in mice and rats. J Ethnopharmacol. 2015; 174:293-8. https://doi.org/10.1016/j.jep.2015.08.022

37.Cooper LA, Roter DL, Johnson RL, Ford DE, Steinwachs DM, Powe NR. Patient-centered communication, ratings of care, and concordance of patient and physician race. Ann Intern Med. 2003; 139:907-15. https://doi.org/10.7326/0003-4819-139-11-200312020-00009

38.Almuttairi RS. The effects of metformin treatment on diabetic albino rats' pancreas, liver and kidney histology. Arch Razi Inst. 2023; 78:459-68.

39.Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008;51:216-26. https://doi.org/10.1007/s00125-007-0886-7

40.Anwer T, Sharma M, Pillai KK, Khan G. Protective effect of Withania somnifera against oxidative stress and pancreatic beta-cell damage in type 2 diabetic rats. Acta Pol Pharm. 2012; 69:1095-101.

41.Udayakumar R, Kasthurirengan S, Mariashibu TS, Rajesh M, Anbazhagan VR, Kim SC, et al. Hypoglycaemic and hypolipidaemic effects of Withania somnifera root and leaf extracts on alloxan-induced diabetic rats. Int J Mol Sci. 2009; 10:2367-82. https://doi.org/10.3390/ijms10052367

42.Gorelick J, Rosenberg R, Smotrich A, Hanuš L, Bernstein N. Hypoglycemic activity of withanolides and elicitated Withania somnifera. Phytochemistry. 2015; 116:283-9. https://doi.org/10.1016/j.phytochem.2015.02.029

43.Kumar V, Dey A, Hadimani MB, Emerald M. Chemistry and pharmacology of Withania somnifera: an update. CellMed. 2015;5:1-17. https://doi.org/10.5667/tang.2014.0030

45.Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles-based drug delivery systems. Colloids Surf B Biointerfaces. 2010; 75:1-18. https://doi.org/10.1016/j.colsurfb.2009.09.001

46.Ene AC, Nwankwo EA, Samdi LM. Alloxan-induced diabetes in rats and the effects of black caraway (Carum carvi L.) oil on their body weight. Res J Med Med Sci. 2007; 2:48-52.

Al-Qadisiyah J. Vet. Med. Sci. 2025; 24 (2). doi.org/10.29079/qjvms.2025.164884.1112

ISSN P: 1818-5746
E: 2313-4429
qjvms.qu.edu.iq

47.Sabry SM. Functional role of ashwagandha (Withania somnifera) leaves on type 2 diabetes rat induced by streptozotocin [dissertation]. 2021.
48.Nagaraj B, Veeresham C. Effect of ashwagandha on pharmacokinetic and pharmacodynamic parameters of glimepiride in streptozotocin-induced diabetic rats. Asian J Pharm Clin Res. 2018;11:207-11. https://doi.org/10.22159/ajpcr.2018.v11i4.23960
49.Bailey CJ, Turner RC. Metformin. N Engl J Med. 1996;334:57.4-9. https://doi.org/10.1056/NEJM199602293340906
50.Chikezie OD, Meludu SC, Ogbu ISI, Egejuru BN, Ude T, Ekuma-Ogbonnia O. Blood lipids and its atherogenic indices in alloxan-induced diabetic male rats. AJARR. 2020; 9:25-33. https://doi.org/10.9734/ajarr/2020/v9i230216
51.Elhassaneen YA, Boraey RA, Nasef AZ. Biological activities of ashwagandha (Withania somnifera L.) roots and their effect on the neurological complications of obesity in rats. Am J Food Nutr. 2023;11:71-88.
52.Derosa G, Romano D, D'Angelo A, Maffioli P. Berberis aristata/Silybum marianum fixed combination (Berberol®) effects on lipid profile in dyslipidemic patients intolerant to statins at high dosages: a randomized, placebo-controlled clinical trial. Phytomedicine. 2015; 22:231-7. https://doi.org/10.1016/j.phymed.2014.11.018
53.Viollet B, Guigas B, Leclerc J, Hébrard S, Lantier L, Mounier R, et al. AMP-activated protein kinase in the regulation of hepatic energy metabolism: from physiology to therapeutic perspectives. Acta Physiol (Oxf). 2009; 196:81-98. https://doi.org/10.1111/j.1748-1716.2009.01970.x
54.Hall JE, Hall ME, editors. Guyton and Hall textbook of medical physiology. 14th ed. Philadelphia: Elsevier; 2020.
55.Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol. 2007; 35:495-516. https://doi.org/10.1080/01926230701320337
56.Takahashi Y, Fukusato T. Histopathology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol. 2014; 20:15539-48. https://doi.org/10.3748/wjg.v20.i42.15539
57.Pu X, Kamendulis LM, Klaunig JE. Acrylonitrile-induced oxidative stress and oxidative DNA damage in male Sprague-Dawley rats. Toxicol Sci. 2009; 111:64-71. https://doi.org/10.1093/toxsci/kfp133
58.Diehl AM, Day C. Cause, pathogenesis, and treatment of nonalcoholic steatohepatitis. N Engl J Med. 2017;377:2063-72. https://doi.org/10.1056/NEJMra1503519
59.Uribarri J, Cai W, Peppa M, Goodman S, Ferrucci L, Striker G, et al. Circulating glycotoxins and dietary advanced glycation endproducts: two links to inflammatory response, oxidative stress, and aging. J Gerontol A Biol Sci Med Sci. 2007; 62:427-33. https://doi.org/10.1093/gerona/62.4.427
60.Singh J, Mishra NS, Banerjee S, Sharma YC. Comparative studies of physical characteristics of raw and modified sawdust for their use as adsorbents for removal of acid dye. BioResources. 2011;6:2732-43. https://doi.org/10.15376/biores.6.3.2732-2743
61.Patel R, Babady E, Theel ES, Storch GA, Pinsky BA, St George K, et al. Report from the American Society for Microbiology COVID-19 International Summit, 23 March 2020: value of diagnostic testing for SARS-CoV-2/COVID-19. mBio. 2020;11: e00722-20. https://doi.org/10.1128/mBio.00722-20
62.Gao M, Monian P, Pan Q, Zhang W, Xiang J, Jiang X. Ferroptosis is an autophagic cell death process. Cell Res. 2016; 26:1021-32. https://doi.org/10.1038/cr.2016.95
63.Navarro-González JF, Mora-Fernández C. The role of inflammatory cytokines in diabetic nephropathy. J Am Soc Nephrol. 2008;19:433-42. https://doi.org/10.1681/ASN.2007091048
64.Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev. 2013; 93:137-88. https://doi.org/10.1152/physrev.00045.2011.