THE IRAQI JOURNAL OF
VETERINARY MEDICINE
eISSN:2410-7409
pISSN: 1609-5693

OPEN ACCESS

Therapeutic Potential of Ginger Ethanolic Extract, Ginger-Loaded Chitosan Nanoparticles, and Chitosan Nanoparticles in Induced Type 2 Diabetes Mellitus in Dogs

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Received: 19 March 2024
Revised: 01 April 2024
Accepted: 08 July 2024
Published: 28 December 2024

DOI:
https://doi.org/10.30539/fae5kc34

Cite:
Hameed RM, Mahmood AK. The Therapeutic Potential of Ginger Ethanolic Extract, Ginger-Loaded Chitosan Nanoparticles, and Chitosan Nanoparticles in Induced Type 2 Diabetes Mellitus in Dogs. Iraqi J. Vet. Med. 2024;48(2):15-25.

ABSTRACT

Type 2 diabetes mellitus (T2DM) in dogs is a complex, multifactorial disease that is characterized by chronic hyperglycemia and insulin resistance. Current therapeutic options are often limited by side effects and variable efficacy, highlighting the need for more effective and safer treatments. This study assessed the therapeutic potential of ginger ethanolic extract (GEE), GEE-loaded chitosan nanoparticles (GEE-CNPs), and chitosan nanoparticles (CNPs) against T2DM in dogs. Twenty adult local breed mongrel dogs of both sexes, aged 7 to 13 months, with an average body weight of 10.4±0.76 kg, were included. The dogs were allocated into five groups (n=4 each): a non-diabetic, untreated Negative Control group, and four diabetic treatment groups, following T2DM induction via a single intravenous alloxan-nicotinamide injection. Each treatment group received daily oral administrations of either saline (Positive Control), GEE, GEE-CNPs, or CNPs at a dosage of 81.7 mg/kg BW over 45 days. Serum glycemic status (fasting serum glucose, insulin, and insulin resistance) was recorded at baseline and on days 7, 14, 21, 28, 35, 42, and 45 post-treatments. Additionally, on day 45, serum lipid profiles, liver function indicators (alanine aminotransferase [ALT], gamma-glutamyl transferase [GGT], and bilirubin), and markers of antioxidant status (glutathione [GSH] and malondialdehyde [MDA]) were assessed. The results showed that dogs in the diabetic Positive Control group exhibited hyperglycemia, dyslipidemia, liver dysfunction, and elevated oxidative stress markers, underscoring the severe impact of T2DM. Compared to the diabetic Positive Control, oral GEE, GEE-CNPs, and CNPs treatments significantly (P<0.05) improved fasting glucose levels, insulin sensitivity, lipid profiles (reduced total cholesterol, triglycerides, and LDL-C), liver function markers, and antioxidant status, indicating enhanced metabolic health and reduced oxidative stress. The findings suggest that GEE, GEE-CNPs, and CNPs offer potential as therapeutic agents for T2DM in dogs, demonstrating significant benefits in glycemic control, lipid normalization, liver function, and oxidative stress reduction. Further investigations with larger cohorts and longer durations are recommended to confirm these results and ascertain the clinical applicability and safety of these natural remedies in managing canine diabetes.

Keywords: ginger, chitosan nanoparticles, type 2 diabetes, canine diabetes

INTRODUCTION

Diabetes mellitus (DM) in dogs is a growing concern within veterinary medicine, reflecting the condition's complexity and escalating prevalence in the human population (1). This endocrine disorder is characterized by chronic hyperglycemia, leading to diverse complications, including cardiovascular diseases, renal dysfunction, neuropathy, and ocular impairments (2). With an estimated prevalence of 0.32-1.33% in the canine

population, the management of DM necessitates lifelong interventions, predominantly relying on exogenous insulin administration (3). This approach, while indispensable, poses substantial challenges in achieving consistent euglycemia, significantly affecting the quality of life for both dogs and their owners due to the rigorous demands of treatment regimens (4, 5).

Current therapeutic strategies for canine DM are limited, mirroring the treatments available for human diabetics but with notable challenges in veterinary implementation (6, 7). Novel insulins and incretin-based drugs offer potential; however, their application in dogs faces obstacles, highlighting a crucial need for innovative, effective, and more easily managed treatments. Owner compliance is a pivotal factor in the successful management of canine diabetes, with the complexity of existing treatments often leading to suboptimal outcomes (8).

The use of medicinal plants in diabetic dogs aims to provide supplementary support to conventional treatments, with the ultimate goal of improving glycemic control, preventing complications, and enhancing the overall quality of life for these animals (9). Amidst this context, herbal remedies emerge as a promising avenue for exploration. Historical and recent studies have demonstrated the potential of various plant-based interventions, including *Andrographis paniculata*, curcuminoid derived from turmeric (*Curcuma longa*), garlic, fenugreek, black seed, *Gongronema latifolium*, and *Bixa orellana*, in improving glycemic control and managing diabetes-related complications in canine models (9-13).

Despite these advances, the exploration of ginger (*Zingiber officinale* Roscoe), particularly in conjunction with nanotechnology to enhance its bioavailability and efficacy, remains scant. Ginger is renowned for its extensive pharmacological properties, including anti-inflammatory, antioxidant, and anti-diabetic effects, attributed to its rich profile of bioactive compounds such as gingerols, shogaols, and paradols (14,15). These compounds present multiple mechanisms for counteracting DM pathogenesis, ranging from modulating autoimmune responses to mitigating obesity-induced inflammation and oxidative stress (16). However, the therapeutic application of ginger in canine diabetes is limited by challenges in bioavailability and stability, which could be significantly improved through the use of chitosan nanoparticles. Derived from chitin, these nanoparticles are distinguished for their biocompatibility, mucoadhesive properties, and potential to enhance the delivery and efficacy of loaded bioactive compounds, offering a controlled release mechanism that could develop the application of herbal remedies in veterinary medicine (17). Additionally, with many different biological functions reported, such as promoting wound healing, protecting pancreatic beta cells, reducing hyperglycemia and hyperlipidemia, and modulating the immune system response, chitosan nanoparticles are gaining attention as a potential new approach to treat DM (18-20).

This study, therefore, seeks to investigate the efficacy of ginger ethanolic extract (GEE), GEE-loaded chitosan nanoparticles (GEE-CNPs), and chitosan nanoparticles (CNPs) in the management of experimentally induced type 2 diabetes mellitus (T2DM) in dogs.

MATERIALS AND METHODS

Ethical Statement

Animal research was conducted in accordance with ethical guidelines from the College of Veterinary Medicine, University of Baghdad's Animal Care and Use Committee. The Research Ethics Committee approved the study (Protocol Number: 549/P.G. dated May 8, 2023), ensuring ethical standards were met in all procedures of the study.

Plant and Extraction

The detailed method for plant material acquisition and authentication, extraction, phytochemical analyses, and the preparation of CNPs for loading with GEE was described in our previous work (21).

Animals

The study incorporated twenty healthy mongrel dogs of mixed genders, aged between 7 and 13 months and weighing between 11 and 19 kg. These dogs were acquired from a commercial vendor in the region of Baghdad and were part of the experiment. Each animal underwent a clinical examination before being placed in the Veterinary College's Department of Surgery and Obstetrics Animal House at the University of Baghdad. The housing facility provided a controlled environment with moderate temperatures and a light-dark cycle of 12 h each. During the two-week acclimatization period before the experiment began, the dogs were kept in cages measuring 1×2×1 m. During the study, they were fed a diet of standard pellets, meat, and bread twice a day and accessed to tap water *ad libitum*.

Induction of Diabetes

Before starting the induction process, all dogs underwent an 18-hour fasting period. According to Buishand (22), normal levels of fasting blood glucose in healthy dogs range from 75 to 120 mg/dL. T2DM was induced using a modified protocol based on earlier studies (23-25). The procedure involved a single intravenous dose of alloxan monohydrate (70 mg/kg BW, CDH, India) and nicotinamide (50 mg/kg BW, Avonchem, UK) mixed in a freshly prepared solution of 0.9% saline. The nicotinamide was given 30 min before the alloxan to help protect the pancreatic beta cells from the damaging effects of alloxan (26). Post-injection, the dogs were given a 20% glucose solution overnight to mitigate potential early mortality due to drug-induced hypoglycemia. Blood glucose levels were monitored 72 h later with an ACCU-CHEK glucometer (China), considering dogs with levels above 120 mg/dL as diabetic.

Experimental Design

The dogs were randomly divided into five groups (n=4). Group 1 acted as Negative Control with no diabetes induction and no treatment administered. The other

sixteen dogs underwent diabetes induction and were classified into four treatment groups. Group 2, the Positive Control, received only saline treatments. Group 3 dogs were given an oral dose of ginger ethanolic extract (GEE) at 81.7 mg/kg BW for 45 days. Group 4 received GEE loaded with CNPs (GEE-CNPs) at the same dosage and duration, while Group 5 was treated with only CNPs at 81.7 mg/kg BW for 45 days.

Glycemic Status

The quantification of serum glucose levels in this study was carried out utilizing the GLUCOSE MR kit (LINEAR CHEMICALS S.L., Spain, REF 1129005). The underlying principle of this assay is the enzymatic colorimetric method, specifically the Trinder reaction. This reaction involves the enzymatic oxidation of glucose to D-gluconate, catalyzed by glucose oxidase (GOD), a biological enzyme serum glucose levels of the dogs were measured.

Determination of serum insulin levels was conducted using the Canine HS-INS (High sensitive Insulin) Accquant® ELISA Kit (Wuhan Fine Biotech Co., Ltd., Wuhan, China, CAT. No., ECA0103), using the sandwich enzyme-linked immunosorbent assay (ELISA) principle. This involves capturing the insulin antigen between two layers of antibodies for sensitive and specific detection.

To estimate insulin resistance (IR), the Homeostasis Model Assessment (HOMA) method was used, which calculates the HOMA-IR index from fasting insulin and glucose levels. The HOMA-IR index is defined as: HOMA−IR=Fasting blood glucose (mg/dL) × Fasting insulin (μIU/mL) / 405. The value 405 in the HOMA-IR equation is a conversion factor that normalizes the units of fasting blood glucose (FBG) and fasting insulin to facilitate the computation of insulin resistance.

Lipid Profile

Serum total cholesterol levels were determined using the CHOLESTEROL MR kit (LINEAR Chemicals S.L.U., Spain, REF 1118005) through an enzymatic colorimetric method involving cholesterol esterase, cholesterol oxidase, and peroxidase. Serum triglyceride levels were quantified with the TRIGLYCERIDES MR kit (LINEAR Chemicals S.L.U., Spain, REF 1155005), which uses a series of enzymatic reactions for precise measurement. For high-density lipoprotein cholesterol (HDL-C), the HDL-CHOLESTEROL kit (LINEAR Chemicals S.L.U., Spain, REF 133010) applied a differential precipitation technique followed by enzymatic analysis of the supernatant. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula, which subtracts the sum of HDL-C and VLDL-C from total cholesterol, reflecting the composition of LDL-C, HDL-C, and VLDL-C in serum. Very low-density lipoprotein cholesterol (VLDL-C) was estimated from triglyceride levels as one-fifth of the concentration, acknowledging the lipid composition of VLDL particles.

Liver Function

Serum alanine aminotransferase (ALT) levels were evaluated using the ALT/GPT BR kit (LINEAR Chemicals S.L.U., Spain, REF 1105000), which relies on an enzymatic kinetic method to quantify ALT/GPT activity based on the catalytic action that facilitates the transfer of an amino group from alanine to oxoglutarate, forming glutamate and pyruvate. Serum gamma-glutamyl transferase (GGT) levels were determined with the Gamma-Glutamyl Transferase sandwich ELISA kit (MyBioSource, USA, Cat. No. MBS162030), employing the Enzyme-Linked Immunosorbent Assay (ELISA) technique for quantitative detection. Additionally, serum bilirubin was quantified using the BILIRUBIN kit (LINEAR Chemicals S.L.U., Spain, REF 1110005), based on a colorimetric method that converts bilirubin to colored azobilirubin using diazotized sulfanilic acid.

Oxidative Status

Oxidative status was evaluated by measuring serum glutathione (GSH) and malondialdehyde (MDA) concentrations. Serum GSH levels were quantified using the GSH (Glutathione) ELISA Kit (Elabscience, USA, Cat No., E-EL-0026), which utilizes the competitive-ELISA principle. The MDA (Malondialdehyde) ELISA Kit (Elabscience, USA, Cat No., E-EL-0060) was used for the quantitative measurement of MDA in serum, based on a competitive-ELISA approach where MDA in the samples competes with a fixed amount of MDA on the solid phase supporter for binding sites on the biotinylated detection antibody specific to MDA.

Statistical Analysis

Statistical analysis was performed using the one-way ANOVA to evaluate the differences between group means. Subsequent post hoc comparisons were performed using the Fisher Least Significant Difference (LSD) test to identify significant mean differences at a significance level of $P \leq 0.05$. All data analyses were conducted using IBM SPSS software version 22.0 (IBM Corporation, Armonk, NY).

RESULTS

Glycemic Status

The results of the serum fasting glucose are presented in **Table 1**. The results demonstrated that there were no significant differences in serum fasting glucose levels across the groups at baseline (day 0), as indicated by the initial serum fasting glucose measurements ($P=0.816$). The following time points (days 7 through 45) revealed statistically significant differences ($P<0.001$), indicative of treatment effects. The Positive Control group had significantly ($P<0.001$) higher serum fasting glucose levels than the Negative Control group. It remained consistently high throughout the experimental period, indicating that the diabetes induction method used was effective and that the disease was not spontaneously reversed.

Oral administration of GEE, GEE-CNPs, and CNPs significantly reduced serum fasting glucose levels compared to the Positive Control group. This demonstrates their hypoglycemic effect in lowering blood sugar. The GEE group had a hypoglycemic effect, as their glucose levels

decreased gradually from 288 mg/dL on day 7 to 116 mg/dL on day 45. Serum fasting glucose in animals of the GEE group became significantly (P<0.001) lower than that recorded in animals of the Positive Control group by day 21. It remained lower until the end of the experiment. The GEE-CNPs group had a hypoglycemic effect, as their glucose levels decreased sharply from 261 mg/dL on day 7 to 114 mg/dL at day 45. They became significantly lower than the Positive Control group on day 14 and reached the lowest levels among all the treatment groups by day 45. This indicates that the CNPs could enhance the delivery and efficacy of the GEE and provide a more potent hypoglycemic effect. The CNPs group had a hypoglycemic effect, as their glucose levels decreased from 286 mg/dL on day 7 to 116 mg/dL on day 45. Similar to the GEE group, serum fasting glucose in animals of the CNPs group became significantly (P<0.001) lower than that recorded in animals of the Positive Control group by day 21 and remained lower until the end of the experiment.

Table 1. Effect of ginger ethanolic extract (GEE), ginger loaded with chitosan nanoparticles (GEE-CNPs), and chitosan nanoparticles (CNPs) on serum fasting glucose of adult dogs induced type 2 diabetes

Days	Negative Control	Positive Control	GEE	GEE-CNPs	CNPs	*P*-value
0	91.9±9.85 ^{a}	83.9±3.48 ^{a}	91.1±4.50 ^{a}	84.9±7.32 ^{a}	92.5±6.01^{a}	0.8160
7	98.3±6.31 ^{b}	280±21.8 ^{a}	288±24.4 ^{a}	261±14.5 ^{a}	286±27.9 ^{a}	<0.001
14	95.7±3.20 ^{c}	267±19.3 ^{a}	223±14.8 ^{ab}	209±6.76 ^{b}	250±26.5 ^{ab}	<0.001
21	96.2±3.66 ^{c}	261±20.2 ^{a}	209±9.52 ^{b}	189±10.0 ^{b}	210±14.2 ^{b}	<0.001
28	96.1±4.38 ^{c}	242±21.4 ^{a}	173±6.43 ^{b}	165±8.16 ^{b}	181±13.5 ^{b}	<0.001
35	97.8±7.91 ^{c}	234±20.0 ^{a}	151±4.03 ^{b}	142±4.31 ^{b}	160±8.64 ^{b}	<0.001
42	90.2±5.71 ^{c}	215±14.2 ^{a}	123±0.73 ^{b}	125±3.24 ^{b}	127±1.73 ^{b}	<0.001
45	94.6±3.39 ^{c}	209±11.4 ^{a}	116±2.49 ^{b}	114±2.74 ^{b}	116±1.46 ^{b}	<0.001

Values are means±SEM, n = 4 per treatment group. Means in a row without a common superscript letter significantly different (P≤0.05)

The results of serum insulin levels among different treatment groups are presented in Table 2. At baseline (day 0), there was no significant difference (P=0.888) among the treatment groups in the level of serum insulin). As with the glucose levels, the results of serum insulin showed that throughout the period of the experiment the Positive Control group had significantly (P=0.002) lower serum insulin levels than that of the Negative Control group, which had stable and normal serum insulin levels. This indicates that the induction of diabetes impacted the secretion of insulin and, consequently, the pancreatic β-cells. Additionally, the results at most time points showed that GEE, GEE-CNPs, and CNPs groups had significantly different serum insulin levels comparing to the Positive Control group, indicating that the treatments had an insulinotropic effect and could improve the insulin secretion of diabetic dogs.

Dogs of the GEE group had an insulinotropic effect, as their insulin levels increased gradually from 6.46 µIU/mL at day 7 to 14.2 µIU/mL at day 45. By day 14, the serum insulin levels became significantly higher (P<0.001) than that of the Positive Control group and remained higher until the end of the experiment. This suggests that the GEE had a beneficial effect on the insulin secretion of diabetic dogs, possibly by stimulating pancreatic cells, enhancing insulin sensitivity, and modulating the oxidative stress and inflammation.

The GEE-CNPs group had an insulinotropic effect, as their insulin levels increased sharply from 6.77 µIU/mL on day 7 to 12.9 µIU/mL on day 45. The serum insulin levels became significantly higher (P<0.001) than that of the Positive Control group by day 21 and remained higher until the end of the experiment.

The CNPs group had an insulinotropic effect, as their insulin levels increased from 6.20 µIU/mL at day 7 to 13.2 µIU/mL at day 45. The serum insulin levels became significantly higher (P<0.001) than that of the Positive Control group by day 28.

Table 2. Effect of ginger ethanolic extract (GEE), ginger loaded with chitosan nanoparticles (GEE-CNPs), and chitosan nanoparticles (CNPs) on serum insulin of adult dogs induced type 2 diabetes

Days	Negative Control	Positive Control	GEE	GEE-CNPs	CNPs	*P*-value
0	13.1±2.61 ^{a}	14.1±1.76 ^{a}	11.7±1.59 ^{a}	14.1±2.03 ^{a}	14.1±1.85 ^{a}	6.025
7	15.9±2.06 ^{a}	6.05±0.86 ^{b}	6.46±0.47 ^{b}	6.77±1.06 ^{b}	6.20±1.29 ^{b}	3.803
14	14.5±1.89 ^{a}	6.97±0.73 ^{c}	10.5±0.95 ^{b}	10.0±0.72 ^{bc}	9.21±0.54 ^{bc}	3.246
21	18.9±3.40 ^{a}	6.77±0.47 ^{c}	11.9±0.47 ^{b}	12.3±0.55 ^{b}	10.6±0.84 ^{bc}	4.868
28	15.2±1.93 ^{a}	5.70±0.64 ^{b}	15.3±2.08 ^{a}	13.7±1.39 ^{a}	14.6±1.27 ^{a}	4.676
35	20.6±3.46 ^{a}	5.86±1.52 ^{b}	17.8±1.63 ^{a}	15.8±2.51 ^{a}	15.5±2.23 ^{a}	7.162
42	16.5±1.07 ^{a}	5.59±0.51 ^{b}	14.2±1.17 ^{a}	14.2±2.78 ^{a}	13.4±1.63 ^{a}	4.892
45	18.4±1.04 ^{a}	7.04±1.10 ^{b}	14.2±2.37 ^{a}	12.9±2.08 ^{a}	13.2±2.52 ^{a}	5.815

Values are means±SEM, n = 4 per treatment group. Means in a row without a common superscript letter significantly different (P≤0.05)

In terms of insulin resistance (Table 3), the results showed that at baseline (day 0), there was no significant difference among the treatment groups in the insulin resistance levels (P=0.873). However, variable results were observed throughout the study, reflecting the complex dynamics of insulin resistance modulation by the treatments. Treatment groups (GEE, GEE-CNPs, and CNPs) exhibited higher insulin resistance levels compared to Negative and Positive controls at some time points,

highlighting the nuanced effect of these interventions on glucose metabolism.

Lipid Profile

The results showed that the Negative Control group (non-diabetic and untreated) had consistently lower lipid values, serving as a baseline for comparison. Particularly, this group exhibited serum cholesterol, triglyceride, and LDL-C levels at 144±0.495 mg/dL, 187±4.75 mg/dL, and 63.6±2.35 mg/dL, respectively (Table 4). These values were significantly lower than those observed in the Control Positive group, underscoring the impact of diabetes on lipid metabolism.

When compared to the Negative Control group, the diabetic Positive Control group showed significantly elevated total serum cholesterol and triglyceride levels at 222±2.8 mg/dL and 228±2.58 mg/dL, respectively (P<0.001). This elevation was accompanied by the highest recorded LDL-C and VLDL-C values in the study, at 138±3.06 mg/dL and 45.6±0.507 mg/dL, respectively, highlighting the dyslipidemia characteristic of untreated diabetes. In contrast, the GEE and GEE-CNPs groups showed a notable reduction in cholesterol (169±6.74 and 168±4.31 mg/dL, respectively) and triglyceride (198±2.64 and 197±3.69 mg/dL, respectively) levels. These reductions were statistically significant (P<0.001) compared to the Positive Control group, indicating the potential lipid-lowering effect of these treatments. The CNPs group also showed a reduction in lipid levels (cholesterol at 153±2.05 mg/dL and triglyceride at 196±2.30 mg/dL). The study also observed a differential impact on HDL-C levels. The Control Negative and CNPs groups displayed significantly higher serum HDL-C levels (43.2±2.05 mg/dL and 45.3 ± 1.82 mg/dL, respectively), as compared to the level observed in the Control Positive group (38.0±0.462 mg/dL) (P<0.036). These findings suggest a beneficial role of CNPs in preserving HDL-C levels in the diabetic state.

Table 3. Effect of ginger ethanolic extract (GEE), ginger loaded with chitosan nanoparticles (GEE-CNPs), and chitosan nanoparticles (CNPs) on serum insulin resistance of adult dogs induced type 2 diabetes

Days	Negative Control	Positive Control	GEE	GEE-CNPs	CNPs	*P*-value
0	2.79±0.30	2.91±0.35	2.65±0.45	2.91±0.40	3.27±0.53	1.251
7	3.77±0.34	4.26±0.89	4.24±0.60	4.12±0.20	4.26±0.87	1.933
14	3.39±0.37 ^{b}	4.68±0.75 ^{ab}	5.76±0.56 ^{a}	5.18±0.44 ^{a}	5.59±0.34 ^{a}	1.552
21	4.46±0.79	4.39±0.54	6.12±0.30	5.77±0.52	5.59±0.76	1.841
28	3.60±0.51 ^{b}	3.46±0.59 ^{b}	6.56±0.94 ^{a}	5.59±0.67 ^{ab}	6.55±0.84 ^{a}	2.188
35	4.90±0.75 ^{ab}	3.53±1.13 ^{b}	6.63±0.64 ^{a}	5.58±1.04 ^{ab}	6.21±1.10 ^{ab}	2.876
42	3.63±0.05	2.99±0.39	4.32±0.34	4.34±0.82	4.23±0.56	1.501
45	4.32±0.39	3.61±0.53	4.00±0.57	3.66±0.67	3.78±0.74	1.178

Values are means±SEM, n = 4 per treatment group. Means in a row without a common superscript letter significantly different (P≤0.05)

Table 4. Effect of ginger ethanolic extract (GEE), ginger loaded with chitosan nanoparticles (GEE-CNPs), and chitosan nanoparticles (CNPs) on serum lipid profile of adult dogs induced type 2 diabetes

Groups	CHL (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
Negative Control	144±0.49 ^{c}	187±4.75 ^{c}	43.2±2.05 ^{a}	63.6±2.35 ^{c}	37.4±0.95 ^{c}
Positive Control	222±2.80 ^{a}	228±2.58 ^{a}	38.0±0.46 ^{b}	138±3.06 ^{a}	45.6±0.51 ^{a}
GEE	169±6.74 ^{b}	198±2.64 ^{b}	42.1±1.72 ^{ab}	87.5±7.21 ^{b}	39.6±0.53 ^{b}
GEE-CNPs	168±4.31 ^{b}	197±3.69 ^{b}	41.3±1.60 ^{ab}	85.4±5.86 ^{b}	40.9±0.90 ^{b}
CNPs	153±2.05 ^{c}	196±2.3 ^{bc}	45.3±1.82 ^{a}	69.9±2.42 ^{c}	39.3±0.46 ^{bc}
P-value	<0.001	<0.001	0.036	<0.001	<0.001

Values are means±SEM, n = 4 per treatment group. Means in a column without a common superscript letter significantly different (P≤0.05). CHL, cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol

Liver Function

In addition to the lipid profile, liver function parameters in serum, including ALT, GGT, and bilirubin were evaluated. Regarding ALT (Figure 1A), a key enzyme indicative of liver health, the Control Positive group had the highest level (86.1±3.44 U/L), reflecting the adverse effects of diabetes on the liver. The lowest ALT level was observed in the CNPs group (33.7±1.83 U/L), followed closely by the GEE-CNPs group (38.9 ± 3.07 U/L), indicating a mitigating effect of these treatments on hepatic stress in diabetic dogs. The GEE and Negative Control groups showed similar ALT levels (45.4±2.70 and 41.7±1.5 U/L, respectively), again highlighting the potential hepatoprotective properties of ginger extract.

For GGT (Figure 1B), another important liver enzyme, the Positive Control group exhibited the highest level (11.6±1.43 U/L), aligning with the observed increase in ALT and bilirubin levels. The other groups, including Negative Control, GEE, GEE-CNPs, and CNPs, demonstrated similar and lower GGT levels, ranging from 7.02±0.373 to 7.77±0.498 U/L. This uniformity across the treated and Control Negative groups suggests a lesser impact of these treatments on GGT levels compared to ALT and bilirubin.

The Positive Control group exhibited the highest serum levels of bilirubin (1.275±0.257 mg/dL), indicating a pronounced impact of diabetes on liver function (Figure 1C). The lowest bilirubin level, however, was recorded in dogs of the CNPs group (0.695±0.071 mg/dL), suggesting a potential protective effect of CNPs against diabetes-induced hepatic dysfunction. The Negative Control and GEE-CNPs groups showed comparable bilirubin levels (0.756±0.035 and 0.748±0.035 mg/dL, respectively), which were lower than the Positive Control but higher than the CNPs group.

The GEE group presented an intermediate level of bilirubin (0.924±0.005 mg/dL).

These findings underscore the significant alterations in liver function parameters due to alloxan-induced diabetes and reveal the potential therapeutic effects of GEE, GEE-CNPs, and CNPs in mitigating these changes. The data contribute valuable insights into the management of diabetes-related liver dysfunction in canine models.

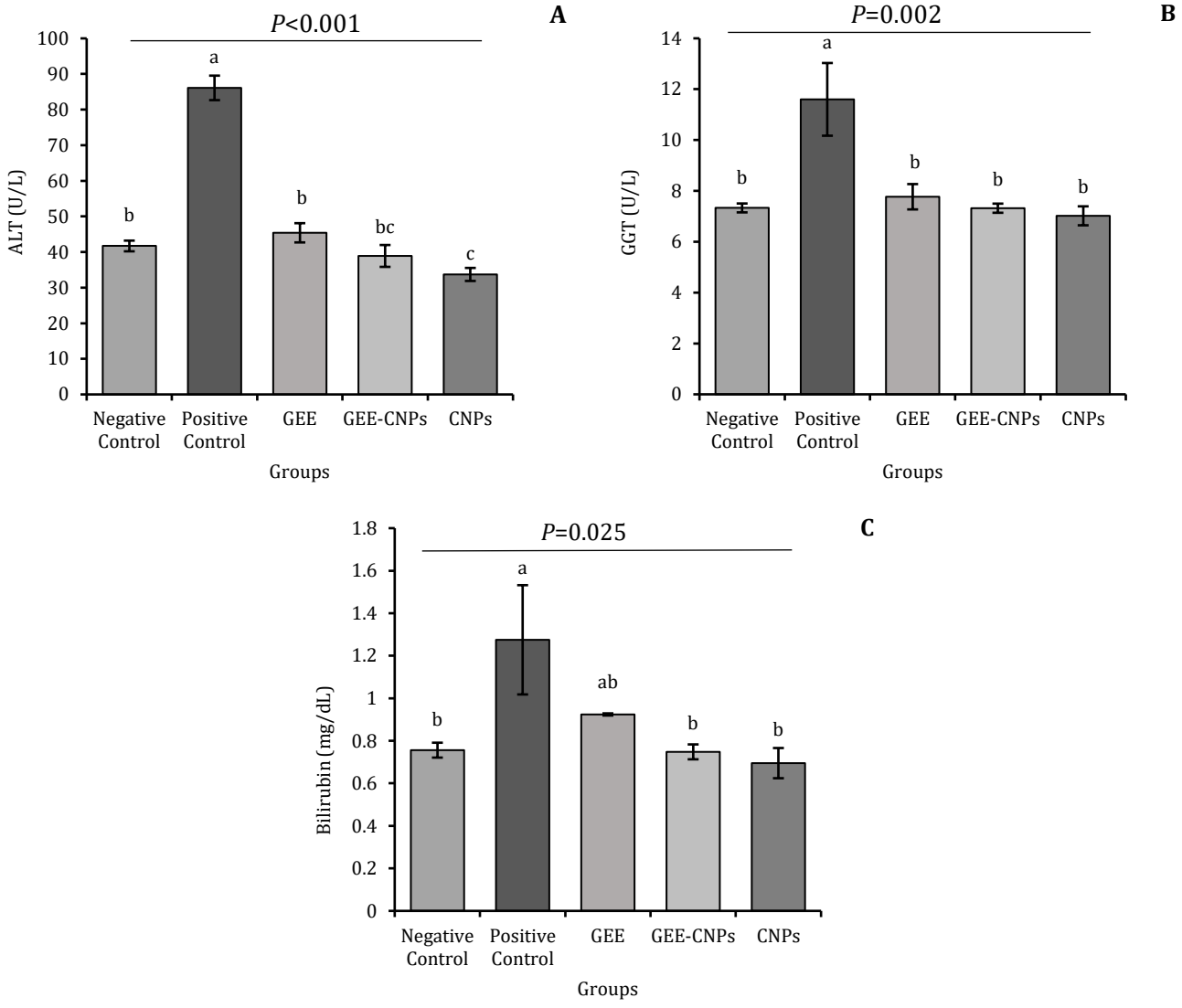

Figure 1. Serum ALT **(A)**, GGT **(B)**, and bilirubin **(C)** levels in adult dogs induced type 2 diabetes and treated by ginger ethanolic extract (GEE), ginger loaded with chitosan nanoparticles (GEE+CNPs), and chitosan nanoparticles (CNPs). Bars (means) with different letters are statistically significant ($P \leq 0.05$). Error bars represent the standard error of mean (SEM), n = 4 per treatment group

Oxidative Status

Diabetic Positive Control dogs exhibited significantly decreased serum GSH (23.4±0.749 µg/mL) ($P<0.001$) and increased ($P<0.001$) MDA levels (835±58.5 µg/mL) compared to non-diabetic Negative Control group (73.7±4.77 µg/mL and 331±37.5 µg/mL, respectively) (Figure 2 A). This indicates the heightened systemic oxidative stress. Treatment with GEE, GEE-CNPs, and CNPs significantly ($P<0.001$) increased serum GSH and reduced MDA when compared to diabetic Positive Control, suggesting attenuation of diabetes-associated oxidative damage (Figure 2 B). However, GEE-CNPs conferred greater amelioration of redox imbalance than GEE alone, as evidenced by GSH and MDA levels closer to the Negative Control group. This points to enhanced bioavailability and antioxidant capacity of GEE when loaded into CNPs.

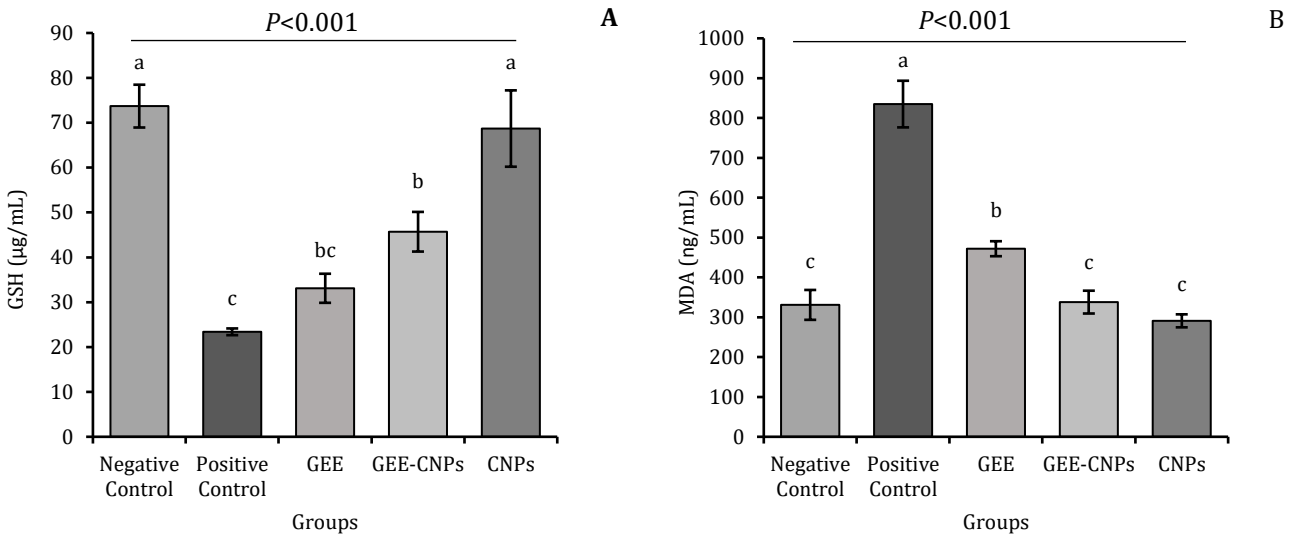

Figure 2. Serum GSH **(A)** and MDA **(B)** levels in adult dogs induced type 2 diabetes and treated by ginger ethanolic extract (GEE), ginger loaded with chitosan nanoparticles (GEE+CNPs), and chitosan nanoparticles (CNPs). Bars (means) with different letters are statistically significant (P≤0.05). Error bars represent the standard error of mean (SEM), n = 4 per treatment group

DISCUSSION

This study aimed to evaluate the potential therapeutic effects of GEE, GEE-CNPs, and CNPs on alloxan-nicotinamide-induced T2DM in dogs. The results show that the Negative Control group, which had no diabetes and no treatment, had stable and normal levels of glucose, insulin, and insulin resistance throughout the experiment, with no significant changes over time at most time points. The Positive Control group, which had diabetes and saline-treated, had significantly higher levels of glucose and significantly lower levels of insulin than the Negative Control group at all time points, indicating that the diabetes induction method was effective, and that the disease was not spontaneously reversed. The results are consistent with previous preclinical studies related to diabetes research, which indicate that a combination of alloxan–nicotinamide can be used to induce T2DM. Alloxan is a toxic compound that mimics glucose and specifically targets the insulin-producing pancreatic β-cells (27). In the present study, intravenous administration of the single dose of alloxan and nicotinamide induced T2DM in dogs in accordance with previous studies (24, 25).

The study found that dogs who received either GEE, GEE-CNP, or CNPs showed significant improvements in their ability to regulate blood sugar levels and their sensitivity to insulin. The fasting glucose levels in the treatment groups decreased gradually and reached nearly normal levels by day 45. Additionally, the circulating insulin levels significantly improved by day 28 and became comparable to the Negative Control group. Partial but non-significant normalization in treatment groups resulted in minor effects on insulin resistance.

The GEE-CNPs demonstrated slight additional benefits in regulating glucose levels compared to the GEE alone, further highlighting the potential of using nanoparticle carriers to enhance biological activity. The marked reduction in fasting glucose levels observed in the treatment groups aligns with previous studies that have reported the hypoglycemic effects of ginger and its bioactive compounds in various experimental models (28-31).

The underlying mechanisms by which ginger exerts its anti-hyperglycemic effects have been attributed to several factors, including enhanced glucose uptake in peripheral tissues, improved insulin sensitivity, inhibition of hepatic gluconeogenesis, and modulation of oxidative stress and inflammation (32-35). The active compounds in ginger, such as 6-gingerol, have been shown to promote glucose uptake in rat skeletal muscle cells and protect pancreatic β-cells from oxidative damage (36, 37). A study by (38) investigated the potential of 6-gingerol in enhancing glucose-stimulated insulin secretion in pancreatic β-cells and promoting glucose disposal in skeletal muscles. The findings revealed that 6-gingerol treatment significantly increased glucose-stimulated insulin secretion and improved glucose tolerance in Leprdb/db in mice with T2DM. Mechanistically, 6-gingerol was found to potentiate the glucagon-like peptide 1 (GLP-1) mediated insulin secretion pathway in pancreatic β-cells by upregulating and activating critical components such as cyclic adenosine monophosphate (cAMP), protein kinase A (PKA), and cAMP response element-binding protein (CREB). Additionally, 6-gingerol facilitated glucose disposal in skeletal muscles by increasing the abundance of glucose transporter type 4 (GLUT4) transporters on the cell membrane, thus enhancing glucose uptake. These findings further underscore the potential of ginger and its bioactive constituents in mitigating hyperglycemia and improving insulin sensitivity in diabetes management.

CNPs have been reported to have antidiabetic properties, such as reducing intestinal glucose absorption,

stimulating insulin secretion, and improving insulin resistance, however, their effect may depend on the dose, size, and surface modification of the nanoparticles (18). The underlying mechanisms by which CNPs exert their anti-diabetic effects may involve the induction of the phosphoinositide-3-kinase–protein kinase B/Akt (PI3K/Akt) pathway, which facilitates glucose uptake and glycogen synthesis (39). Additionally, chitosan has been shown to inhibit carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase, which can reduce glucose absorption in the small intestine and postprandial blood glucose levels (39).

The GEE-CNPs formulation exhibited a minor potent hypoglycemic effect compared to GEE alone, suggesting that the CNPs enhanced the bioavailability and delivery of the GEE. This finding aligns closely with previous studies that have demonstrated the potential of nanoparticle carriers to improve the therapeutic efficacy of natural compounds (40, 41).

The observed increase in serum insulin levels and improvement in insulin sensitivity indices in the treatment groups further support the insulin-sensitizing and insulinotropic properties of GEE, GEE-CNPs, and CNPs. Ginger has been reported to enhance insulin release, potentially through interaction with the 5-hydroxytryptamine type 3 (5-HT3) receptor (42), while chitosan and its derivatives have been shown to activate insulin signaling pathways, such as the PI3K-PKB/Akt pathway, which is involved in glucose uptake and glycogen synthesis (39). In our previous work (21), the phytochemical analysis of the GEE using analytical techniques HPLC and GC-MS revealed the presence of a range of bioactive components that may contribute to the observed antidiabetic effects. HPLC fingerprinting detected phenolic acids like gallic acid, caffeic acid, and ferulic acid, while GC-MS identified various compounds, including fatty acids such as palmitic acid, oleic acid, linoleic acid and vitamin E-like anti-inflammatory oleic acid esters. Many phenolics and fatty acids exert well-established hypoglycemic, antioxidant and anti-inflammatory activities relevant in diabetes through mechanisms like peroxisome proliferator-activated receptor gamma (PPAR-γ) agonism (33). The enrichment of these ginger phytocompounds could, therefore, provide a mechanistic basis for the promising outcomes seen in the diabetic models. Additionally, in our previous work, as earlier mentioned, DPPH radical scavenging assays verified potent antioxidant potential in vitro that aligned with in vivo redox-sensitive improvements, further linking biochemical composition to bioactivity.

Regarding insulin resistance, the fluctuating results across the treatment period highlight the complexity of this metabolic parameter's response to therapeutic interventions. Although treatments seemed to enhance insulin sensitivity in general, the varying levels of insulin resistance indicate a complex interplay between different physiological mechanisms affected by these treatments. This variation may be partially attributed to the functions of glucagon and the protective impact of nicotinamide on pancreatic β-cells, which were essential elements of the diabetes induction model in the present study.The variability emphasizes the importance of having a detailed understanding of how these interventions affect insulin resistance in the context of T2DM.

The improvements in lipid profile parameters are significant as dyslipidemia is a common complication in diabetes mellitus and contributes to the development of cardiovascular diseases. The study's findings revealed a significant improvement in the lipid profile of diabetic dogs treated with GEE, GEE-CNPs, and CNPs. The observed reductions in total cholesterol, triglycerides, and LDL-C levels, coupled with increased HDL-C levels, suggest the potential lipid-lowering and cardioprotective effects of these treatments.

These results are consistent with previous studies that have reported the hypolipidemic effects of ginger and its bioactive compounds in various experimental models (43, 44). The mechanisms underlying the lipid-modulating effects of ginger may involve the inhibition of cholesterol biosynthesis, increased excretion of cholesterol and bile acids, and modulation of lipid metabolism enzymes (45).

Additionally, CNPs demonstrated potential lipid-lowering effects, as evidenced by the reduction in total cholesterol, triglyceride levels and the preservation of HDL-C levels observed in the CNPs group. These findings align with previous studies that have reported the anti-obesity and anti-dyslipidemic effects of chitosan and its derivatives, mediated through the modulation of adipogenesis, inhibition of lipid accumulation, and regulation of genes involved in lipid metabolism, such as PPAR-α (46, 47). The combination of ginger and chitosan nanoparticles may have synergistic effects in improving lipid homeostasis, thereby mitigating the risk of cardiovascular complications associated with diabetes.

The normalization of liver enzyme activities is essential for maintaining liver function and preventing liver damage in diabetic dogs. In this study, the results demonstrated a significant improvement in liver function parameters, including ALT, GGT, and bilirubin levels, in diabetic dogs treated with GEE, GEE-CNPs, and CNPs. These results suggest the potential hepatoprotective effects of these treatments against diabetes-induced liver injury. Diabetes is known to adversely affect liver function, as evidenced by the elevated liver enzyme levels and increased bilirubin observed in the untreated diabetic Positive Control group. The hepatoprotective effects of ginger and its bioactive compounds have been previously reported and attributed to their antioxidant and anti-inflammatory properties (16). Ginger has been shown to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are implicated in various liver diseases, and to exhibit protective effects against hepatic oxidative damage and inflammation (48). Furthermore, chitosan and its derivatives have been reported to possess hepatoprotective properties by modulating oxidative stress, inflammation, and apoptosis in the liver (40). The combination of ginger and CNPs may synergistically

enhance these protective mechanisms, leading to improved liver function in diabetic dogs.

The changes in GSH and MDA levels were examined as markers of oxidative stress in response to the treatments. Oxidative stress plays a crucial role in the pathogenesis of diabetes and its complications, contributing to cellular dysfunction and tissue damage (49). The observed increase in GSH levels and decrease in MDA levels in the treatment groups suggest an improvement in the antioxidant defense mechanisms and a reduction in lipid peroxidation, respectively. Ginger and its bioactive compounds, such as 6-gingerol, have been extensively studied for their antioxidant properties and their ability to scavenge free radicals, inhibit lipid peroxidation, and modulate antioxidant enzyme activities (36, 50). These antioxidant effects may contribute to the protection of pancreatic β-cells and the amelioration of oxidative stress-induced insulin resistance observed in the study.

CNPs have also been reported to possess antioxidant properties and to enhance the bioavailability and stability of antioxidant compounds (40, 41). The combination of ginger extract and CNPs may have synergistic antioxidant effects, contributing to the observed improvements in oxidative status and potentially mitigating the progression of diabetes-related complications.

An important limitation of this study is the small sample size, with only four animals per treatment group. Although significant differences were observed between groups, a larger sample size would increase statistical power and confidence in observed effects. In addition, a larger sample size would better account for individual differences and enhance generalizability to the population. In addition, the study assessed treatment effects over 45 days. This timeframe permits observation of acute and subacute effects on metabolic and biochemical parameters but does not reveal the long-term efficacy and safety of interventions. To effectively manage chronic metabolic disorders like T2DM, it is important to assess the long-term benefits, potential side effects, and impact on disease progression and complications. Long-term studies with larger sample sizes are needed to evaluate the lasting effects, optimize dosing regimens, and prevent or delay diabetic complications, including nephropathy, neuropathy, and cardiovascular disease.

In conclusion, the findings of this study provided compelling evidence for the potential therapeutic benefits of ginger ethanolic extract and its nanoparticle formulations in the management of T2DM and its associated complications. The observed improvements in glycemic control, lipid metabolism, liver function, and oxidative status highlighted the multifaceted mechanisms of action and the potential for synergistic effects when combined with CNPs as a delivery system. While further research is warranted, these findings contributed to the growing body of knowledge on the therapeutic potential of natural compounds and their nanoparticle formulations in the management of chronic metabolic disorders.

ACKNOWLEDGEMENTS

N/A

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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الإمكانات العلاجية لمستخلص الزنجبيل الإيثانولي، والجسيمات النانوية المحملة بالزنجبيل، والجسيمات النانوية الشيتوزان في داء السكري من النوع الثاني المستحث في الكلاب

راسمة مجيد حميد، علاء كامل محمود

فرع الطب الباطني و الوقائي، كلية الطب البيطري، جامعة بغداد، بغداد، العراق

الخلاصة

داء السكري من النوع الثاني في الكلاب هو مرض معقد ومتعدد العوامل يتميز بارتفاع السكر في الدم المزمن ومقاومة الأنسولين. غالبًا ما تكون الخيارات العلاجية الحالية محدودة بالآثار الجانبية والفعالية المتغيرة، مما يسلط الضوء على الحاجة إلى علاجات أكثر فعالية وأمانًا. قيمت هذه الدراسة الإمكانات العلاجية لمستخلص الزنجبيل الإيثانولي، وجسيمات الشيتوزان النانوية المحملة بمستخلص الزنجبيل الإيثانولي، وجسيمات الشيتوزان النانوية ضد داء السكري من النوع الثاني في الكلاب. تم استخدام عشرين كلبًا هجينًا من السلالات المحلية البالغة من كلا الجنسين، تتراوح أعمارهم بين ٧ إلى ١٣ شهرًا، ويبلغ متوسط وزن الجسم ١٠,٤±٠,٧٦ كجم. تم تقسيم الكلاب إلى خمس مجموعات (عدد كل منها ٤): مجموعة مراقبة سلبية غير مصابة بالسكري وغير معالجة، وأربع مجموعات لعلاج مرض السكري، بعد استحداث السكري من النوع الثاني عن طريق حقنة واحدة من الألوكسان-نيكوتيناميد في الوريد. تلقت كل مجموعة علاجية جرعات يومية عن طريق الفم إما من المياه المالحة (التحكم الإيجابي)، أو GEE، أو GEE-CNPs، أو CNPs بجرعة قدرها ٨١,٧ ملجم/كجم من وزن الجسم على مدار ٤٥ يومًا. تم تسجيل حالة نسبة السكر في الدم (الجلوكوز في الدم الصائم، الأنسولين، ومقاومة الأنسولين) عند خط الأساس وفي الأيام ٧، ١٤، ٢١، ٢٨، ٣٥، ٤٢، و ٤٥ بعد العلاج. فضلاً عن ذلك، في اليوم ٤٥، قامت الدراسة بتقييم مستويات الدهون في الدم، ومؤشرات وظائف الكبد (ألانين أمينوترانسفيراز [ALT]، غاما جلوتاميل ترانسفيراز [GGT]، والبيليروبين)، وعلامات حالة مضادات الأكسدة (الجلوتاثيون [GSH] و [malondialdehyde [MDA). أظهرت النتائج أن الكلاب في مجموعة التحكم الإيجابية المصابة بالسكري أظهرت ارتفاعًا في السكر في الدم، واضطراب شحوم الدم، وخللًا في وظائف الكبد، وارتفاعًا في علامات الإجهاد التأكسدي، مما يؤكد التأثير الشديد لـ T2DM . بالمقارنة مع التحكم الإيجابي في مرض السكري، تحسنت علاجات GEE وGEE-CNPs وCNPs عن طريق الفم بشكل ملحوظ ($P < 0.05$) من مستويات الجلوكوز في الصيام وحساسية الأنسولين وملامح الدهون (انخفاض إجمالي الكوليسترول والدهون الثلاثية وLDL-C)، وعلامات وظائف الكبد، وحالة مضادات الأكسدة، مما يشير إلى تعزيز الصحة الأيضية وانخفاض الإجهاد التأكسدي. تشير النتائج إلى أن GEE وGEE-CNPs وCNPs توفر إمكانات كعوامل علاجية لـ T2DM في الكلاب، مما يدل على فوائد كبيرة في التحكم في نسبة السكر في الدم، وتطبيع الدهون، ووظيفة الكبد، وتقليل الإجهاد التأكسدي. يوصى بإجراء مزيد من الدراسات على مجموعات أكبر ولفترات أطول لتأكيد هذه النتائج والتأكد من إمكانية التطبيق السريري وسلامة هذه العلاجات الطبيعية في إدارة مرض السكري لدى الكلاب.

الكلمات المفاحية: الزنجبيل، جزيئات الشيتوزان النانوية، داء السكري من النوع الثاني، داء السكري في الكلاب