Sains Malaysiana 54(4)(2025): 1077-1088
http://doi.org/10.17576/jsm-2025-5404-09

Synergistic Effect of Fenugreek-Zinc Oxide Nanoparticles for Managing Diabetes Mellitus in Experimental Animals

(Kesan Nanozarah Zink Oksida Fenugreek untuk Mengawal Diabetes Melitus dalam Haiwan Uji Kaji)

HALA S. ABDEL FATAH[1,*], MOHAMED A. EL-BADRY[2], ABDULLAH ALSUBAIE[3] & YASER A. EL-BADRY[4]

[1]*Biochemistry Laboratory, Biochemistry and Nutrition Department, Faculty of Women`s for Arts, Science and Education, Ain Shams University, 11767 Cairo, Egypt*
[2]*Biochemistry Department, Research Institute of Medical Entomology, General Organization for Teaching Hospitals and Institutes (GOTHI), Giza, Egypt*
[3]*Physics Department, AlKhurma University College, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia*
[4]*Chemistry Department, Faculty of Science, Taif University, Khurma, P.O. Box 11099, Taif 21944, Saudi Arabia*

Received: 22 June 2024/Accepted: 16 December 2024

ABSTRACT

Fenugreek extract and zinc oxide nanoparticles were prepared using ultrasonication and chemical methods, respectively. The physico-chemical characterization of fenugreek extract and zinc oxide nanoparticles was performed using FT-IR and UV spectroscopy. The phase formation, morphology, and size of particles were investigated by scanning electron microscope and X-ray diffraction. The antidiabetic activities of both Fenugreek extract and zinc oxide nanoparticles were performed using the inhibition assays of α-glucosidase and α-amylase. A mixture of fenugreek and ZnO nanoparticles was prepared under a chemical food strategy and then its efficacy has been examined in preclinical studies on experimental animals. As projected, the fenugreek extract and zinc oxide nanoparticles exerted remarkable antidiabetic activities. It was concluded that they possess a concentration-dependent reduction in the % of inhibition for the α-glucosidase and α-amylase activities. Oral administration of fenugreek extract, zinc oxide nanoparticles, and a mixture of both for 45 days in streptozotocin-prompted diabetic rats exerted a hypoglycemic impact. An expressive reduction in biochemical parameters has been realized with a non-toxic nature of the fenugreek extract, zinc oxide nanoparticles, and the mixture. Conclusively, the superior scope of the nano-mixture for excellent hyperglycemic improvement in contrast to fenugreek extract or zinc oxide nanoparticles supplementation was illuminated via its synergistic effect on diabetic persistence.

Keywords: Diabetes; fenugreek extract; fenugreek-zinc oxide mixture; synergistic effect; zinc oxide nanoparticles

ABSTRAK

Ekstrak fenugreek dan nanozarah zink oksida telah disediakan masing-masing menggunakan kaedah ultrasonik dan kimia. Pencirian fiziko-kimia ekstrak fenugreek dan nanozarah zink oksida dilakukan menggunakan spektroskopi FT-IR dan UV. Pembentukan fasa, morfologi, dan saiz zarah telah dikaji dengan mengimbas mikroskop elektron dan pembelauan sinar-X. Aktiviti antidiabetik kedua-dua ekstrak Fenugreek dan nanozarah zink oksida telah dilakukan menggunakan ujian perencatan α-glucosidase dan α-amilase. Campuran nanozarah fenugreek dan ZnO telah disediakan di bawah strategi makanan kimia dan kemudian keberkesanannya telah diperiksa dalam kajian praklinikal ke atas haiwan uji kaji. Seperti yang diunjurkan, ekstrak fenugreek dan nanozarah zink oksida memberikan aktiviti antidiabetik yang luar biasa. Disimpulkan bahawa mereka mempunyai pengurangan yang bergantung kepada kepekatan dalam % perencatan untuk aktiviti α-glukosidase dan α-amilase. Pemberian lisan ekstrak fenugreek, nanozarah zink oksida dan campuran kedua-duanya selama 45 hari dalam tikus diabetes yang didorong oleh streptozotosin telah menimbulkan kesan hipoglisemik. Pengurangan ekspresif dalam parameter biokimia telah direalisasikan dengan sifat bukan toksik ekstrak fenugreek, nanozarah zink oksida serta campuran. Secara kesimpulannya, skop unggul campuran nano untuk peningkatan hiperglisemik yang sangat baik berbanding dengan ekstrak fenugreek atau suplemen nanozarah zink oksida telah diterangi melalui kesan sinergistiknya terhadap kegigihan diabetes.

Kata kunci: Campuran fenugreek-zink oksida; diabetes; ekstrak fenugreek; kesan sinergistik; nano zarah zink oksida

INTRODUCTION

Diabetes mellitus, endocrine syndrome, is known as one of the most exciting actual health problems that humanity suffers from. The challenge of Type 2 Diabetes mellitus (T2DM) is proposed to expand from the accompanying several complications and is announced as a multifunctional

disease. Reports are suggesting diabetes mellitus will be an essential reason for injury and death in the next two decades (Hbika et al. 2022; Saraste, Knuuti & Bax 2023).

This disease and its complications till now did not have effective management plans; acquired finding new medications possess multiple modes of action (Alkaladi, Abdelazim & Afifi 2014). It was reported that the founded treatments are boring and likely attitude numerous clinical queries (Jin et al. 2008). The most replacer of traditional medications is medicinal plants which play a promising excellent role via extending a supreme degree of efficacy without or even reducing the so many undisciplined side effects. Several decades went and natural products are played as a significant diabetes manager (Gobert & Duncan 2009).

Although numerous medicinal plants like Aloe vera, garlic, and ginger are provided for diabetes mellitus management. A remarkable amount of research papers suggest that fenugreek seeds are the emerging source to cure diabetes and are superior in protection and competency (Bordolo & Dutta 2014; Tak et al. 2024). Recently, fenugreek has been defined as one of the oldest medicinal plants found in ancient Egypt as a seasoning food product. Fenugreek seeds are enriched in soluble fibers and possess multiple benefits for diabetes (El-Nagdy et al. 2024). Such fibers are a good aid for striking the rate of ingestion and consequently affect sugar absorption reduction and insulin elevation. Furthermore, fenugreek seeds contain galactomannan which has the role of decreasing blood sugar levels as well as improving both insulin secretion and sensitivity. Fenugreek seeds have been claimed as a hyperglycemic nutraceutical (Kumar, Bhandari & Jamadagni 2014; Luo et al. 2023). A recent study showed the importance of fenugreek extracts resembles a class of drugs which traditionally available due to biguanidine contents (Perla & Jayanty 2013).

Additionally, advances in nanoparticles are being a focal point of technical and precise research for the interdisciplinary domain of bio-medical medicines in full extension. Nowadays, nanoparticles (NPs) stuff are playing an expressive role in the life span of human beings owing to their privileged features (Ashwini & Mahalingam 2020; Samadder 2014). Along with NPs, metal oxide nanoparticles have earned enhanced significance and are enclosed into a multiplicity of products created on their catalytic capability and medicinal activities (Akintelu & Folorunso 2020).

Among various metal oxide nanoparticles, zinc oxide nanoparticles (ZnONPs) are announced as the major promising nanoparticles and they have a medicinal significance role in the field of biology and medicine owing to their broad availability, low costs, less injuriousness, and high stability (Umrani & Paknikar 2014). ZnONPs are stated to possess anti-diabetic, antioxidant, larvicidal toxicity, and anti-biofilm properties as well as are used as a source of zinc delivery (Vinotha et al. 2019). Recently, the enhanced hemolytic, antimicrobial, antiproliferative, and photocatalytic activities of zinc oxide nanoparticles have been reported (Vardatsikos, Pandey & Srivastava 2013). On the other hand, the crucial effect of zinc as antidiabetic as well as its insulin-like effects and α-amylase inhibition has been established in numerous studies (Hbika et al. 2022).

In continuation of our program at the present study, we intended to pay attention to the exploration of the synergistic role of fenugreek seeds extract combined with ZnONPs and ZnONPs themselves on diabetes mellitus type II and its complications as well as checking these materials for their anti-diabetic assessment for α-amylase and α-glucosidase inhibitions.

MATERIALS AND METHODS

MATERIALS AND MAIN REAGENTS

All chemical reagents and solvents required for the synthesis of nano oxide particles and the chemicals for supporting anti-diabetic activity assays like α-amylase and α-glucosidase inhibitions are procured from Sigma-Aldrich, Germany. They are analytical grades and were used without further purification. Fenugreek seeds were purchased from farms at EL-Fayoum Governorate, Egypt. Streptozotocin is procured from Cornell lab. Co., Giza, Egypt.

SYNTHESIS OF ZINC OXIDE NANOPARTICLES (ZnONPs)

Synthesis of zinc oxide nanoparticles was enrolled using a chemical oxidation method. Typically, A 20 mL solution of 0.1 M KOH in deionized water was added dropwise under vigorous magnetic stirring at 70 °C to a 20 mL solution of 0.5 M zinc acetate in deionized water, the stirring was continued till a consistency was obvious (2-3 h). Then, the homogenous solution formed was left to cool down and kept at r.t for 1 h and the formed precipitate was centrifuged, filtered off, and splashed several times using deionized water and EtOH. The precipitate obtained was air-dried followed by annealing at 500 °C for 2 h to get ZnO nanoparticles as a white powder.

FENUGREEK EXTRACT (FE)

The seeds were washed, dried in the sun then roasted at low temperatures (70-100 °C). Thereafter grounded and reserved for further use. Dried, powdered fenugreek seeds (10 g) and 50 mL of ethanol were ultrasonicated at 30 °C for 45 min. The formed extract was percolated formerly, concentrated, and stored at 4 °C till be exploited.

PHYSICO-CHEMICAL CHARACTERIZATION

ZnO nanoparticles were characterized using infra-red and ultra-violet spectral techniques. The phase formation,

morphology, and size of particles were investigated by X-ray diffraction (Bruker system XRD) and scanning electron microscope (SEM, Hitachi SU6600). The chemical composition of fenugreek extract (FE) was deduced from its IR (infra-red) spectrum.

ANTI-DIABETIC ACTIVITY ASSAY

The anti-diabetic activities of FE and ZnONPs were demonstrated using the α-amylase and α-glucosidase inhibition assays.

α-Glucosidase Inhibition Assay

At first, a mixture of α-Glucosidase (10 µL, 0.025 M) and different concentrations (50-300 µg/mL of FE and/or 20-100 µg/mL of ZnONPs) was sonicated at r.t for 30 min (to prevent nanoparticles accumulation). The reaction was started by adding the substrate p-nitrophenyl-α-D-glucopyranoside (1.5 mM) followed by incubation at 37 °C for 25 min and was terminated using sod. carbonate (2 mL, 0.1 M). Measurements were carried out at 420 nm and the inhibition rates were calculated as a percentage (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

α-Amylase Inhibition Assay

The α-amylase inhibition assays of FE and ZnONPs were executed according to the starch-iodine procedure with slight modification (Clark & Hales 1991). Concisely, a mixture of 10 µL α-amylase (0.025 M) and different concentrations (50-300 µg/mL of FE and/or 20-100 µg/mL of ZnONPs) was sonicated at r.t for 30 min (to prevent nanoparticles accumulation). Adding a solution of 250 µL starch (0.5%) to the reaction mixture, incubated at 37 °C for 20 min, then, termination of the reaction was sequenced using 2 mL of 1% 3,5-dinitrosalicylic acid. Then, the determinations of the sample substrate, the positive control (acarbose), and the α-amylase blank were performed under the same conditions by measuring the absorbance at 540 nm. Inhibition of the enzyme activity was calculated as (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$.

EXPERIMENTAL ANIMALS

The experiment was conducted with sixty white male albino rats of Sprague-Dawley strain of 150 ± 20 g approximate weight, attained from EOBPV, Helwan, Cairo, Egypt. The randomization and maintaining rats in the specific pathogen-free environment were exposed to a 12:12 h daylight/darkness and supported the basal diet devoid of any treatment for one week for adaptation.

INDUCTION OF DIABETES MELLITUS

Diabetes mellitus was induced in forty-eight rats using a single intraperitoneal injection of STZ (40 mg/kg) in a newly formulated citrate buffer (0.1 mol/L, PH 4.5). Rapidly after injection, rats were given 5% glucose solution for 48 h. After three days, the fasting blood glucose was measured to ascertain the diabetic status.

The experiment was designed for 45 days of daily oral gavage and experimental animals were collected into the following five groups: Group 1 (Twelve rats were served as control and were obtained an equivalent amount of citrate buffer (vehicle), a normal control group (NC)); Group 2 (Twelve rats were indicating STZ-induced diabetic rats, diabetic control (DC)); Group 3 (Twelve rats were diabetic and obtained fenugreek extract (FE) (100 mg/kg body weight) (D+(FE)); Group 4 (Twelve rats were diabetic and obtained ZnONPs (10 mg/kg body weight) (D+NPs); and Group 5 (Twelve rats were diabetic and obtained FE (100 mg/kg body weight) and ZnONPs (10 mg/kg body weight) (D+FE-NPs)).

BLOOD SAMPLES COLLECTION

A periodical fasting blood samples were vacated from the tail and plasma glucose, insulin, and HbA1c were estimated. After 45 days, the planned scarification time, the vacated fasting blood samples were obtained from the retro-orbital vein and divided into three tubes instantly, a fluoride tube for instant fasting plasma glucose determinations, the second was EDTA tube for isolation of plasma, and the third without anticoagulant was used for biochemical parameters determinations.

BIOCHEMICAL ASSAYS

The glucose oxidase procedure was followed for fasting plasma glucose assay and a formerly pronounced ELISA procedure was followed for plasma insulin estimation (Manita et al. 2023). Determination of HbA1c has followed a known procedure (Tietz 1976). The serum lipid profile, serum liver functions (AST, ALT, and albumin), blood urea, and serum creatinine were investigated following reported standard techniques (Brod & Sirota 1948; King 1965; Lowry et al. 1951; Natelson, Scott & Beffa 1951; Steel & Torri 1980).

STATISTICAL ANALYSIS

The statistical exploration was performed using an announced software (edition 16.0; SPSS Inc, Chicago, USA). The stated results as mean ± SD and the one-way analysis of variance (ANOVA) were established and exploited to study whether there are any statistically significant differences between the means of two or more independent (unrelated) groups. The Bonferroni-Dunn test was used to determine differences between means ($P < 0.05$) (Uribe-López et al. 2021).

RESULTS

SPECTRAL DATA AND STRUCTURAL ANALYSIS OF ZnONPs

The Uv-vis. data of the synthesized ZnONPs exhibited a characteristic absorption peak at 385 nm attributed to the absorption of ZnONPs wide band-gap; owing to the electronic transition of the valence band into the conduction band (O2p → Zn3d) (Benayad et al. 2014). The FT-IR spectra of the synthesized ZnONPs confirm its formation where it exhibited absorption bands at 454 and 3413 cm^{-1} attributed to $\nu_{Zn\text{-}O}$ bending and ν_{OH} stretching, respectively.

Figure 1 shows the XRD pattern of the synthesized ZnONPs, it exhibited diffraction peaks at values 31.90, 34.65, 36.41, 56.80, 63.05, 67.95, and 69.40 corresponding to the plane of reflections (100), (002), (101), (102), (110), (112), and (201), respectively. These results are in good agreement with the standard data obtained from the Joint Committee on Powder Diffraction Standard (JCPDS); such agreement emphasizes the wurtzite phase formation of the synthesized ZnONPs.

SEM image of the synthesized ZnONPs shown in Figure 2 exhibited the particle size around 20 nm with nearly monodispersed in nature. As well as the particles were in excellent connection with a uniform matrix of ZnO material.

CHEMICAL COMPOSITION OF FENUGREEK SEED EXTRACT (FE)

The phenolic composition of FE originating from Egypt (Fayoum Government) has been investigated. Its FT-IR spectra showed strong absorption bands for alcoholic O-H, carbonyl groups as well as for olefinic, CH-aliphatic and aromatic, and S-S bond.

ANTI-DIABETIC ACTIVITIES OF FE AND ZnONPs

Figures 3 and 4 show the *in vitro* α-glucosidase and/or α-amylase inhibitory findings using FE and ZnONPs. Both FE and ZnONPs displayed remarkably elevated antidiabetic activities against α-glucosidase and/or α-amylase incomparable to the therapeutic drug acarbose. The fenugreek extract produced the inhibition % varied from 10.2% to 42.1% and 8.9% to 54.3% for α-glucosidase and/or α-amylase activities, respectively. On the other hand, ZnONPs flashed a considerably higher inhibition % varied from 19.1% to 73.8% and 21.8% to 49.6% for α-glucosidase and/or α-amylase activities, respectively.

EFFECT OF ORAL ADMINISTRATION OF FENUGREEK EXTRACT (FE), ZnO NANOPARTICLES (ZnONPs), AND FENUGREEK+ZnO NANOPARTICLES (FE-NPS) ON FOOD INTAKE, BODY WEIGHT GAIN, AND FOOD EFFICIENCY RATIO FOR INVESTIGATED GROUPS (45 DAYS)

Data derived from Figure 5 recorded a food intake significant difference between the normal group G1 (19.24 g/day) and positive control G2 (14.06 g/day), at $p < 0.05$. Diabetic groups G3 (17.23 g/day) and G5 (16.23 g/day) showed an expressive difference ($p < 0.05$) regarding the diabetic control G2 (14.06 g/day). In contrast, diabetic group G4 did not show any meaningful difference, ($p < 0.05$).

Body weight gain has displayed a suggestive weight loss ($p < 0.05$) in diabetic control group G2 (43.61 g) regarding negative control G1 (69.41 g) has been observed. On the other hand, there is a significant enhancement of the body weight gain for the treated diabetic groups G3, G4, and G5 at $p < 0.05$, 65.13, 53.47, and 58.86 g, respectively, in comparison to the diabetic control group 'DC' (43.61 g). Also, data summarized in Figure 5 showed that food efficiency ratio (FER) was significantly increased at ($p < 0.05$) by 1.37% and 1.42%, respectively, after treatment for [D+(FE-NPs)] and diabetic control as contrasted with negative control (0.61%).

BIOCHEMICAL EFFECT OF ORAL ADMINISTRATION OF FENUGREEK EXTRACT (FE), ZnO NANOPARTICLES (ZnONPs), AND FENUGREEK+ZnO NANOPARTICLES (FE-NPS) ON EXPERIMENTALLY INDUCED DIABETIC RATS

Effect of Fenugreek Extract (FE), ZnO Nanoparticles (ZnONPs), and Fenugreek+ZnO Nanoparticles (FE-NPs) on Blood Glucose Level, Serum Insulin Level, and Percent of Glycosylated Hemoglobin (HbA1c) for the Investigated Groups (45 Days)

Figures 6 and 7 counted the influence of fenugreek extract (FE), ZnO nanoparticles (ZnONPs), and fenugreek+ZnO nanoparticles (FE-NPs) on blood glucose level, serum insulin level, and percent of Glycosylated hemoglobin (HbA1c) for normal and diabetic groups. We found that serum glucose level was extensively reduced ($p < 0.05$) with the medians 207.62, 228.36, and 180.03 mg/dL for the diabetic groups G3, G4, and G5, respectively; in comparison with the mean value recorded in the diabetic control group G2 (426.74 mg/dL).

Alternatively, the mean value of serum insulin level in diabetic groups G3, G4, and G5 has markedly significant elevation ($p < 0.05$) 8.24, 8.65, and 9.35 U/L, respectively, regarding diabetic control (5.98 U/L). The percent of Glycosylated hemoglobin has been counted as a statistically notable dropping ($p < 0.05$) in G3 (6.74%), G4 (7.08%), G7 (7.08%) in comparison to diabetic group G2 (11.62%).

Effect of Fenugreek Extract (FE), ZnO Nanoparticles (ZnONPs), and Fenugreek+ZnO Nanoparticles (FE-NPs) on Serum Lipid Profile for Investigated Groups (45 days)

The validity of FE, ZnONPs, and FE-NPs on serum lipid profiles for investigated groups was pronounced in Figure 8. There is no significant effect ($p < 0.05$) of the F-ZnONPs and ZnONPs oral administration in the treated diabetic groups G3 and G5 on the lipid profile assays concerning the negative control (G1).

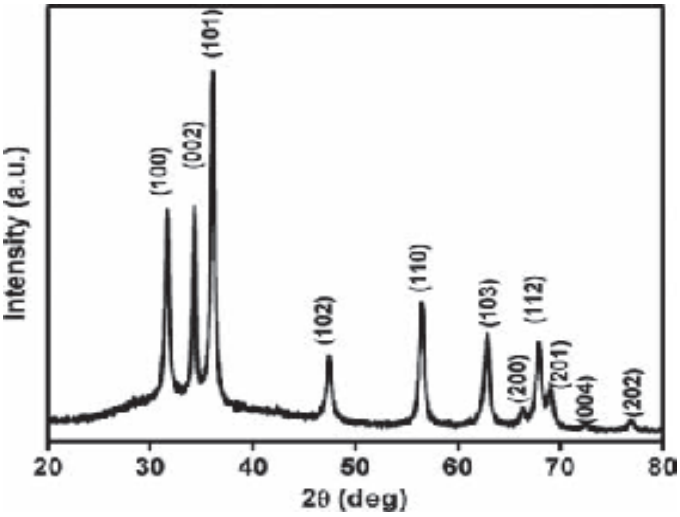


FIGURE 1. XRD data of ZnO nanoparticles

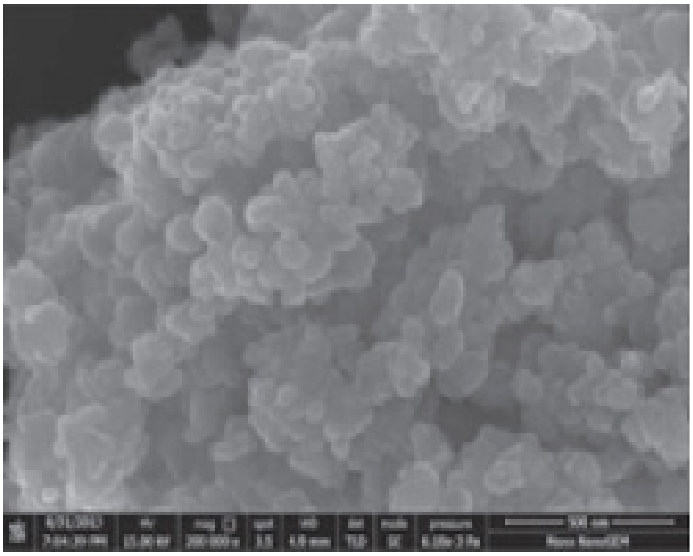


FIGURE 2. SEM imaging for ZnO nanoparticles

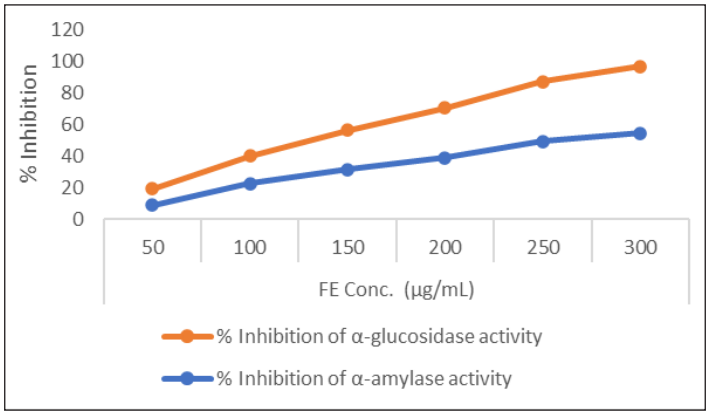


FIGURE 3. Inhibitory effect of FE on α-glucosidase and α-amylase activities

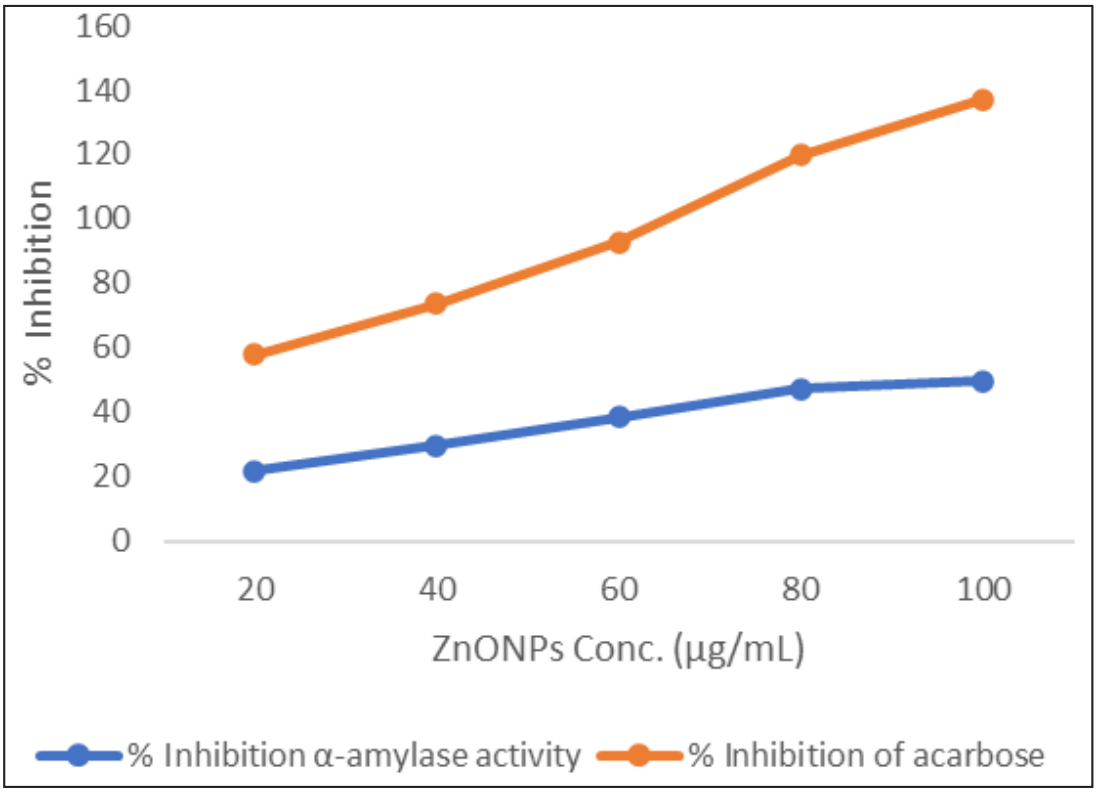
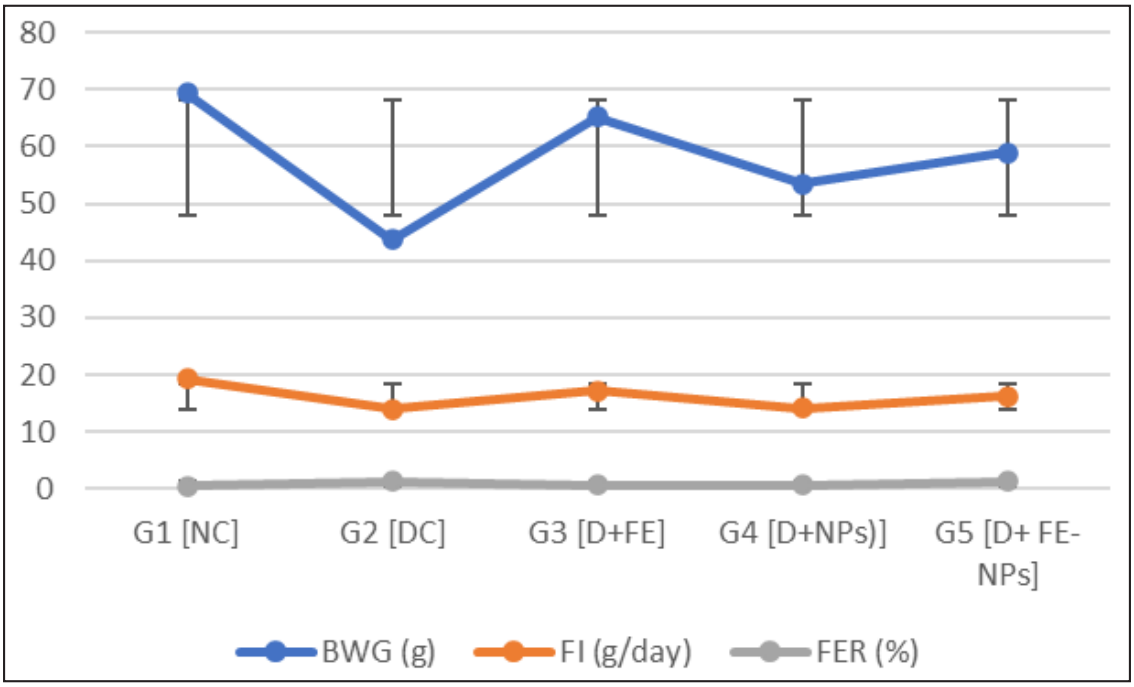
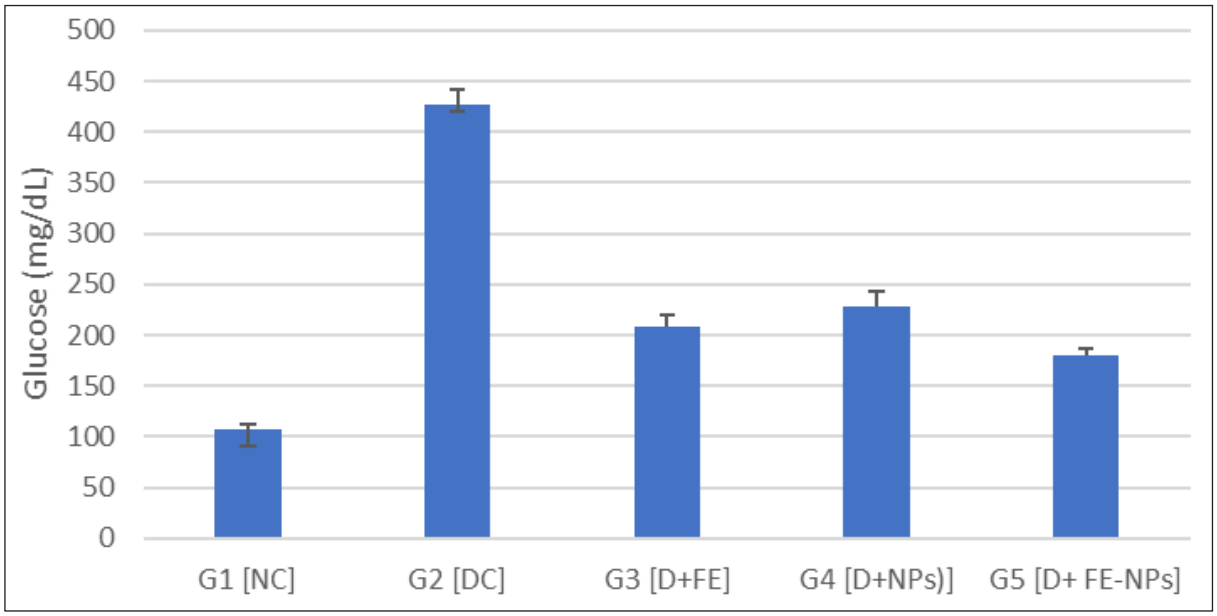


FIGURE 4. Inhibitory effect of ZnO nanoparticles on α-glucosidase and α-amylase activities



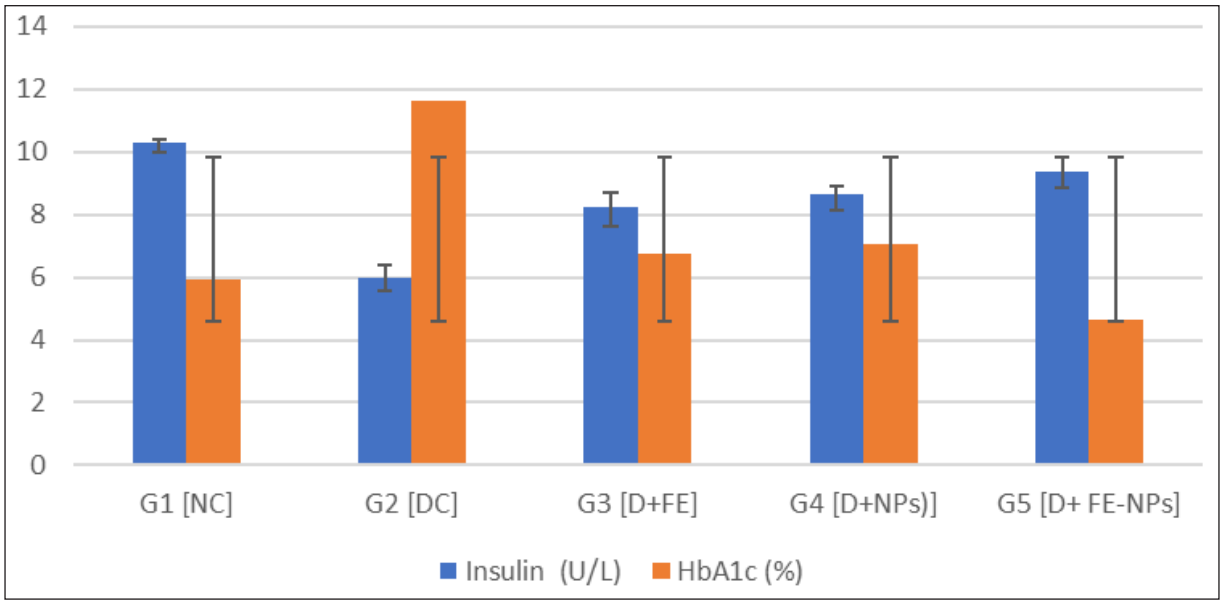
Values are given as Mean ± SD for groups of twelve rats: Each value is considered statistically significant at $p < 0.05$. Statistical significant was compared within groups as follows: a) Groups vs. normal control 'G1' and b) Groups vs. diabetic control 'G2'

FIGURE 5. Effect of FE, ZnONPs, and FE-NPs on BWG, FI, and FER for normal and diabetic rats (45 days)



Values are given as Mean ± SD for groups of twelve rats: Each value is considered statistically significant at $p < 0.05$. Statistical significant was compared within groups as follows: a) Groups vs. normal control 'G1' and b) Groups vs. diabetic control 'G2'

FIGURE 6. Effect of FE, ZnONPs, and FE-NPs on blood glucose level (45 days)



Values are given as Mean ± SD for groups of twelve rats: Each value is considered statistically significant at $p < 0.05$. Statistical significant was compared within groups as follows: [a)] Groups vs. normal control 'G1' and b) Groups vs. diabetic control 'G2'

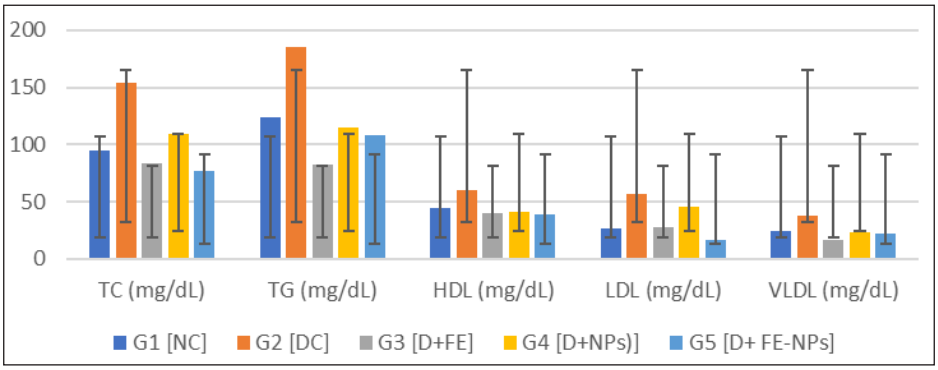
FIGURE 7. Effect of FE, ZnONPs, and FE-NPs on serum insulin level and percent of Glycosylated hemoglobin (HbA1c) (45 days)

The mean value of TC in diabetic groups G3, G4, and G5 was reduced significantly ($p < 0.05$) to the mean values 83.25, 109.65, and 76.38 mg/dL, respectively, in comparison to the diabetic control G2 (154.32 mg/dL). In the same fashion, TG levels for G3, G4, and G5 were significantly decreased ($p < 0.05$) to record the individual values 82.35, 114.65, and 107.88 mg/dL, concerning the diabetic control (185.62 mg/dL).

Similarly, the achieved findings shown in Figure 8 showed a meaningful reduction ($p < 0.05$) of HDL (39.43, 41.38, and 38.26 mg/dL), LDL (27.35, 45.34, and 16.54 mg/dL), and vLDL levels (16.47, 22.93, and 21.58 mg/dL) for the treated diabetic groups G3, G4, and G5, respectively, in respect to the diabetic control 'DC' (60.51, 56.69, and 37.12 mg/dL) for HDL, LDL, and vLDL, respectively.

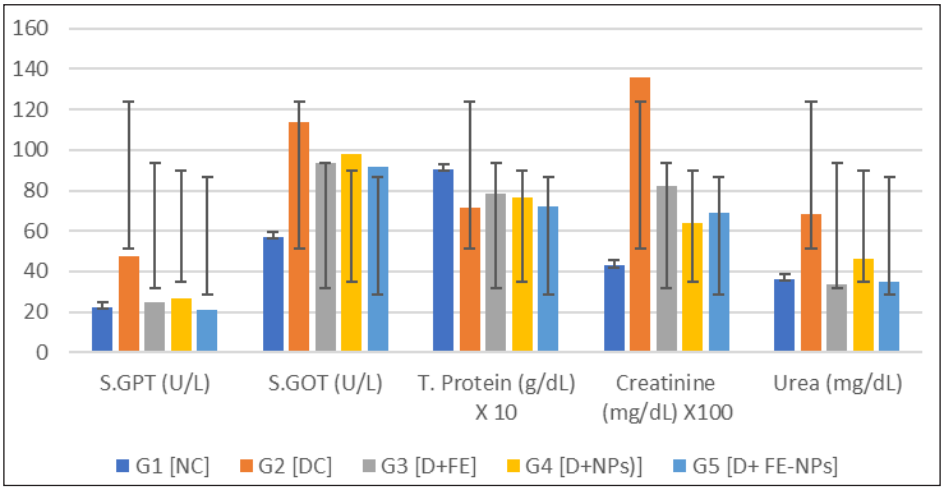
Effect of Fenugreek Extract (FE), ZnO Nanoparticles (ZnONPs), and Fenugreek+ZnO Nanoparticles (FE-NPs) on Liver and Kidney Functions for the Investigated Groups (45 days)

The selected liver functions results are shown in Figure 9. The oral processing of FE, ZnONPs, and FE-NPs in G3, G4, and G5 prompted an expressive decrease ($p < 0.05$) on the enzyme's serum activities, S.GPT (24.63, 26.38, and 21.00 U/L), S.GOT (93.50, 98.28, 91.38 U/L); regarding G2 group, S.GPT (47.38 U/L), S.GOT (113.88 U/L), respectively. Furthermore, total protein findings exhibited an expressive enhancement ($p < 0.05$) in the respective treated groups G3, G4, and G5 (7.85, 7.68, and 7.22 mg/dL) in comparable to positive control G2 (7.14mg/dL).



Values are given as Mean ± SD for groups of twelve rats: Each value is considered statistically significant at $p < 0.05$. Statistical significant was compared within groups as follows: a) Groups vs. normal control 'G1' and b) Groups vs. diabetic control 'G2'

FIGURE 8. Effect of FE, ZnONPs, and FE-NPs on serum lipid profile (45 days)



Values are given as Mean ± SD for groups of twelve rats: Each value is considered statistically significant at $p < 0.05$. Statistical significant was compared within groups as follows: a) Groups vs. normal control 'G1' and b) Groups vs. diabetic control 'G2'

FIGURE 9. Effect of FE, ZnONPs, and FE-NPs on liver and kidney functions (45 days)

Additionally, Figure (9) shows the influence of oral processing of FE, ZnONPs, and FE-NPs on the kidney functions of the groups under investigation. The diabetic groups G3, G4, and G5 showed a meaningful reduction ($p < 0.05$) of serum creatinine readings 0.82, 0.64, and 0.69 mg/dL, respectively, in comparison with the mean value recorded in the positive control G2 (1.36 mg/dL). An expressive statistical shrinkage ($p < 0.05$) was observed in the blood urea levels for diabetic groups G3 (33.77 mg/dL), G4 (46.13 mg/dL), and G5 (34.60 mg/dL) when compared with 'DC' group (68.60 mg/dL).

DISCUSSION

The affordability of functional foods as a source of natural medications possesses smaller unpleasant consequences than synthetic drugs. Particularly, numerous diabetes mellitus synthetic drugs are easily available but still unsafe, not cheap, and have unclear nonexpert effectivity.

Nanoparticles (NPs) possess exceptional properties and behaviors owing to their well-known specific dimensional feature. Moreover, they are conducting several innovative scientific and technical applications indebted to their distinctive physical, chemical, or biological properties. A small number of research papers are informed by the effectiveness of ZnONPs on insulin secretion or even its levels. As well as ZnO nanoparticles are suggested to enhance glucose-stimulated insulin secretion (Debele & Park 2022).

The metabolic chronic hyperglycaemic disorder, Diabetes mellitus, is indebted impairment the insulin secretion, action, or jointly and is accomplished beyond insufficient carbohydrate, protein, or fat metabolism (Drent et al. 2002). The process of breaking down oligosaccharides and disaccharides into monosaccharides (glucose synthesis) took place via the dual inhibition action of the intestinal α-glucosidase enzyme and pancreatic α-amylase enzyme (Hbika et al. 2022).

The *in vitro* inhibitory studies of α-glucosidase and α-amylase for FE and ZnONPs showed a great beneficial effect of both reducing the rates of absorption and digestion of carbohydrates and consequently affording an effective diabetes management scenario, which is in agreement with the previous findings by Rehana et al. (2017) who established that, the *in vitro* α-glucosidase and α-amylase inhibitory effects of FE and ZnONPs were useful to investigate their antidiabetic activity and excluding substantial time and money.

Our efforts showed a resultant suggestive enhancement for both plasma glucose and glycosylated hemoglobin percent and an expressive reduction of plasma insulin levels for the treated groups regarding the basal levels. One can interpret such results as the diabetic animal prototypes show a proper elevated oxidative stress indebted for persistent and chronic hyperglycemia. In that way, the antioxidative defense system activity has been depleted, accordingly, the generation of free radicals was promoted. Previous results proved the more effective antidiabetic and antioxidant activities for some ZnO nanocomposites (Vinotha et al. 2019). Rochette et al. (2014) have primarily screened diabetes, oxidative stress, and therapeutic approaches. One can envisage that the glucose injuriousness was a β-cell dysfunction caused particularly by oxidative stress in agreement with recent literature (Ravi Kiran, Subramanyam & Asha Devi 2004).

Administration of the F-ZnONPs mixture, as well as ZnONPs, precedes a restored dispersal of the tissues' blood flow and is devoid of meaningful discrepancies in further metabolites, consequently, minimization of the injury magnitude produced from the generated reactive oxygen species has been achieved. We can suggest the role of ZnO nanoparticles in delivery; they have an important task, facilitating glucose penetration in the cell tissues; ZnO nanoparticles are an easy crosser of biological membranes and can be accumulated in the blood. Moreover, zinc has also been suggested to interact with cell membranes to stabilize them against oxidative damage (Suwalsky et al. 2009). Occasionally, we can say the plant-ZnONPs mixture resulted in higher antidiabetic and antioxidant activities owing to the presence of amino acids and proteins besides others like phytochemical constituents, is in agreement with Rehana et al. (2017).

A former study was matched with us and has announced that fenugreek seeds retain an encouraging antioxidant property and have been considered a treasured aspirant for diabetes impediment management and prevention (Hanna, Sándor & Wink 2022). Our findings came following the judgments of Hamza et al. (2012); who conveyed that the oral administration of fenugreek extract (2 g/kg b.w. daily) for 17 weeks reduced the serum glucose level.

Sayed, Khalifa and Abd El-Latif (2012) showed that fenugreek seeds significantly reduced the elevated glucose level in experimental diabetic animals linked to the untreated diabetic group. Furthermore, it has been spotted that the increase in serum glucose was the salient feature chronicled in diabetic control rats which significantly decreased the glucose level in diabetic-fenugreek supplemented rats (1 g/kg b.w.) (Marzouk, Soliman & Omar 2013).

Also, serum insulin level variation is considered for conveying the glucose level. Our conclusions emphasize that oral administration of fenugreek extract mixed with ZnONPs and ZnONPs itself has significantly exerted a hypoglycemic effect, which enriches insulin secretion hyperglycemia and improves insulin sensitivity. It is presumed to proceed via motivating the pancreatic β-cells to release the glucose-dependent insulin, which agrees with previous results recorded by Sauvaire, Petit and Broca (1998). Such findings were also confirmed by Dhull et al. (2024) who established the fenugreek seeds effect on the serum insulin of human subjects.

Characteristic protein glycation is a common feature in diabetes mellitus attributed to irreversible glycation, and consequently, a drastic reduction in hemoglobin levels has been attained. Oral supplementation of F-ZnONPs

composite and ZnONPs resulted in a marked reduction in the HbA1c percentage of the diabetic groups. Neelakantan et al. (2014) found a lessened percentage of glycated hemoglobin for the administration of fenugreek seeds and extracts of the dose ranging from 5 to 100 g/day in human diabetes trials.

The non-esterified fatty acids are one of the diabetes accompanying complications. A hyperlipidemia case is pronounced by diabetic dyslipidemia and indebted elevated coronary heart disease risk factors. The findings in the present study are in correlation with the findings of the literature (Sharma, Balomajumder & Roy 2008; Srivastava 2018; Vijayaraghavan 2010).

The remarked elevated liver enzymes AST and ALT which were observed in diabetic rats are suggested to raise from the liver cytosol insufficient enzymes paths through the bloodstream; a result of the hepatic tissue damage (Scott et al. 1984). The setback of the investigated liver enzyme levels in F-ZnONPs mixture and ZnONPs treated diabetic animals approaching the typical emphasis for the origin of hepatic protection (Arvind et al. 2002).

Fascinatingly, our findings documented a significant ameliorating effect of fenugreek-ZnONPs and ZnONPs on liver insult in diabetic rats. This effect is signposted by the abrogation of liver enzymes and the regularization of the virtual liver weight-to-bodyweight ratio. The hepatoprotective power of the F-ZnONPs mixture established in the present study might be due to the antioxidant properties of the fenugreek extract incorporated via the provocation of endogenous antioxidants resistance and their antiradical effect which decreasing STZ-induced intrusion of lipid profile, decreasing STZ-induced elevation of blood glucose level.

Renal dysfunctions are one of the accompanying diabetes complications and are known as diabetic proteolysis; there is an extensive proliferation in the serum creatinine and blood urea concentrations. The noteworthy influence of the fenugreek-ZnONPs supplementation was superior in reducing the serum creatinine levels than other investigated treatments ascertained the modest adaptation of protein and carbohydrate metabolism for the fenugreek-ZnONPs mixture treated group.

Finally, the upraised urea levels monitored in diabetic-treated groups have returned closely to the ordinary levels for the fenugreek-ZnONPs nano mixture treated group because of progressed glycemic management. Ajaya, Shetty and Salimath (2009) surveyed the enhanced renal enzyme endeavors concerned with glycosaminoglycans synthesis/degradation were notably reduced by fenugreek administration (Kodumuri et al. 2019).

CONCLUSIONS

Fenugreek and ZnONPs have exerted a synergistic role in diabetes, where the treasured force of fenugreek extract in glycemic significance was investigated similarly as the exploit of nano mixture in cell penetration as well as in insulin maintenance. Moreover, a release or reduction in hyperglycemia complications and getting rid of toxic effects has succeeded. Also, FE and ZnONPs have been explored as promising antidiabetic agents and showed a concentration-dependent reduction against both α-glucosidase and α-amylase.

ACKNOWLEDGMENTS

We would like to extend our appreciation to Taif University, Saudi Arabia, for supporting this work through project number (TU-DSPP-2024-222).

REFERENCES

Ajaya, K., Shetty, P. & Salimath, V. 2009. Reno-protective effects of fenugreek (*Trigonella foenum greacum*) during experimental diabetes. *E. J. Clin. Nutr. Metab.* 4(3): 137-142.

Akintelu, S.A. & Folorunso, A.S. 2020. A review on green synthesis of zinc oxide nanoparticles using plant extracts and its biomedical applications. *BioNanoSci.* 10: 848-863.

Alkaladi, A., Abdelazim, A.M. & Afifi, M. 2014. Antidiabetic activity of zinc oxide and silver nanoparticles on streptozotocin-induced diabetic rats. *Int. J. Mol. Sci.* 15: 2015-2023.

Arvind, K., Pradeepa, R., Deepa, R. & Mohan, V. 2002. Diabetes & coronary artery disease. *Ind. J. Med. Res.* 116: 163-176.

Ashwini, D. & Mahalingam, G. 2020. Green synthesized metal nanoparticles, characterization and its antidiabetic activities- A review. *Res. J. Pharm. Tech.* 13(1): 468-474.

Benayad, Z., Martinez-Villaluenga, C., Frias, J., Gomez-Cordoves, C. & Es-Safi, N. 2014. Phenolic composition, antioxidant and anti-inflammatory activities of extracts from Moroccan *Opuntia ficus-indica* flowers obtained by different extraction methods. *Ind. Crops Prod.* 62: 412-420.

Bordolo, R. & Dutta, N. 2014. A review on herbs used in the treatment of diabetes mellitus. *J. Pharm. Chem. Biol. Sci.* 2(2): 86-92.

Brod, J. & Sirota, J.H. 1948. The renal clearance of endogenous creatinine in man. *J. Clin. Invest.* 27: 645-654.

Clark, P.M.S. & Hales, C.N. 1991. Assay of insulin. In *Textbook of Diabetes,* edited by Pickup, P.C. & Williams, G. Blackwell Scientific Publications. 1: 335-347.

Debele, T.A. & Park, Y. 2022. Application of nanoparticles: Diagnosis, therapeutics, and delivery of insulin/anti-diabetic drugs to enhance the therapeutic efficacy of diabetes mellitus. *Life (Basel, Switzerland)* 12(12): 2078.

Dhull, S.B., Bamal, P., Chandak A., Nain, K.B. & Malik, A. 2024. Fenugreek (*Trigonella foenum-graecum*): An herb with impressive nutritional and antidiabetic properties. In *Antidiabetic Medicinal Plants,* edited by Naeem, M. & Aftab, T. Massachusetts: Academic Press. pp. 305-325.

Drent, M.L., Tollefsen, A.T., van Heusden, F.H., Hoenderdos, E.B., Jonker, J.J. & van der Veen, E.A. 2002. Dose-dependent efficacy of miglitol, an alpha-glucosidase inhibitor, in type 2 diabetic patients on diet alone: results of a 24-week double-blind placebo-controlled study. *Diabetes Nutr. Metab.* 15(3): 152-159.

El-Nagdy, S.A., Elfakharany, Y.M., Morsy, M.M., Ahmad, M.M., Abd El-Fatah, S.S. & Khayal, E.E. 2024. The role of fenugreek seed extract in alleviating pancreatic toxic effects and altering glucose homeostasis induced by acetamiprid via modulation of oxidative stress, apoptosis, and autophagy. *Tissue Cell* 86: 102265.

Gobert, C.P. & Duncan, A.M. 2009. Consumption, precipitations and knowledge of soy among adults with type 2 diabetes. *J. Am. Coll. Nutr.* 28(2): 203-318.

Hamza, N., Berke, B., Cheze, C., Le Garrec, R., Umar, A., Agli, A., Lassalle, R., Jové, J., Gin, H. & Moore, N. 2012. Preventive and curative effect of *Trigonella foenum-graecum* L. seeds in C57BL/6J models of type 2 diabetes induced by high-fat diet. *J. Ethnopharmacol*. 142(2): 516-522.

Hanna, L., Sándor, M. & Wink, M. 2022. Effect of ethanol solvents on total phenolic content and antioxidant properties of seed extracts of fenugreek (*Trigonella foenum-graecum* L.) varieties and determination of phenolic composition by HPLC-ESI-MS. *Diversity* 14(1): 7.

Hbika, A., Daoudi, N.E., Bouyanzer, A., Bouhrim, M., Mohti, H., Loukili, E.H., Mechchate, H., Al-Salahi, R., Nasr, F.A., Bnouham, M. & Zaid, A. 2022. *Artemisia absinthium* L. aqueous and ethyl acetate extracts: Antioxidant effect and potential activity *in vitro* and *in vivo* against pancreatic α-amylase and intestinal α-glucosidase. *Pharmaceutics* 14(3): 481.

Jin, L., Xue, H.Y., Jin, L.Y. & Li, S.Y. 2008. Antioxidant and pancreas-protoctive effect of aucubin on rats with streptozotocin-induced diabetes. *Eur. J. Pharmacol.* 582: 162-167.

King, J. 1965. Transaminases: Alanine and aspartate transaminases. In *Practical Clinical Enzymology*, edited by Van D. London: Van Nostrand. pp. 363-395.

Kodumuri, P.K., Thomas, C., Jetti, R. & Pandey, A.K. 2019. Fenugreek seed extract ameliorates cognitive deficits in streptozotocin-induced diabetic rats. *J. Basic Clin. Physiol. Pharmacol.* 30(4): 20180140.

Kumar, P., Bhandari, U. & Jamadagni, S. 2014. Fenugreek seed extract inhibit fat accumulation and ameliorates dyslipidemia in high fat diet-induced obese rats. *Biomed. Res. Int.* 2014: 606021.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.

Luo, W., Deng, J., He, J., Yin, L., You, R., Zhang, L., Shen, J., Han, Z., Xie, F., He, J. & Guan, Y. 2023. Integration of molecular docking, molecular dynamics and network pharmacology to explore the multi-target pharmacology of fenugreek against diabetes. *J. Cell. Mol. Med.* 27: 1959-1974.

Manita, D., Ogino, S., Marivoet, S. & Ogura, M. 2023. Detectability of and interference by major and minor hemoglobin variants using a new-generation ion-exchange HPLC system with two switchable analysis modes. *Pract. Lab. Med.* 38: e00346.

Marzouk, M., Soliman, A.M. & Omar, T.Y. 2013. Hypoglycemic and antioxidative effects of fenugreek and termis seeds powder in streptozotocin-diabetic rats. *Eur. Rev. Med. Pharmacol. Sci.* 17(4): 559-565.

Natelson, S., Scott, M.L. & Beffa, C. 1951. A rapid method for the estimation of urea in biologic fluids. *Am. J. Clin. Pathol.* 21: 275-281.

Neelakantan, N., Narayanan, M., de Souza, R.J. & van Dam, R.M. 2014. Effect of fenugreek (*Trigonella foenum-graecum* L.) intake on glycemia: A meta-analysis of clinical trials. *Nutr. J.* 13: 7.

Perla, V. & Jayanty, S.S. 2013. Biguanide related compounds in traditional antidiabetic functional food. *Food Chem.* 138: 1574-1580.

Ravi Kiran, T., Subramanyam, M.V. & Asha Devi, S. 2004. Swim exercise training and adaptations in the antioxidant defense system of myocardium of old rats: Relationship to swim intensity and duration. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* 137(2): 187-196.

Rehana, D., Mahendiran, D., Kumar, R.S. & Rahiman, A.K. 2017. *In vitro* antioxidant and antidiabetic activities of zinc oxide nanoparticles synthesized using different plant extracts. *Bioprocess Biosyst. Eng*. 40(6): 943-957.

Rochette, L., Zeller, M., Cottin, Y. & Vergely, C. 2014. Diabetes, oxidative stress and therapeutic strategies. *Biochim. Biophys. Acta* 1840(9): 2709-2729.

Samadder, A. 2014. Nanotechnological approaches in diabetes treatment: A new horizon. *World J. Transl. Med.* 3(2): 84-95.

Saraste, A., Knuuti, J. & Bax, J. 2023. Screening for coronary artery disease in patients with diabetes. *Curr. Cardiol. Rep.* 25: 1865-1871.

Sauvaire, Y., Petit, P. & Broca, C. 1998. 4-Hydroxyisoleucine: A novel amino acid potentiator of insulin secretion. *Diabetes* 47: 206-210.

Sayed, A.A.R., Khalifa, M. & Abd El-Latif, F.F. 2012. Fenugreek attenuation of diabetic nephropathy in alloxan-diabetic rats. *J. Physiol. Biochem*. 68: 263-269.

Scott, F.W., Trick, K.D., Lee L.P., Hynie, I., Heick, H.M. & Nera, E.A. 1984. Serum enzymes in the BB rat before and after onset of the overt diabetic syndrome. *Clin. Biochem.* 17(4): 270-275.

Sharma, B., Balomajumder, C. & Roy, P. 2008. Hypoglycemic and hypolipidemic effects of flavonoid rich extract from *Eugenia jambolana* seeds on streptozotocin induced diabetic rats. *Food Chemi. Toxicol*. 46: 2376-2383.

Srivastava, R.A.K. 2018. Dysfunctional HDL in diabetes mellitus and its role in the pathogenesis of cardiovascular disease. *Mol. Cell. Biochem.* 440: 167-187.

Steel, R.G. & Torri, J.H. 1980. *Principle and Procedures of Statistical Biometrical Approach*. 2nd ed. New York: McGrew Hill Book Company.

Suwalsky, M., Novoa, V., Villena, F., Sotomayor, C.P., Aguilar, L.F., Ronowska, A. & Szutowicz, A. 2009. Structural effects of Zn^{2+} on cell membranes and molecular models. *J. Inorg. Biochem.* 103(5): 797-804.

Tak, Y., Kaur, M., Chitranashi, A., Samota, M.K., Verma, P., Bali, M. & Kumawat, C. 2024. Fenugreek derived diosgenin as an emerging source of diabetic therapy. *Front. Nutr.* 11: 1280100.

Tietz, N.W. 1976. *Fundamentals of Clinical Chemistry*, 1st ed. Philadephia: W.B. Saunders Co.

Umrani, R.D. & Paknikar, K.M. 2014. Zinc oxide nanoparticles show anti-diabetic activity in streptozotocin-induced type 1 and 2 diabetic rats. *Nanomedicine (Lond Engl)* 9(1): 89-104.

Uribe-López, M., Hidalgo-López, M.D., López-Gónzalez, R., Frías-Márquez, D.M., Núñez-Nogueira, G., Hernández-Castillo, D. & Álvarez-Lemus, M.A. 2021. Photocatalytic activity of ZnO nanoparticles and the role of the synthesis method on their physical and chemical properties. *J. Photochem. Photobiol. A Chem.* 404: 112866.

Vardatsikos, G., Pandey, N. & Srivastava, A. 2013. Insulino-mimetic and anti-diabetic effects of zinc. *J. Inorg. Biochem.* 120: 8-17.

Vijayaraghavan, K. 2010. Treatment of dyslipidemia in patients with type 2 diabetes. *Lipids Health Dis*. 9: 144.

Vinotha, V., Iswarya, A., Thaya, R., Govindarajan, M., Alharbi, N.S., Kadaikunnan, S., Khaled, J.M., Al-Anbr, M.N. & Vaseeharan, B. 2019. Synthesis of ZnO nanoparticles using insulin-rich leaf extract: Anti-diabetic, antibiofilm and anti-oxidant properties. *J. Photochem. Photobiol. B.* 197: 111541.

*Corresponding author; email: hala_elmohamdy@yahoo.com