RESEARCH PAPER

From date seed to functional bread: zein nanoparticles boost antidiabetic properties in an animal model

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ABSTRACT

Objective (s): This study investigated the antioxidant properties of Rabbi date seed ethanolic extract, evaluated the physicochemical properties of extract-loaded zein nanoparticles, and examined the antidiabetic effects of dry lavash bread enriched with date seed powder (5%, 10%) or extract-loaded zein nanoparticles (5%, 10%) on fasting blood glucose in normal and streptozotocin-induced diabetic rats.

Materials and Methods: The ethanolic extract was prepared from date seed powder via ultrasound-assisted extraction and nanoencapsulated within a zein biopolymer matrix using an antisolvent precipitation technique. Fasting blood glucose levels in male rats were monitored and quantified using a standard glucometry assay.

Result: This study found that the encapsulation efficiency (EE), mean size, and Zeta potential of the zein nanoparticles loaded with the extract were equal to 95.42%, 188.35 nm, and +10.24 mV, respectively. The nanoparticles had semi-spherical morphology. The dietary intervention significantly reduced fasting blood glucose levels in both rat models. In normal control rats, the mean level decreased from 58.81 mg/dl to 35.06 mg/dl after 15 days. In diabetic rats, levels rose from a baseline of 70.47 mg/dl to 233.67 mg/dl after STZ induction, then declined to 159.67 mg/dl following 15 days of treatment with the enriched lavash bread.

Conclusion: Dry lavash bread enriched with zein nanoparticles exhibited a greater potential for reducing fasting blood glucose levels in both normal and diabetic rats compared to bread enriched directly with date seed powder.

Keywords: *Diabetes mellitus; Seeds; Bread; Nanoparticles; Zein.*

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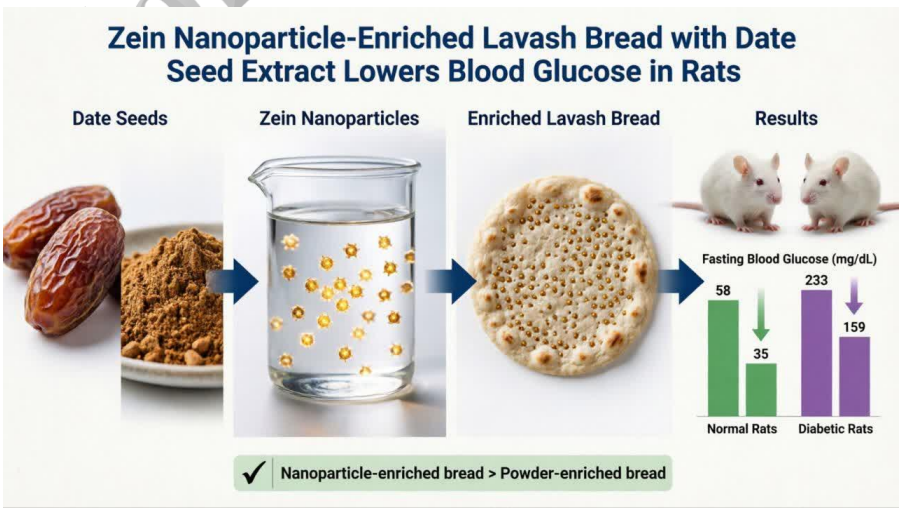
GRAPHICAL ABSTRACT



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INTRODUCTION

Diabetes mellitus is a prevalent metabolic disease of significant global concern, imposing substantial economic burdens and profound physiological impacts. The condition is characterized by acute symptoms including polyuria, polydipsia, and polyphagia, and can progress to severe, life-threatening complications. These encompass diabetic ketoacidosis, cardiovascular disorders, nephropathy, diabetic foot ulcers, retinopathy, and increased mortality risk [1]. Type II diabetes accounts for over 90% of global diabetes cases, with its prevalence experiencing a significant increase. This rise is strongly associated with key modifiable risk factors, including obesity, physical inactivity, poor dietary patterns, and sedentary lifestyles [2].

The Rabbi date cultivar originates from and is traditionally cultivated in Iran's Sistan-and-Baluchistan province. Characterized by its dark brown to black hue, this semi-dry variety typically contains moisture levels below 14%. It is relatively long, with a normal length of 3-5 cm [3].

Previous evidence indicates that date seeds can lower blood glucose, alleviate diabetes-related complications, help regenerate pancreatic cells, block lipase activity, and promote the body's own insulin release. In addition, date seeds may boost the action of natural antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase. This leads to reduced levels of free-radical oxidation products and less lipid peroxidation in various cell membranes [4]. Therefore, date seed powder and extract can be utilized as antioxidant and antidiabetic ingredients with nutritional value in food formulations [5].

Because they are rich in phenolics and other antioxidants, date seed extracts can be easily degraded by heat, changes in pH, oxygen, or light [6]. Such instability makes it difficult to add them directly to food products. However, breakthroughs in nanotechnology now enable the food sector to bypass these challenges, facilitating the utilization of plant-derived extracts as multifunctional preservatives that offer both nutritional advantages and health-promoting properties [7].

Zein, a polyamine and the primary storage protein found in corn, acts as an effective biopolymer for encapsulation. It offers notable benefits such as high resistance to digestive enzymes and poor breakdown within the gut. Additionally, because zein can strongly encapsulate water-insoluble bioactive substances via hydrophobic forces, it is a promising candidate for delivering therapeutic compounds [8].

Current literature reveals a significant gap in research on the antioxidant potential of Rabbi date seed extracts, with existing studies sparse. One notable study by Radfar et al. [9] quantified key antioxidant parameters in an 80% ethanolic macerated extract, reporting a total phenolic content (TPC) of 24.23 mg GAE/g dry weight and half maximal inhibitory concentration (IC_{50}) value of 20.4 µg/ml. Importantly, the scientific exploration of zein biopolymer as an encapsulation system for date seed extracts remains entirely unexplored. Consequently, the physicochemical characteristics of zein-based particles containing such extracts have not yet been described. Nevertheless, Jivan et al. [10] employed a microemulsion approach to encapsulate Kabkab date seed extract in starch nanocrystals, reporting the following characteristics for loaded particles: mean size=198 nm, size distribution=105-376 nm, polydispersity index=0.162, and EE=62%. Morphological analysis showed semi-spherical shapes for both empty and loaded nanocrystals. In another study, Sadeghi et al. [11] utilized a whey protein microemulsification and cold gelation technique to encapsulate an aqueous extract from Kabkab date seeds. Their analysis revealed distinct mean particle sizes: 23 nm for the heat-treated whey protein isolate, 304 nm for the empty particles, and 230 nm for the capsules containing the extract. The research further confirmed that the encapsulation process yielded particles with a predominantly spherical morphology, while leaving their thermal properties unchanged. On the other hand, no study has been performed so far on the antidiabetic effect of nanofood produced with date seed extracts. At the same time, few studies to date have focused on the hypoglycemic properties of dietary formulations supplemented with date seed powder. In a study, Mostafa et al. [12] evaluated the effect of yogurt direct enrichment with date fruit, seed powder, or palm leaves at a concentration of 10% on lowering blood glucose levels in diabetic rats. They declared that the yogurt with 10% date seed powder had the greatest effect on the response (44.65%). Halaby et al. [13] studied the antidiabetic impact of pan bread enriched with 10 and 15% date seed powder on the reduction of blood glucose level in diabetic rats. They claimed that the blood glucose level was equal to

119.85 and 105.60 mg/dl in the diabetic rats fed with the pan bread enriched with 10 and 15% date seed powder for 48 days, respectively.

Nanotechnology holds significant promise for nanofood enrichment due to its capacity to preserve the bioactive compounds in date seed extracts. Furthermore, it can enhance the bioavailability, enable targeted delivery, and improve the therapeutic efficacy of these extracts in disease prevention and management. Against this background, the present research aims to evaluate the efficacy of dry lavash bread enriched with Rabbi date seed powder and its ethanolic extract-loaded zein nanoparticles in reducing fasting blood glucose levels in both diabetic and non-diabetic rat models.

MATERIALS AND METHODS

Chemicals and reagents

The experimental procedures utilized analytical-grade materials sourced from commercial suppliers. Key reagents, including the Folin-Ciocalteu reagent, gallic acid monohydrate, anhydrous sodium carbonate, ethanol (96%), methanol (99.9%), and glacial acetic acid, were procured from Merck KGaA (Darmstadt, Germany). Double-distilled water was obtained from Zolal Teb Co. (Tehran, Iran). Sigma-Aldrich Co. (Massachusetts, USA) supplied the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and zein powder. Additional specialized materials, such as streptozocin (STZ) powder from ZellBio (Germany), ketamine hydrochloride from Rotex medica (Germany), diazepam ampoules from Caspian Supply Co. (Iran), and standard rodent diet from Behparvar Co. (Tehran, Iran), were also acquired for specific protocols.

Lavash bread ingredients

Whole wheat flour with an extraction percentage of 95-97% was obtained from Isfahan Atlas Flour Co., Iran. Non-fat dry milk was purchased from Pegah Co. (Tehran, Iran). Instant yeast was supplied by Iran-Molasses Co. (Fariman, Iran), and bakery salt was bought from Tehran-Chemical Co., Iran.

Preparation of date seed powder

The Rabbi dates used in this study were harvested in September 2022 from groves in Zabol, Sistan and Baluchistan, Iran. The seeds were first removed from the fruit flesh and rinsed with water to eliminate any remaining pulp. They were then left to dry in air at ambient temperature for one week. After complete drying, the seeds were ground into a fine powder using a vacuum-driven industrial mill (Flavina Co., Iran) equipped with an internal cooling system to avoid heat-induced damage. The powder was finally sieved through a 40-mesh screen [3].

Ultrasound-Assisted (UA) preparation of date seed ethanolic extract

First, 25 g of date seed powder was mixed with 100 ml of 70% ethanol (1:4 w/v) and stirred continuously at 500 rpm for 30 min (Heidolph, Germany). The blend was then placed into a closed 500 ml vessel to reduce solvent evaporation and exposed to ultrasound in a Bandelin bath (DT 102H, Germany; 35 kHz, 480 W) for 45 min while keeping the temperature at 65°C. Following sonication, the crude extract was clarified by centrifugation (7800 rpm, 30 min; ATP, RF14000, Germany) and filtered through Whatman No. 1 filter paper. The filtrate was concentrated using a Heidolph rotary evaporator (Hei-VAP Value Digital, Germany) under reduced pressure at 40°C and 200 rpm to preserve thermolabile phenolic constituents. To eliminate any residual moisture, the concentrate was spread on a plate and completely dried in a 40°C vacuum oven (SHEL LAB, 1425-2, USA). The final dried extract was stored at -18°C for subsequent analyses [14].

Antioxidant tests

Determination of TPC

TPC was determined using the Folin-Ciocalteu method [14]. Initially, a 1000 ppm stock solution of the extract was made in 70% ethanol. From this, 100 µl was pipetted into a tube, mixed with 500 µl of Folin-Ciocalteu reagent and 1 ml of distilled water. After 1 min, 1.5 ml of 20% sodium carbonate was added. The tube was vortexed and left for 2 h at room temperature in the dark. Finally, absorbance of the blue product was read at 760 nm with a UNICO 2150 spectrophotometer (USA). The TPC was calculated from a gallic acid standard curve based on the linear regression equation derived from the calibration data (Equation 1):

$$Y = 2.9507\,X - 0.039 \qquad R^2 = 0.9951 \quad (1)$$

In this equation, Y corresponds to the absorbance reading, while X corresponds to the concentration expressed as mg of gallic acid equivalents per milliliter (mg GAE/ml). The final TPC was expressed as mg GAE/g dry extract and calculated based on the following equation (Equation 2):

$$TPC = \frac{X \times V}{m} \quad (2)$$

Where X is the concentration obtained from the calibration curve (mg GAE/ml), V is the volume of the extract (ml), and m is the dry weight of the extract (g).

Measurement of free radical scavenging activity

To measure radical-scavenging activity, the DPPH assay was performed as described previously [14]. Briefly, 50 µl of methanolic extract at different concentrations (10, 20, 40, 80, 160, and 320 ppm) was mixed with 5 ml of 0.004% DPPH in methanol. After 30 min of dark incubation at ambient temperature, absorbance was measured at 517 nm for both the sample and a blank control. The radical scavenging activity, expressed as a percentage, was determined using Equation (3):

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Blank absorbance value} - \text{Sample absorbance value}}{\text{Blank absorbance value}} \times 100 \quad (3)$$

Encapsulation of date seed extract

A modified antisolvent precipitation approach was used to encapsulate the ethanolic extract of Rabbi date seeds inside a zein carrier [15]. The solvent phase was prepared by dissolving weighed quantities of the dry extract (0 g and 0.2 g) in 15 ml of 70% (v/v) ethanol under magnetic stirring at 40°C, yielding final extract concentrations of 0 and 13.3 mg/ml, respectively. Upon cooling to ambient temperature, 0.5 g of zein powder was incorporated into each solution, resulting in a constant zein concentration of 33.3 mg/ml (extract-to-zein ratios of 0 and 0.4). These mixtures were stirred for 1.5 hours at 25°C and then homogenized at 12,000 rpm for 5 minutes using an IKA T18 Digital Ultra-Turrax homogenizer. To promote nanoparticle formation, the dispersions were subjected to probe ultrasonication (Bandelin Sonopuls HD 2070, Germany; 20 kHz) for six 30-second pulses with 15-second intervals, while maintained in an ice bath. This solvent phase was then introduced dropwise via burette at approximately 1 drop per second into 40 mL of continuously stirred deionized water (the antisolvent phase) at 1500 rpm. The immediate formation of a precipitate at room temperature indicated nanoparticle generation. The resulting aqueous suspensions of zein nanoparticles were promptly aliquoted into centrifuge tubes and pelleted at 10,000 rpm for 20 minutes at 4°C. The collected solids were separated, spread onto plates, and lyophilized (Zirbus VaCo2, Germany) for 48 hours at -57°C and 0.017 mPa. The freeze-dried material was subsequently milled using a combination of an electric grinder and an agate mortar and pestle to produce a fine nanoparticulate powder. The final samples were stored at 4°C pending further characterization.

Encapsulation tests

Evaluation of EE

EE was determined by measuring the TPC entrapped within the particles [16]. For this analysis, 200 mg of each powdered capsule sample was dispersed in 2 mL of a ternary solvent mixture comprising methanol, acetic acid, and water in a 42:8:50 (v/v/v) ratio. The dispersion was vortexed for one minute and then subjected to ultrasonic extraction (480 W, 35 kHz) for 20 minutes at 25°C. The resulting mixture was centrifuged at 5,000 rpm for 10 minutes to separate the solids. The TPC in the clear supernatant was then measured via the Folin-Ciocalteu colorimetric assay [14]. The EE percentage was derived from the following equation:

$$\text{EE (\%)} = \frac{\text{Encapsulated TPC}}{\text{Total TPC}} \times 100 \quad (4)$$

Measurement of particle size and Zeta potential

Dynamic light scattering (DLS) using an HD NanoPlus device (Particulate Systems, USA) was employed to determine the mean particle size and Zeta potential (surface charge) of the nanoparticles. Before analysis, the freeze-dried powder was re-dispersed in 70% ethanol (1:100 w/v). Measurements were performed at 25°C with a 657 nm laser and a scattering angle of 90° [17].

Morphological analysis of nanoparticles

The surface morphology of the nanoparticles was examined using field emission scanning electron microscopy (FE-SEM; model EM8000F, KYKY, China) [18]. Sample preparation involved dusting a thin layer of the freeze-dried powder onto a silicon substrate, followed by sputter coating with a gold film. Micrographs were acquired at an accelerating voltage of 10 kV to assess particle characteristics.

Preparation of traditional dry lavash bread enriched with date seed powder and zein nanoparticles loaded with the extract

Bread dough was prepared from 500 g whole wheat flour, 300 ml water, 67 g non-fat dry milk, 10 g salt, and 7 g instant yeast. To activate the yeast, it was first blended into a mixture of lukewarm water and milk powder. Once gas formation was evident, this yeast solution was incorporated into the dry blend of flour and salt.

After that, the date seed powder was directly added at 5 and 10% to the bread dough to produce two bread samples (F_1 and F_2), and the date seed ethanolic extract-loaded zein nanoparticles were added at 5 and 10% to produce two other bread samples (F_3 and F_4). The bread sample free of both the date seed powder and the zein nanoparticles (F_5) was considered to be the control. The lavash bread samples were produced following a modified protocol adapted from the standard method described by Maleki et al. [19], followed by baking at 130 °C for 15 minutes to obtain the traditional dry lavash bread.

Application of the prepared traditional lavash bread in the diet of rats to investigate its effect on reducing blood glucose level

The animal experimental protocol received review and approval from the Animal Ethics Committee of Zahedan University of Medical Sciences (ethical code: IR.ZAUMS.AEC.1401.013). The study utilized 100 male Wistar rats (approximately 2–3 months old, weighing 200–250 g), which were sourced from and housed at the university's Laboratory Animal Research Center. Animals were acclimatized in standard polycarbonate cages under controlled conditions (22°C, 50–60% relative humidity, 12-hour light/dark cycle) for a period of ten days before experimentation. The rats were subsequently randomly allocated into four groups:

The first group: the normal control group comprised of 10 rats fed with the control lavash bread (F_5).

The second group: the normal control group fed with the lavash bread diets enriched with the date seed powder and the date seed ethanolic extract-loaded zein nanoparticles, consisting of 40 rats in 4 subgroups each composed of 10 rats (the first subgroup: the rats fed with the lavash bread containing 5% date seed powder (F_1); the second subgroup: the rats fed with the lavash bread containing 10% date seed powder (F_2); the third subgroup: the rats fed with the lavash bread containing 5% zein nanoparticles loaded with the date seed ethanolic extract (F_3); and the fourth subgroup: the rats fed with the lavash bread containing 10% zein nanoparticles loaded with the date seed ethanolic extract (F_4)).

The third group: the diabetic control group including 10 rats fed with F_5.

The fourth group: the diabetic group fed with the lavash bread diets enriched with the date seed powder and the date seed ethanolic extract-loaded zein nanoparticles, consisting of 40 rats in 4 subgroups each composed of 10 rats (the first subgroup: the rats fed with F_1; the second subgroup: the rats fed with F_2; the third subgroup: the rats fed with F_3; and the fourth subgroup: the rats fed with F_4).

In order to induce diabetes in the rats, STZ was used at 60 mg/kg by dissolving it in 0.1 M sodium citrate buffer and intraperitoneal injection as a single dose [4]. In order to ensure that the animals became diabetic, blood was taken from their tails 72 h after the injection, and the blood glucose level was measured with a glucometer (Emperor, Prodigy, Taiwan). A blood glucose level of more than 200 mg/dl was regarded as a criterion for becoming diabetic. After the rats became diabetic, each diabetic and non-diabetic rat was daily fed with the lavash bread at 2000 mg/kg, which was included in the animal diet comprising 30 g of standard food. The standard rodent diet (rat feed) provided to the control and experimental groups consisted of the following composition: crude protein (23%), salt (0.5%), calcium (1%), phosphorus (0.65%), tryptophan (0.25%), methionine (0.33%), lysine (1.15%), threonine (0.7%), crude fat (minimum 4%), and crude fiber (maximum 4%). This diet was selected to ensure adequate nutrition and consistency across all experimental groups. After two weeks of receiving the determined diets, in order to check the possible effect of the received diets on the weight of the animals, all the groups were weighed on the 15th day with a digital laboratory scale (Sartorius, Practum 5101-1S, Germany). For subsequent procedures, rats were anesthetized via intraperitoneal injection of ketamine (100 mg/kg) and diazepam (2 mg/kg). The depth of anesthesia was confirmed by the absence of pedal and corneal reflexes. Initial blood glucose measurements (72 hours post-STZ injection) were performed by collecting blood samples from the tail vein using a glucometer (Tajhiz Sanjesh, Clinic III, Iran) to assess baseline glucose levels.

For the final blood glucose measurement (on the 15th day), the animals were placed under deep anesthesia (as described above), and terminal blood samples were collected via cardiac puncture to ensure sufficient volume for biochemical analysis. Immediately following blood collection, euthanasia was performed by guillotine decapitation to ensure a rapid and humane endpoint. Blood glucose levels were quantified using a biochemical photometer (Tajhiz Sanjesh, Clinic III, Iran).

2.10. Statistical Analysis. All mean comparisons were performed using the Tukey

multiple-range test at a 99% confidence level (α = 1%) for between-group analyses, and paired t-tests (or repeated measures ANOVA, as appropriate) were conducted to assess changes in blood glucose levels within each subgroup from baseline to the end of the intervention, with a p-value of < 0.01 considered statistically significant for within-group analyses; all statistical analyses were performed using SPSS version 28.

RESULTS AND DISCUSSION

Antioxidant activity of ethanolic extracts of Rabbi date seed

The study revealed that Rabbi date seed ethanolic extract, obtained using 70% ethanol at 65°C with 45 minutes of sonication, contained 272.24±0.44 mg GAE/g dry extract of TPC and exhibited antioxidant activity with an IC_{50} of 4.23±0.06 µg/ml. Since medicinal plant extracts possess varying antioxidant and antidiabetic properties due to differences in phenolic compound content, evaluating the phenolic compound levels and free radical scavenging ability (IC_{50}) of date seed extract is of particular importance. The IC_{50} value denotes the extract concentration required to achieve a 50% inhibition of the DPPH radical activity. DPPH radicals are neutralized into stable molecules through electron donation by antioxidant compounds. There exists an inverse correlation between TPC and IC_{50} values - extracts with higher concentrations of antioxidant compounds demonstrate greater radical scavenging capacity, resulting in significantly lower IC_{50} measurements [14, 20]. A comparison with prior studies reveals that both the extraction technique and the date cultivar are critical determinants of antioxidant potential. For the Rabbi cultivar, the ultrasonic-assisted (UA) method employed in this work yielded a date seed extract with superior antioxidant activity (indicated by a higher TPC and a lower IC_{50}) compared to the macerated extract reported by Radfar et al. [9]. This demonstrates the enhanced efficiency of UA extraction for this specific seed matrix. In contrast, Afshari et al. [21] reported that macerated extracts from the Estameran cultivar exhibited substantially higher TPC and lower IC_{50} values than those achieved for Rabbi seeds in the present study. This divergence highlights the significant intrinsic influence of the plant genotype on phenolic composition and bioactivity, independent of the extraction method. Variations in these results may be attributed to multiple factors including: (1) cultivar-specific characteristics and maturation stage, (2) agroecological conditions (climate, soil properties, fertilization practices), (3) pre-harvest factors (pest/disease incidence), (4) post-harvest handling (processing techniques, storage parameters), and (5) extraction variables (solvent selection, time-temperature optimization, ultrasonication efficacy) [22]. The time parameter extends the mass transfer duration, while the intensity of sound waves, due to their high energy content, creates shear forces that fracture and disrupt cell walls. This process significantly improves the liberation of intracellular phytochemicals into the solvent, accelerates the mass transfer kinetics, and shortens the required extraction duration [14, 23]. Elevated temperature improves polyphenol solubility while simultaneously facilitating their diffusion from plant matrices into the solvent system [24]. The application of thermal energy improves extraction yield through multiple mechanisms: cellular matrix disruption increases membrane permeability, dissociation of polyphenol-lipoprotein complexes enhances metabolite solubility, and reduced solvent surface tension and viscosity facilitate improved plant material wetting and deeper penetration into cellular structures, collectively optimizing the extraction kinetics [25]. Increasing the time and temperature of ultrasonic extraction results in higher phenolic compound yields and a lower IC_{50} value of the extract compared to traditional maceration methods. A comparative assessment underscores the enhanced efficiency of modern extraction techniques. Where a prior maceration study reported a TPC of 175.4 mg GAE/g for a date seed methanolic extract, the ultrasonic-assisted method utilized in this research yielded a significantly higher phenolic content [26]. This marked improvement is primarily attributed to the disruptive power of ultrasonic waves, which facilitate a more efficient and rapid release of bioactive compounds from the plant matrix compared to conventional maceration. Additionally, the variation in phenolic compound levels among different date seed varieties can be influenced by two main factors: genetics and soil conditions. Genetically, each date variety possesses a unique biochemical profile that determines its capacity to produce phenolic compounds. Some varieties inherently produce higher levels of phenolic compounds. On the other hand, soil conditions such as pH, organic matter, moisture, and nutrient availability can also affect the plant's secondary metabolism and, consequently, the production of phenolic

compounds. Al-Farsi et al. [27] demonstrated that genetic differences between date varieties play a primary role in the variation of phenolic compounds. Furthermore, Amira et al. [28] emphasized that soil conditions, particularly mineral and organic content, can significantly influence the phenolic compound levels in date seeds. Additionally, Zarie et al. [29] revealed that the interaction between genetic factors and environmental conditions, such as soil type, can lead to significant variations in phenolic compound content.

EE of Rabbi date seed extract in zein particles

EE quantitatively represents the proportion of bioactive compounds successfully incorporated and maintained within a delivery system [30]. In this research, the EE of Rabbi date seed ethanolic extract in zein carrier (extract-to-zein ratio of 0.4) was found to be 95.42 ± 0.04%. The EE observed in this study aligns with the performance of protein-based carriers documented in prior literature, particularly zein. Calliari et al. [15], utilizing a similar antisolvent precipitation method with zein, reported high EE values (66.1–100%), demonstrating its efficacy as a carrier matrix. This consistency supports the reliability of the chosen methodology. Further comparative analysis reveals a critical advantage of protein-based wall materials over polysaccharides. For instance, Sassi et al. [31] utilized a composite carrier of egg yolk protein and gum arabic to encapsulate date seed polyphenols, observing a decline in EE with increasing gum arabic content. The lowest EE was recorded when gum arabic served as the sole wall material. This trend aligns with the enhanced performance of zein observed in our study. The authors attributed this phenomenon to the fact that protein-polyphenol interactions, governed by stronger hydrophobic forces and hydrogen bonding, typically result in more effective entrapment than those occurring between polyphenols and carbohydrate matrices.

Size and Zeta potential of zein nanoparticles loaded with Rabbi date seed extract

Nanoparticle characteristics, particularly size and surface charge, play pivotal roles in bioactive compound encapsulation and delivery. The Zeta potential, a fundamental surface property measured at the interface between the nanoparticle's diffuse ion layer and bulk solution, serves as a critical identifier of nanoparticle stability and behavior [20]. In the present study, the encapsulation of the date seed extract within zein nanoparticles via the antisolvent method resulted in an increase in particle mean size (from 102.23±8.24 nm to 188.35±4.21 nm) and a decrease in Zeta potential (from +20.83±0.07 mV to +10.24±0.05 mV) compared to blank zein particles (Table 1). This trend aligns with the behavior observed in similar zein-based encapsulation studies, such as those by Calliari et al. [15] and Luque-Alcaraz et al. [32], and can be mechanistically explained by nucleation and growth phenomena during precipitation, as well as surface modifications induced by polyphenolic compounds [33].

Table 1. Comparative analysis of particle size and surface charge (Zeta potential) between blank zein particles and those loaded with date seed extract.

Zein particle type	Particle size (nm)	Zeta potential (mV)
Blank zein particles	102.23±8.24^{a}	+20.83±0.07^{a}
Zein particles with encapsulated extract	188.35±4.21^{b}	+10.24±0.05^{b}

Values are reported as the mean ± standard deviation (SD) from triplicate measurements.
Within a given column, means superscripted with distinct letters differ significantly ($p < 0.01$).

Notably, the nanoparticles produced here demonstrated smaller dimensions than those reported for other carrier systems encapsulating date seed extracts (e.g., starch nanocrystals or whey protein) [10, 11]. This enhanced size profile is attributed to the optimized ultrasonication step and the specific zein concentration employed, which effectively mitigated excessive particle growth and aggregation. The observed inverse relationship between particle size and surface charge is consistent with established colloid science, where the adsorption of extract components (particularly hydroxyl-rich polyphenols) onto the zein surface modulates the interfacial charge, thereby influencing colloidal stability and potential biointeractions [32, 34].

Morphological analysis of unloaded and loaded zein nanoparticles

Representative FE-SEM micrographs of the lyophilized zein nanoparticles, both unloaded and containing the extract, are presented in Figure 1. The images reveal that particles in both samples predominantly exhibit a semi-spherical morphology and tend to agglomerate or overlap in the dried state [10]. Microstructural analysis revealed intermolecular crosslinking and aggregation phenomena among both unloaded and extract-encapsulated zein particles [35]. The enhanced stability and abundance of extract-encapsulated zein nanoparticles results from synergistic non-covalent interactions, including van der Waals forces, hydrogen bonding, and hydrophobic effects between the date seed polyphenols and zein's protein network [11].

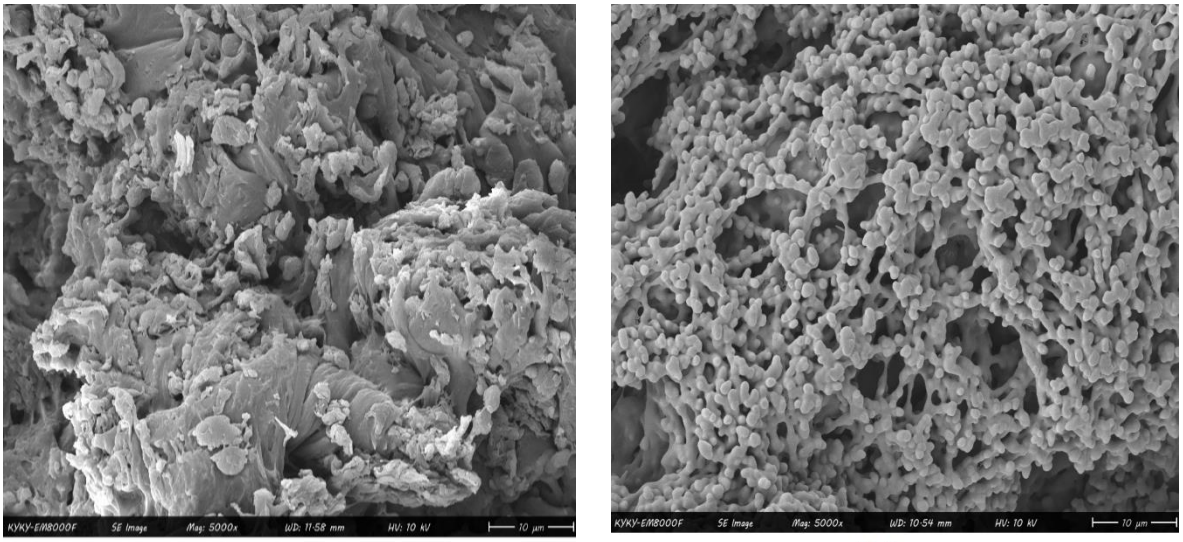

Fig. 1. FE-SEM analysis of zein nanoparticles: (a) blank particles and (b) particles loaded with the date seed extract.

Table 2. Weight and fasting blood glucose level of normal control rats fed with diets containing lavash bread with different formulations.

Group/Subgroup	First day (before receiving the diet containing lavash bread)		15th day (after two weeks of receiving the diet containing lavash bread)	
	Body weight (g)	Fasting blood glucose level (mg/dl)	Body weight (g)	Fasting blood glucose level (mg/dl)
Normal control rats fed with the diet containing control lavash bread (F_5)	207.30±11.95^{c}	46.00±12.72^{a}	232.80±13.91^{d}	36.50±8.58^{a}
Normal control rats fed with the diet containing lavash bread with 5% date seed powder (F_1)	206.70±13.50^{c}	46.75±14.24^{a}	231.00±8.56^{d}	33.00±2.70^{a}
Normal control rats fed with the diet containing lavash bread with 10% date seed powder (F_2)	204.50±14.47^{c}	63.00±6.73^{a}	229.60±14.00^{d}	38.50±3.31^{b}
Normal control rats fed with the diet containing lavash bread with 5% zein nanoparticles loaded with the ethanolic extract of Rabbi date seed (F_3)	205.60±10.96^{c}	51.50±6.45^{a}	243.10±11.30^{d}	33.75±5.18^{b}
Normal control rats fed with the diet containing lavash bread with 10% zein nanoparticles loaded with the ethanolic extract of Rabbi date seed (F_4)	205.70±12.10^{c}	74.00±18.05^{a}	249.40±8.64^{d}	35.00±4.08^{b}

*Within a row, means assigned different superscript letters are significantly different ($p < 0.01$).

Effect of diets containing lavash bread with different formulations on weight of rats

The weight changes of the normal control rats fed with the diets containing lavash bread with different formulations are presented in Table 2. It was observed that all the normal control rats in the first group and the four subgroups of the second group showed an increasing trend in weight on the 15th day, after receiving F_5, F_1 and F_2, and F_3 and F_4, compared to the first day. The mean weight of the normal control rats varied between 204.50-207.30 g on the first day and 229.60-249.40 g on the 15th day. The mean weight of the normal control rats of the first group fed with F_5 was equal to 207.30±11.95 and 232.80±13.91 g on the first and 15th days, respectively, which were significantly differ from one another (P<0.01). The mean weight of the normal control rats of the second group fed with F_1, F_2, F_3, and F_4 was found to be 205.62±12.36 and 238.27±13.39 g on the first and 15th days, respectively, which were significantly different from each other (P<0.01). Moreover, there was a significant increase in the weight of the normal control rats fed with F_3 and F_4 on the 15th day compared to the first day (P<0.01).

The changes in the weight of the diabetic control and diabetic rats fed with the diets containing lavash bread with different formulations are summarized in Table 3. It was found out that all the diabetic control and diabetic rats in the third group and the four subgroups of the fourth group showed a decrease in weight on the first day after

becoming diabetic (72 h after STZ injection) compared to the 0th day (before injecting STZ). Only the diabetic rats fed with F_2 and F_4 were able to recover and increase their weight until the 15th day compared to the first day. The mean weight of the diabetic and diabetic control rats ranged from 225.30-247.90, 200.60-245.77 and 178.00-253.00 g on the 0th, first, and 15th days, respectively. The mean weight of the diabetic control rats fed with F_5 (third group) was equal to 225.30±6.39, 200.60±12.85, and 178.00±14.84 g on the 0th, first, and 15th days, respectively, which were significantly different with each other ($P<0.01$). The mean weight of the diabetic rats fed with F_1, F_2, F_3, and F_4 was 240.20±10.56 g on Day 0, 227.87±15.35 g on the first day, and 225.50±21.85 g on the 15th day. Significant differences were found between Day 0 and the first day, and between Day 0 and the 15th day ($P<0.01$). There was no significant difference between the first and the 15th days.

The observed weight loss in the diabetic control group (DC+F5) is consistent with the metabolic disturbances characteristic of uncontrolled diabetes, including hyperglycemia, insulin deficiency, and increased catabolism of proteins and fats. These factors contribute to a negative energy balance and subsequent weight loss in diabetic individuals. In contrast, the treated diabetic sub-groups (e.g., those receiving lavash bread enriched with date seed powder or zein nanoparticles loaded with the ethanolic extract) exhibited attenuated weight loss, suggesting a potential protective effect of the formulated nanofood on metabolic homeostasis. This reduction can be explained by multiple potential mechanisms, such as enhanced glycemic regulation, increased insulin sensitivity, and a decrease in catabolic processes mediated by the bioactive constituents of the nanofood. For instance, the dietary fiber in date seed powder and the antioxidant properties of the ethanolic extract may help stabilize blood glucose levels and reduce oxidative stress, thereby mitigating the metabolic derangements associated with diabetes. Similarly, the zein nanoparticles may enhance the bioavailability and efficacy of the bioactive compounds, further contributing to the observed metabolic improvements. These findings highlight the potential of the formulated nanofood as a therapeutic intervention for managing diabetes-related weight loss and metabolic dysfunction. Future studies should explore the long-term effects of this intervention on body composition and metabolic parameters in diabetic models.

One of the determining parameters of becoming diabetic or improving blood sugar in diabetic rats is the control of weight changes. Mostafa et al. [12] reported that the initial and final weights of diabetic Albino rats fed with a basic diet and a cup of yogurt enriched with 10% date seed powder for 8 weeks were 178.16±1.01 and 191.33±2.09 g, respectively.

Table 3. Weight and fasting blood glucose level of diabetic and diabetic control rats fed with diets containing lavash bread with different formulations.

Group/Subgroup	0th day (before STZ injection)		First day (72 h after injection of STZ)		15th day (after two weeks of receiving diet containing lavash bread)	
	Body weight (g)	Fasting blood glucose level (mg/dl)	Body weight (g)	Fasting blood glucose level (mg/dl)	Body weight (g)	Fasting blood glucose level (mg/dl)
Diabetic control rats fed with the diet containing control lavash bread (F_5)	225.30±6.39^{a}	65.90±5.82^{d}	200.60±12.85^{b}	267.60±10.63^{e}	178.00±14.84^{c}	311.70±17.70^{f}
Diabetic rats fed with the diet containing lavash bread with 5% Rabbi date seed powder (F_1)	241.40±5.31^{a}	73.80±8.41^{d}	213.20±13.58^{bc}	278.20±9.11^{e}	204.20±10.97^{bc}	327.80±28.97^{f}
Diabetic rats fed with the diet containing lavash bread with 10% Rabbi date seed powder (F_2)	242.90±6.35^{a}	68.70±6.46^{d}	227.90±8.00^{bc}	211.80±13.31^{e}	233.60±6.72^{bc}	100.50±17.58^{f}
Diabetic rats fed with the diet containing lavash bread with 5% zein nanoparticles loaded with the ethanolic extract of Rabbi date seed (F_3)	228.60±9.09^{ab}	71.00±5.85^{d}	226.40±8.00^{ab}	220.60±9.16^{e}	211.20±12.05^{c}	121.70±15.03^{f}
Diabetic rats fed with the diet containing lavash bread with 10% zein nanoparticles loaded with the ethanolic extract of Rabbi date seed (F_4)	247.90±10.37^{a}	68.40±6.51^{bd}	245.77±11.58^{a}	224.10±10.56^{c}	253.00±10.67^{a}	88.70±25.53^{bd}

*Within a row, means assigned different superscript letters are significantly different ($p < 0.01$).

They ascribed the weight gain of the diabetic rats to the effect of the diet enriched with date seed powder. In the present study, the initial weight of the diabetic rats before receiving F_2 and their final weight after two weeks of receiving the diet were found to be 227.90±8.00 and 233.60 ±6.72, respectively. This is consistent with the above-mentioned research in terms of weight gain as a result of receiving a diet containing enriched food. Imran et al. [36] claimed the weights of normal control and diabetic control rats fed with normal saline through intraperitoneal injection and diabetic rats fed with a pellet composed of 1.5 g Ajwa date seed powder and 100 g standard food for 6 weeks to be 209.5±9.4, 140.6±17.3, and 176.4±22.0 g, respectively. In the present study, the weights of the normal control and diabetic control rats fed with a diet consisting of 30 g standard food (in the form of pellets) and 0.5 g F_5 for two weeks were respectively equal to 232.80±13.91 and 178.00±14.84. On the other hand, the weight of the diabetic rats fed with a diet comprising 30 g standard food and 0.5 g F_2 for two weeks was found to be 233.60±6.72 g. These results agree with those of the aforementioned study in terms of improvement in the growth and weight of diabetic rats compared to diabetic control ones as a result of treatment with a diet containing date seed powder. The significant weight loss of the diabetic rats on the first day after becoming diabetic (72 h after STZ injection) is due to the loss of protein tissues, and the relationship between severe hyperglycemia and weight loss has been proven [36]. Severe weight loss, polyphagia, polydipsia, and polyuria are among the early symptoms of diabetes [4]. Halaby et al. [13] stated that the weight gains of normal control and diabetic control rats fed with a basic diet for 48 days were respectively equal to 63.64 and 53.55%, while those of the diabetic rats fed with pan bread enriched with 10 and 15% date seed powder for the same period of time were reported to be 60.02 and 64.18%, respectively. In our research, the weight gains of the normal control rats (daily fed with a diet containing 30 g standard food and 0.5 g F_5 for two weeks) and the diabetic ones (daily fed with a diet containing 30 g standard food and 0.5 g F_2 for two weeks) were 12.30% and 2.50% less than those reported in the above-mentioned study. Furthermore, the rate of weight loss of the diabetic control rats was 11.27% which did not conform to the weight gain effect declared in the aforementioned research. The difference in the reported values can be caused by the difference in the amount of the daily diet and the duration of receiving the diet, as well as the difference in the extent of the hyperglycemia created in the rats due to the injection of alloxan and STZ.

Effect of diets containing lavash bread with different formulations on blood glucose level of rats

The changes in the fasting blood glucose level of normal control rats fed with the diets comprising lavash bread with different formulations are shown in Table 2. It was found out that all the normal control rats had a lower blood glucose level on the 15th day (after two weeks of receiving the diet) compared to the first day (before receiving the diet). The mean fasting blood glucose level of the normal control rats varied between 46.00-74.00 and 33.00-38.50 mg/dl on the first and 15th days, respectively. The mean fasting blood glucose level of the normal control rats of the first group fed with F_5 was equal to 46.00±12.72 and 36.50±8.58 mg/dl on the first and 15th days, respectively, which did not have a significant difference with each other (P>0.01). The mean fasting blood glucose level of the normal control rats of the second group fed with F_1, F_2, F_3, and F_4 was 58.81±15.57 and 35.06±4.13 mg/dl on the first and 15th days, respectively, which had a significant difference with one another (P<0.01).

Table 3 denotes the changes in the fasting blood glucose level of the diabetic and diabetic control rats on the 0th (before STZ injection), first (after becoming diabetic and before receiving the diet) and 15th (after two weeks of receiving the diet) days. It was observed that all the diabetic and diabetic control rats showed a higher blood glucose level on the first day after becoming diabetic (72 h after STZ injection) than on the 0th day (before STZ injection). Except for the diabetic control rats fed with F_5 and the diabetic ones fed with F_1, the other diabetic rats had a lower blood glucose level on the 15th day compared to the first day. The mean fasting blood glucose level of the diabetic and diabetic control rats varied between 65.90-73.80, 211.80-278.20, and 88.70-327.80 mg/dl on the 0th, first, and 15th days, respectively. The mean fasting blood glucose level of the diabetic control rats (third group fed with F_5) was found to be 65.90±5.82, 267.60±10.63, and 311.70±17.70 mg/dl on the 0th, first, and 15th days, respectively, which significantly differed from one another (P<0.01). The mean fasting blood glucose level of the diabetic rats (fourth group fed with F_1, F_2, F_3, and F_4) was obtained to be 70.47±6.96, 233.67±28.34, and 159.67±101.34 mg/dl on the 0th, first, and 15th days, respectively, which were significantly different from each other (P<0.01). Additionally, there was a significant decline in the blood glucose level of the

normal control and diabetic rats fed with F_2, F_3, and F_4 on the 15th day relative to the first day ($P<0.01$). By increasing the amount of the date seed powder or the date seed ethanolic extract-loaded zein nanoparticles in the formulation of dry lavash bread from 5 to 10%, an increasing trend was observed in reducing the blood glucose level of the normal control and diabetic rats. The lavash bread samples enriched with the zein nanoparticles were more efficient in lowering the blood glucose level of the normal control rats than those enriched with the date seed powder.

The effect of F_5 on decreasing the blood glucose level of the normal control rats can be due to the presence of whole wheat flour and milk powder in its formulation. Meanwhile, the gluten and starch present in wheat flour elevate blood glucose level [37, 38]. The fiber-rich polysaccharides found in whole wheat bran can improve pancreatic beta-cell health through several routes: they increase beta-cell mass, enhance insulin receptor signalling, and activate the PI3K/Akt pathway. They also influence ERK/JNK/MAPK cascades, which helps restore beta-cell function [39]. In general, plant polysaccharides lower blood sugar by protecting beta cells, boosting insulin sensitivity, slowing starch digestion, reducing inflammation, altering fat metabolism, and adjusting gut microbiota [40]. Dairy proteins (caseins and whey) and their breakdown products also help prevent and manage type 2 diabetes. They act by inhibiting DPP-IV, stimulating IRS/PI3K/Akt signalling, reducing blood glucose and insulin resistance, lowering HbA1c, activating AMPK, suppressing Pck1 and G6PC, increasing glucose use and insulin release, decreasing fasting glucose, improving beta-cell performance, and upregulating GLUT-4 [41].

The presence of phenolic compounds, abundant fiber, and minerals in the composition of date seed powder can be the reason for the greater effects of F_1 and F_2 on reducing the fasting blood glucose level of the normal control rats compared to F_5, in addition to the greater effect of F_2 on lowering the fasting blood glucose level of the diabetic rats than that of F_5. Date seed powder polyphenols have a high potential of inhibiting digestive enzymes related to hyperglycemia, including α-amylase and α-glucosidase [42]. These substances have a major role in regenerating pancreatic cells, inhibiting lipase enzymes, stimulating endogenous insulin secretion, and giving rise to the activity of endogenous antioxidants such as catalase, superoxide dismutase, and glutathione peroxidase [4, 43, 44]. Date seed contains more dietary fiber than cereals [45]. The dietary fiber of date seed powder cleans the digestive system by removing strong carcinogenic substances from the body and prevents the absorption of excess cholesterol. It has a positive effect on controlling diabetes by controlling hyperglycemia after eating, which is performed by delaying the stomach depletion or by increasing the volume of the digestive tract. As a result, it prevents carbohydrate digestion and retards its absorption [46]. The presence of manganese, magnesium, and zinc in date seed powder also contributes to its antidiabetic action. Manganese, a cofactor for several glycolytic enzymes, helps prevent glucose intolerance, increases insulin synthesis and sensitivity, and facilitates glucose entry into cells; it also stimulates insulin receptors, which are hormone-dependent kinases [47]. Magnesium supports ATP-kinase transporters (including those for insulin), activates protein-kinase tyrosine, and promotes phosphorylation of insulin intermediates, thereby lowering blood glucose and enhancing insulin production and sensitivity [48]. Zinc improves insulin-receptor binding, insulin sensitivity, and serum insulin levels; its antioxidant properties protect pancreatic beta cells and further reduce blood glucose [49, 50].

According to Halaby et al. [13], after 48 days, normal control rats on a standard diet had blood glucose of 66.05±3.49 mg/dl; diabetic controls on the same diet reached 152.59±3.41 mg/dl; while diabetic animals fed pan bread containing 10% and 15% date seed powder showed 119.85±4.73 and 105.60±4.15 mg/dl, respectively. In our experiment, healthy rats receiving 30 g standard chow plus 0.5 g F_5 (control bread) daily for two weeks had a mean glucose level of 36.50±8.58 mg/dl, and diabetic rats given the same amount of standard chow plus 0.5 g F_2 (bread with 10% date seed powder) had 100.50±17.58 mg/dl. Both values are considerably lower than those reported by Halaby et al., indicating a stronger glucose-lowering effect of our lavash bread formulation. This is despite the fact that the blood glucose level of the diabetic control rats in this study (311.70±17.70 mg/dl) is more than twice the corresponding value of the aforementioned study. This reveals the greater anti-diabetic effect of traditional dry lavash bread enriched with 10% date seed powder on diminishing the blood glucose level of diabetic rats, compared to pan bread enriched with 10 and 15% date seed powder. Mostafa et al. [12] claimed that the blood glucose levels of normal control and diabetic control rats fed with a basic diet and diabetic rats fed with a basic diet and a cup of yogurt enriched with 10% date seed powder for eight weeks were equal to 122.22±2.42, 277.40±3.63, and 153.52±2.96 mg/dl, respectively.

In the present study, the blood glucose levels of the normal control rats (daily fed with the diet containing 30 g standard food and 0.5 g F_5 for two weeks) and the diabetic ones (daily fed with the diet containing 30 g standard food and 0.5 g F_2 for two weeks) were lower than the corresponding values reported in the aforementioned study. At the same time, the blood glucose level of the diabetic control rats (daily fed with the diet containing 30 g standard food and 0.5 g F_5) was higher than that of the above-mentioned study.

The greater effectiveness of the lavash bread enriched with the date seed ethanolic extract-loaded zein nanoparticles in reducing the fasting blood glucose level of the normal control and diabetic rats than that of the lavash bread enriched with the date seed powder can be attributed to the antioxidant properties of zein and the phenolic compounds of the extract, in addition to the extraordinary efficiency of zein in creating strong non-covalent van der Waals, hydrogen, and hydrophobic interactions with the hydroxyl groups of the phenolic compounds, protecting the phenolic compounds against the bread processing conditions (fermentation pH and high baking temperatures) and the environmental conditions of the digestive system (stomach acidic pH and digestive enzymes), creating a targeted delivery system for the encapsulated extract in the alkaline environment of the small intestine, raising the bioavailability of the extract phenolic and bioactive compounds, as well as their absorption and entry into the circulatory system. Zein and the peptides resulting from its enzymatic hydrolysis have antioxidant activity [51]. While the observed reduction in blood glucose levels in the Diabetic+F_3 and Diabetic+F4 groups is consistent with the therapeutic potential of the experimental diets, the magnitude of this effect is noteworthy and warrants careful consideration. STZ-induced diabetes typically results in irreversible beta-cell destruction and sustained hyperglycemia, making the observed reduction in blood glucose levels somewhat unexpected. However, the bioactive compounds in date seed powder and zein nanoparticles may exert multiple beneficial effects, including antioxidant and anti-inflammatory activities, enhanced insulin sensitivity, and modulation of gut microbiota, which could collectively contribute to the observed hypoglycemic effect. Nevertheless, it is important to acknowledge the limitations of the STZ model and the fact that complete reversal of diabetes is unlikely. Further studies are needed to validate these findings and to explore the long-term effects of the experimental diets on glucose homeostasis and beta-cell function.

Zein exerts antioxidant activity primarily through two pathways: direct scavenging of free radicals and suppression of radical generation [52]. Native zein has relatively weak antioxidant power compared to its hydrolyzed form. Hence, enzymatically digested zein can act as a metal chelator, hydrogen donor, or radical trapper, thereby protecting bioactive molecules from oxidation [53]. Zein bioactive peptides have excellent antioxidant activity. However, their effects on the oxidative stress associated with hyperglycemia have remained unclear.

Date seed extracts may lower blood glucose through several possible mechanisms: 1) Insulin-mimetic activity: They may act like insulin by blocking K^+-ATP channels, leading to membrane depolarisation, increased Ca^{2+} influx, and the initial burst of insulin secretion [54]. 2) Glucose-fatty acid interaction in beta cells: Glucose enters pancreatic β-cells, is metabolised via glucokinase, raises the ATP/ADP ratio, and triggers Ca^{2+}-dependent exocytosis of insulin [55]. 3) Enhanced tissue sensitivity: The extract may stimulate insulin release from remaining or regenerated β-cells (possibly via saponins). An extra-pancreatic effect includes increased insulin receptor binding and glucose transport across cell membranes, promoting glycogen synthesis [56]. 4) Bioactive phytochemicals: Flavonoids, fatty acids, amino acids, tannins, and steroids all contribute to hypoglycaemia. Flavonoids act both on the pancreas and peripherally, inhibiting intestinal glucosidases [54, 57-59]. Fatty acids can acutely enhance insulin secretion, though chronic elevation leads to insulin resistance [60-62].

In a related study, Ahmad and Gani [63] developed an extruded snack containing resveratrol nanoparticles encapsulated in starch (0.4% w/w). After extrusion at 65-100°C, resveratrol retention was 43–53% for the encapsulated form versus 42.5% for the free form. The resulting nanofood inhibited α-glucosidase by 23–63% in vitro. These findings mirror our own observations with zein: both starch and zein protect bioactive compounds against high-temperature processing, and the nano-encapsulated forms show improved antidiabetic activity, likely due to inhibition of intestinal digestive enzymes. Meanwhile, the greater effect of the encapsulated extract on reducing the blood glucose level of the diabetic rats compared to the non-encapsulated extract in the aforementioned study is consistent with the bigger antidiabetic effects of F_3 and F_4 compared to F_1 and F_2 in the present research. The decrease in the blood glucose level of the diabetic rats can be caused by the protective effect of the bioactive

compounds present in the extracts on pancreatic beta cells and the improvement of insulin secretion from these cells, or by the increase in the environmental use of glucose by stimulating the direct absorption of glucose and prevention of the glucose transporter activity in the intestine [64].

Although plant-based extracts are extensively utilized in food and pharmaceutical sectors for their diverse bioactive properties-including antimicrobial, antifungal, antioxidant, and antidiabetic activities-their broader implementation is constrained by several limitations. Key challenges include poor aqueous solubility, high volatility, and instability within food matrices, which hinder their effective incorporation. Additionally, these extracts can impart undesirable flavors to foods and negatively affect the sensory quality of products. Furthermore, plant extracts are sensitive and unstable under processing conditions and environmental stresses [6].

The direct incorporation of extract-derived bioactive compounds, particularly phenolics, into food products is often constrained by their inherent thermolability, which leads to degradation under conventional high-temperature processing. Moreover, polyphenols present in foods, such as curcumin, resveratrol, quercetin, apigenin, baicalein, luteolin, morin, catechins, and naringenin, exhibit antidiabetic effects but are poorly absorbed, often digested, and excreted. Over 90% of polyphenols are digested, and only about 5-10% are absorbed, eventually reaching the intestine [65]. Therefore, nanoencapsulation is a suitable technique for enriching nano-food materials and producing nano-foods, aiming to protect plant-derived extracts from processing, evaporation, and oxidation, while ensuring their long-term activity, bioavailability, and controlled release [7]. Zein nanoparticles protect the antidiabetic bioactive compounds present in date seed extract, including polyphenols, flavonoids, and saponins, from rapid degradation in the body. They enable controlled and gradual release of these compounds in the gastrointestinal tract. As a result, these compounds maintain their therapeutic effects for a longer duration in the body and prevent fluctuations in blood sugar levels by ensuring a stable reduction. On the other hand, zein nanoparticles can enhance the absorption of bioactive compounds in the gastrointestinal tract. The bioactive compounds in date seed extract are easily absorbed through the small intestine wall and enter the bloodstream. This helps maintain the stability and increase the bioavailability of the antidiabetic compounds in the date seed extract, thereby enhancing their effectiveness in lowering blood sugar levels and managing diabetes [66].

While plant-based extracts are valued in food and pharmaceutical applications for their multi-functional bioactivity-encompassing antimicrobial, antifungal, antioxidant, and antidiabetic effects-their full-scale adoption faces significant obstacles. These barriers primarily involve inherent physicochemical limitations, including inadequate solubility in aqueous systems, susceptibility to volatilization, and insufficient stability during food processing and storage. Despite the numerous benefits of protein-based nanocarriers, their industrialization is hindered by the application of complex thermal or chemical processes that cannot be fully controlled [67]. Despite its low cost and simplicity, the antisolvent precipitation method has drawbacks: zein does not dissolve in water, so organic solvents (e.g., alcohols) are required, and the process is batch-wise, limiting industrial scale-up [68]. Moreover, zein is nutritionally incomplete because it contains insufficient amounts of essential amino acids such as lysine and tryptophan. Its water insolubility stems from the high proportion of hydrophobic residues (glutamic acid, leucine, proline, alanine) [69]. During the feeding regimen of laboratory mice, no instances of nanoparticle toxicity were observed, and the biocompatibility of zein nanoparticles within the animal's bodies was confirmed. Zein, being food-grade and classified as a GRAS (Generally Recognized As Safe) additive, has diverse applications in the food industry, including the production of candies and chewy chocolates, the encapsulation and targeted release of bioactive compounds, the fabrication of electrospun nanofibers for delivering bioactive compounds with high-temperature stability, and its use in food packaging. Additionally, it is utilized in other industries such as pharmaceuticals for the targeted delivery and release of drugs in the form of microcapsules, films, and gels; in the packaging industry for producing safe and non-toxic plastics; in the ceramics industry; in ink production; and in the adhesive industry [70, 71]. Zein nanoparticles generally exhibit low toxicity in the body and are recognized as safe and biocompatible materials due to their natural origin and biodegradability. Various studies have shown that these nanoparticles do not cause significant toxicity in cells or animal models at low to moderate concentrations. For example, Podaralla and Perumal [72] demonstrated that zein nanoparticles at concentrations below 100 µg/ml did not induce significant cytotoxicity in Caco-2 cells (an intestinal model). Furthermore, Luo et al. [73] confirmed that these nanoparticles, due to their

proteinaceous nature, are easily degraded in the body without producing toxic byproducts. However, at very high concentrations, toxic effects may be observed, making it essential to conduct thorough toxicity assessments under different conditions. Zein nanoparticles exhibit high biocompatibility in the body due to their natural origin and biodegradability. These nanoparticles are derived from zein, a plant-based protein obtained from corn, and are well-tolerated by cells and tissues. Various studies, including research by Zhang et al. [74], have shown that zein nanoparticles completely degrade in biological environments without leaving toxic residues. Additionally, Pascoli et al. [75] confirmed that these nanoparticles, owing to their biocompatibility and low toxicity, hold significant potential for drug delivery and medical applications.

Limitations of study

This study has certain limitations that should be acknowledged. While the achieved encapsulation efficiency (95.42%) is notably high, it is scientifically justifiable due to the strong hydrophobic interactions and hydrogen bonding between the zein matrix and the date seed polyphenols, combined with the optimized extract-to-zein ratio. However, it is important to acknowledge the limitations of using the Folin-Ciocalteu (TPC) assay for EE determination instead of chromatographic methods like HPLC. The TPC method provides a cumulative measure of the total phenolic payload but lacks chemical specificity, as it reacts with any reducing substances. Furthermore, it cannot profile individual phenolic compounds to determine if specific bioactives were encapsulated preferentially or to detect potential molecular degradation. Therefore, while TPC is the standard for evaluating the total payload of complex plant extracts, future studies should employ HPLC-MS to precisely quantify individual encapsulated phenolics and confirm the structural integrity of specific bioactive compounds within the zein nanoparticles. A notable limitation of the present study is the absence of an in vivo control group fed with empty zein nanoparticles. Although the compelling evidence strongly points to the date seed polyphenols as the primary hypoglycemic agents, zein itself possesses mild intrinsic antioxidant and bioactive properties. Consequently, the exact physiological contribution of the carrier matrix to the observed reduction in blood glucose cannot be entirely isolated in the current experimental design. Future investigations should incorporate an empty carrier control to definitively evaluate and rule out any potential minor glycemic effects attributable solely to the zein nanoparticles. It is important to clarify that the concentrations of 5% and 10% used in this study were based strictly on the weight percentage of the additive incorporated into the bread dough (i.e., 5% and 10% date seed powder vs. 5% and 10% zein nanoparticle powder), rather than being normalized to an equal phenolic content. Consequently, the actual amount of active phenolic compounds delivered to the animals was not identical across the F_1/F_2 and F_3/F_4 groups. This discrepancy in the delivered phenolic dose is a critical factor that influenced the comparative results. Specifically, while the nanoparticle-based breads delivered a lower total phenolic load, they exhibited comparable or superior antidiabetic effects, which further underscores the enhanced bioavailability and dose-sparing effect of the nanoencapsulation system. Future studies should aim to compare these formulations based on iso-phenolic doses to isolate the exact contribution of the nanocarrier to the observed bioefficacy. The 15-day intervention period, while sufficient for evaluating acute effects on blood glucose levels and weight changes, is insufficient to assess long-term outcomes such as chronic toxicity, sustained glucose control, and potential side effects. Future studies should incorporate longer intervention durations to provide a more comprehensive understanding of the nanofood's efficacy and safety profile. Additionally, further investigations could explore dose-response relationships, mechanisms of action, and the impact of prolonged exposure in both diabetic and non-diabetic models.

It is important to note that the STZ-induced diabetic model used in this study, while useful for mimicking certain aspects of Type 2 diabetes, does not fully replicate the complexity of the disease. Specifically, this model may not adequately capture the multifactorial etiology of Type 2 diabetes, including the role of obesity, chronic inflammation, and hormonal dysregulation. Additionally, the STZ model primarily focuses on β-cell dysfunction and may not fully represent the insulin resistance observed in human Type 2 diabetes. Future studies could consider alternative or complementary models, such as high-fat diet-induced insulin resistance or genetically modified rodent models, to better approximate the pathophysiology of Type 2 diabetes.

While the findings of this study provide promising insights into the anti-diabetic potential of the developed nanofood, it is important to acknowledge that results obtained from rat models may not directly translate to humans due to species-specific differences in physiology and metabolism. Therefore, further clinical trials are

necessary to confirm the efficacy and safety of this formulation in human subjects. Additionally, molecular studies to elucidate the precise mechanism of action, including signaling pathways and molecular targets, would provide a more comprehensive understanding of the observed effects and should be considered in future research.

CONCLUSION

Emerging non-thermal technologies, notably ultrasonication, offer substantial advantages over conventional extraction methods. These include markedly shortened processing times, enhanced preservation of thermolabile bioactives, and improved recovery rate of antioxidants from date seeds. Concurrently, the intrinsic hydrophobicity of zein facilitates robust encapsulation. By forming strong non-covalent interactions-including van der Waals forces, hydrogen bonds, and hydrophobic associations-with the phenolic constituents of the extract, zein enhances encapsulation efficiency (EE) and provides effective protection to the core material against environmental and processing stressors. The successful encapsulation was corroborated by several key findings: FE-SEM micrographs, an increase in particle size, and a reduction in Zeta potential for the extract-loaded zein nanoparticles. These results collectively demonstrate zein's strong affinity for date seed polyphenols, its efficacy in forming stable complexes with hydrophobic bioactives, and its promising utility in developing enriched foods and novel nanofood products. Lavash bread enriched with zein nanoparticles loaded with the ethanolic extract of Rabbi date seed were effective in reducing the blood glucose levels of normal control and diabetic rats than the one enriched with date seed powder. This multiplies the necessity of food nano-enrichment. Because results obtained in rodent models do not always translate to human physiology, future clinical trials are needed to confirm the antidiabetic efficacy of this nanofood in people. Moreover, additional investigations should examine its potential toxicity, rheological behavior, digestibility, and the stability of the nanoparticles under storage and gastrointestinal conditions. Complementary molecular studies are also suggested, including measuring insulin resistance, oxidative-antioxidant balance, and examining liver, muscle, and adipose tissues in both human and animal samples.

DATA AVAILABILITY STATEMENT

Data will be available upon request.

ETHICAL CODE

This study was approved via IR.ZAUMS.AEC.1401.013 ethical code.

CONFLICTS of INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Abdolvahed Safarzaei: Conception and drafting the manuscript. Hamed Fanaei: Analysis and interpretation of data. Ahmad Mehravaran: Collected data. Reza Farahmandfar: Statistical analysis. Reza Esmaeilzadeh Kenari: Critical revision of the manuscript for important intellectual content and supervision of the work. All authors read and approved the final manuscript.

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We employed DeepSeek AI for grammar checking and editing the text of this manuscript. All changes were carefully reviewed by the authors to ensure accuracy and adherence to the journal's guidelines.

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During the preparation of this manuscript, the authors used DeepSeek AI, an artificial intelligence-assisted writing tool, to support language editing, grammar checking, and paraphrasing of the English text. The authors reviewed and edited all AI-generated suggestions and take full responsibility for the content, accuracy, and originality of the final manuscript.

REFERENCES

1. Abdel-Shaheed MM, Abdalla ES, Khalil AF, El-Hadidy EM. Effect of Egyptian date palm pollen (Phoenix dactylifera L.) and its hydroethanolic extracts on serum glucose and lipid profiles in induced diabetic rats. Food Nutr Sci. 2021;12(2):147-161.
2. Shivmore K, Johar SK. A Comparative Evaluation of the Effect of Metformin and Voglibose Individually and in Combination on Serum Insulin of Diabetic Patients. Int J Health Environ Res. 2023;1:6-10.
3. Safarzaei A, Farahmandfar R, Kenari RE, Mehravaran A, Fanaei H. Optimization of Ultrasound-Assisted Extraction of Antioxidants From Rabbi Date Seed and Identification of Fatty Acid and Phenolic Compound Profiles of the Seed Extracts. J Food Process Preserv. 2025;2025(1):1599368.
4. Bikri S, Aboussaleh Y, Berrani A, Louragli I, Hafid A, Chakib S, et al. Effects of date seeds administration on

anxiety and depressive symptoms in streptozotocin-induced diabetic rats: biochemical and behavioral evidences. J Basic Clin Physiol Pharmacol. 2021;32(6):1031-1040.
5. Platat C, Habib H, Al Maqbali F, Jaber N, Ibrahim W. Identification of date seeds varieties patterns to optimize nutritional benefits of date seeds. J Nut Food Sci. 2014;8(2):1-8.
6. Kenari RE, Amiri ZR, Motamedzadegan A, Milani JM, Farmani J, Farahmandfar R. Optimization of Iranian golpar (Heracleum persicum) extract encapsulation using sage (Salvia macrosiphon) seed gum: chitosan as a wall materials and its effect on the shelf life of soybean oil during storage. J Food Meas Charact. 2020;14(5):2828-2839.
7. Pateiro M, Gómez B, Munekata PE, Barba FJ, Putnik P, Kovačević DB, et al. Nanoencapsulation of promising bioactive compounds to improve their absorption, stability, functionality and the appearance of the final food products. Molecules. 2021;26(6):1547.
8. Razavi R, Kenari RE, Farmani J, Jahanshahi M. Fabrication of zein/alginate delivery system for nanofood model based on pumpkin. Int J Biol Macromol. 2020;165:3123-3134.
9. Radfar R, Farhoodi M, Ghasemi I, Khaneghah AM, Shahraz F, Hosseini H. Assessment of phenolic contents and antioxidant and antibacterial activities of extracts from four varieties of Iranian date Palm (Phoenix dactylifera L.) seeds. Appl Food Biotechnol. 2019;6(3):173-184.
10. Jivan MJ, Yarmand M, Madadlou A. Short communication Encapsulation of date palm pit extract via particulation of starch nanocrystals in a microemulsion. Int J Food Sci Technol. 2014;49:920-923.
11. Sadeghi S, Madadlou A, Yarmand M. Microemulsification–cold gelation of whey proteins for nanoencapsulation of date palm pit extract. Food Hydrocoll. 2014;35:590-596.
12. Mostafa OE, Rabeh NM, Ibrahim G, Arafat A. Effect of Yogurt Supplemented with Date Fruit, Seeds and Leaves on Blood Sugar of Diabetic Rats. J Res Fields Spec Educ. 2018;4(17):161-176.
13. Halaby MS, Farag MH, Gerges AH. Potential effect of date pits fortified bread on diabetic rats. Int J Nutr Food Sci. 2014;3(2):49-59.
14. Safarzaei A, Sarhadi H, Khodaparast MHH, Shahdadi F, Dashipour AR. Optimization of aqueous and alcoholic extraction of phenolic and antioxidant compounds from Caper (Capparis spinosa L.) Roots assisted by ultrasound waves. zjrms. 2020;22(4):e100747.
15. Calliari CM, Campardelli R, Pettinato M, Perego P. Encapsulation of hibiscus sabdariffa extract into zein nanoparticles. Chem Eng Technol. 2020;43(10):2062-2072.
16. Robert P, Gorena T, Romero N, Sepulveda E, Chavez J, Saenz C. Encapsulation of polyphenols and anthocyanins from pomegranate (Punica granatum) by spray drying. Int J Food Sci Technol. 2010;45(7):1386-1394.
17. Esmaeilzadeh Kenari R, Razavi R. Phenolic profile and antioxidant activity of free/bound phenolic compounds of sesame and properties of encapsulated nanoparticles in different wall materials. Food sci nutr. 2022;10(2):525-535.
18. Zheng H, Wang J, Zhang Y, Xv Q, Zeng Q, Wang J. Preparation and characterization of carvacrol-loaded caseinate/zein-composite nanoparticles using the anti-solvent precipitation method. Nanomaterials. 2022;12(13):2189.
19. Maleki M, Vetter JL, Hoover WJ. The effect of emulsifiers, sugar, shortening and soya flour on the staling of barbari flat bread. J Sci Food Agric. 1981;32(12):1209-1212.
20. Inam W, Bhadane R, Akpolat RN, Taiseer RA, Filippov SK, Salo-Ahen OM, et al. Interactions between polymeric nanoparticles and different buffers as investigated by zeta potential measurements and molecular dynamics simulations. View. 2022;3(4):20210009.
21. Afshari K, Javanmard Dakheli M, Ramezan Y, Bassiri A, Ahmadi Chenarbon H. Physicochemical and control releasing properties of date pit (Phoenix dactylifera L.) phenolic compounds microencapsulated through fluidized-bed method. Food sci nutr. 2023;11(3):1367-1382.
22. Alem C, Ennassir J, Benlyas M, Mbark AN, Zegzouti YF. Phytochemical compositions and antioxidant capacity of three date (Phoenix dactylifera L.) seeds varieties grown in the South East Morocco. J Saudi Soc Agric Sci. 2017;16(4):350-357.
23. Fuad FM, Nadzir MM. Ultrasound-assisted extraction of asiaticoside from Centella asiatica using betaine-based natural deep eutectic solvent. Ind Crops Prod. 2023;192:116069.
24. Zheng B, Yuan Y, Xiang J, Jin W, Johnson JB, Li Z, et al. Green extraction of phenolic compounds from foxtail millet bran by ultrasonic-assisted deep eutectic solvent extraction: Optimization, comparison and bioactivities. Lwt. 2022;154:112740.
25. Oroian M, Ursachi F, Dranca F. Influence of ultrasonic amplitude, temperature, time and solvent concentration on bioactive compounds extraction from propolis. Ultrason Sonochem. 2020;64:105021.
26. Dehghanian F, Kalantaripour TP, Esmaeilpour K, Elyasi L, Oloumi H, Pour FM, et al. Date seed extract ameliorates β-amyloid-induced impairments in hippocampus of male rats. Biomed Pharmacother. 2017;89:221-226.
27. Al-Farsi M, Alasalvar C, Morris A, Baron M, Shahidi F. Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (Phoenix dactylifera L.) varieties grown in Oman. J Agric Food Chem. 2005;53(19):7592-7599.
28. Amira EA, Behija SE, Beligh M, Lamia L, Manel I, Mohamed H, et al. Effects of the ripening stage on phenolic profile, phytochemical composition and antioxidant activity of date palm fruit. J Agric Food Chem. 2012;60(44):10896-10902.
29. Zarie AA, Hassan AB, Alshammari GM, Yahya MA, Osman MA. Date industry by-product: date seeds

(Phoenix dactylifera L.) as potential natural sources of bioactive and antioxidant compounds. Applied Sciences. 2023;13(21):11922.
30. López de Dicastillo C, Velásquez E, Rojas A, Garrido L, Moreno MC, Guarda A, et al. Developing Core/Shell Capsules Based on Hydroxypropyl Methylcellulose and Gelatin through Electrodynamic Atomization for Betalain Encapsulation. Polymers. 2023;15(2):361.
31. Sassi CB, Marcet I, Rendueles M, Díaz M, Fattouch S. Egg yolk protein as a novel wall material used together with gum Arabic to encapsulate polyphenols extracted from Phoenix dactylifera L pits. Lwt. 2020;131:109778.
32. Luque-Alcaraz AG, Velazquez-Antillón M, Hernández-Téllez CN, Graciano-Verdugo AZ, García-Flores N, Iriqui-Razcón JL, et al. Antioxidant Effect of Nanoparticles Composed of Zein and Orange (Citrus sinensis) Extract Obtained by Ultrasound-Assisted Extraction. Materials. 2022;15(14):4838.
33. Campardelli R, Oleandro E, Reverchon E. Supercritical assisted injection in a liquid antisolvent for PLGA and PLA microparticle production. Powder Technol. 2016;287:12-19.
34. Brotons-Canto A, Gonzalez-Navarro CJ, Gurrea J, González-Ferrero C, Irache JM. Zein nanoparticles improve the oral bioavailability of resveratrol in humans. J Drug Deliv Sci Technol. 2020;57:101704.
35. Zhong Q, Jin M. Zein nanoparticles produced by liquid–liquid dispersion. Food Hydrocoll. 2009;23(8):2380-2387.
36. Imran I, Butt IM, Naqvi F, Mansoor M, Chiragh S, editors. Antidiabetic and antinephropathic potential of Ajwa pit & pulp (Phoenix dactylifera) in alloxanized diabetic rats. Proceedings; 2020.
37. Chen X, He X, Fu X, Huang Q. In vitro digestion and physicochemical properties of wheat starch/flour modified by heat-moisture treatment. J Cereal Sci. 2015;63:109-115.
38. Eland I, Klieverik L, Mansour AA, Al-Toma A. Gluten-free diet in co-existent celiac disease and type 1 diabetes mellitus: is it detrimental or beneficial to glycemic control, vascular complications, and quality of life? Nutrients. 2022;15(1):199.
39. Ganesan K, Xu B. Anti-diabetic effects and mechanisms of dietary polysaccharides. Molecules. 2019;24(14):2556.
40. Wang H, Li H, Hou Y, Zhang P, Tan M. Plant polysaccharides: Sources, structures, and antidiabetic effects. Curr Opin Food Sci. 2023;51:101013.
41. Zhou X, Chai L, Wu Q, Wang Y, Li S, Chen J. Anti-diabetic properties of bioactive components from fish and milk. J Funct Foods. 2021;85:104669.
42. Khan SA, Al Kiyumi AR, Al Sheidi MS, Al Khusaibi TS, Al Shehhi NM, Alam T. In vitro inhibitory effects on α-glucosidase and α-amylase level and antioxidant potential of seeds of Phoenix dactylifera L. Asian Pac J Trop Biomed. 2016;6(4):322-329.
43. El Fouhil AF, Ahmed AM, Atteya M, Mohamed RA, Moustafa AS, Darwish HH. An extract from date seeds stimulates endogenous insulin secretion in streptozotocin-induced type I diabetic rats. Funct Foods Health Dis. 2013;3(11):441-446.
44. Saryono S, Rahmawati E, Proverawati A, Hisni D. Effect of antioxidant status and oxidative stress products in pre-menopausal women after treatment with date seed powder (Phoenix dactylifera L.): a study on women in Indonesia. Pak J Nutr. 2017;16(6):477-481.
45. Alkhoori MA, Kong AS-Y, Aljaafari MN, Abushelaibi A, Erin Lim S-H, Cheng W-H, et al. Biochemical composition and biological activities of date palm (Phoenix dactylifera L.) seeds: a review. Biomolecules. 2022;12(11):1626.
46. Abiola T. Assessment of the Antidiabetic Potential of the Ethanolic Extract of Date Palm (Phoenix Dactylifera) Seed in Alloxan-Induced Diabetic Rats. J Diabetes Metab. 2018;9(1):784.
47. Baly DL, Schneiderman JS, Garcia-Welsh AL. Effect of manganese deficiency on insulin binding, glucose transport and metabolism in rat adipocytes. J Nutr. 1990;120(9):1075-1079.
48. Salvemini D, Cuzzocrea S. Therapeutic potential of superoxide dismutase mimetics as therapeutic agents in critical care medicine. Crit Care Med. 2003;31(1):S29-S38.
49. Wang P, Yang Z. Influence of insufficient zinc on immune functions in NIDDM patients. Bull Hunan Med Univ. 1998;23(6):599-601.
50. Chen M-D, Song Y-M, Lin P-Y. Zinc effects on hyperglycemia and hypoleptinemia in streptozotocin-induced diabetic mice. Horm Metab Res. 2000;32(3):107-109.
51. Díaz-Gómez JL, Ortíz-Martínez M, Aguilar O, García-Lara S, Castorena-Torres F. Antioxidant activity of zein hydrolysates from Zea species and their cytotoxic effects in a hepatic cell culture. Molecules. 2018;23(2):312.
52. Zhao C, Zhu J, Zhang H, Qi Q, Hu N, Han R, et al. Postharvest ripening of two varieties of corns: Structure, antioxidant activities and physicochemical properties of zein. Food Chem X. 2023;18:100680.
53. Kong B, Xiong YL. Antioxidant activity of zein hydrolysates in a liposome system and the possible mode of action. J Agric Food Chem. 2006;54(16):6059-6068.
54. Abdullah A, Al-bokai N. Effect of Methanolic Extract of Phoenix dactylifera L. Seeds (PDS) on Blood Glucose levels of Normoglycemic and Dexamethasone induced Diabetic Rabbits. Res Sq. 2022.
55. McGarry J, Dobbins R. Fatty acids, lipotoxicity and insulin secretion. Diabetologia. 1999;42(2):128-138.
56. Ibrahim KM, Al-Shawi NN, Al-Juboury RA. A study on the hypoglycemic effect of ficus carica L. Leaves aqueous extract against alloxan-induced diabetes in rabbits. Med j Babylon. 2009;6(3-4).
57. Kim HY, Moon BH, Lee HJ, Choi DH. Flavonol glycosides from the leaves of Eucommia ulmoides O. with glycation inhibitory activity. J Ethnopharmacol. 2004;93(2-3):227-230.
58. Narváez-Mastache JM, Garduño-Ramírez ML, Alvarez L, Delgado G. Antihyperglycemic activity and chemical constituents of Eysenhardtia platycarpa. J Nat Prod. 2006;69(12):1687-1691.

59. Gu H-J, Lv J-C, Yong K-L, Chen X, Liu P-P, Zhang X-B. Antidiabetic effect of an active fraction extracted from dragon's blood (Dracaena cochinchinensis). J Enzyme Inhib Med Chem. 2009;24(1):136-139.
60. Dobler CC, Flack JR, Marks GB. Risk of tuberculosis among people with diabetes mellitus: an Australian nationwide cohort study. BMJ open. 2012;2(1):e000666.
61. Sivabalan S, Renuka S, Menon VP. Fat feeding potentiates the diabetogenic effect of dexamethasone in Wistar rats. Int Arch Med. 2008;1(1):1-12.
62. Al-Farsi MA, Lee CY. Nutritional and functional properties of dates: a review. Crit Rev Food Sci Nutr. 2008;48(10):877-887.
63. Ahmad M, Gani A. Development of novel functional snacks containing nano-encapsulated resveratrol with anti-diabetic, anti-obesity and antioxidant properties. Food Chem. 2021;352:129323.
64. Usman UZ, Bakar ABA, Mohamed M. Propolis improves pregnancy outcomes and placental oxidative stress status in streptozotocin-induced diabetic rats. BMC Complement Altern Med. 2018;18(1):1-6.
65. Islam F, Khadija JF, Islam MR, Shohag S, Mitra S, Alghamdi S, et al. Investigating polyphenol nanoformulations for therapeutic targets against diabetes mellitus. Evid Based Complement Alternat Med. 2022;2022(1):5649156.
66. Luo Y, Wang Q. Zein-based micro-and nano-particles for drug and nutrient delivery: A review. J Appl Polym Sci. 2014;131(16).
67. Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A. Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules. Innov Food Sci Emerg Technol. 2013;19:29-43.
68. Yan X, Li M, Xu X, Liu X, Liu F. Zein-based nano-delivery systems for encapsulation and protection of hydrophobic bioactives: A review. Front Nutr. 2022;9:999373.
69. Lawton JW. Zein: A history of processing and use. Cereal Chem. 2002;79(1):1-18.
70. Sousa F, Luzardo-Alvarez A, Pérez-Estévéz A, Seoane-Prado R, Blanco-Méndez J. Development of a novel AMX-loaded PLGA/zein microsphere for root canal disinfection. Biomed Mater. 2010;5(5):055008.
71. Labib G. Overview on zein protein: A promising pharmaceutical excipient in drug delivery systems and tissue engineering. Expert Opin Drug Deliv. 2018;15(1):65-75.
72. Podaralla S, Perumal O. Influence of formulation factors on the preparation of zein nanoparticles. Aaps Pharmscitech. 2012;13:919-927.
73. Luo Y, Zhang B, Cheng W-H, Wang Q. Preparation, characterization and evaluation of selenite-loaded chitosan/TPP nanoparticles with or without zein coating. Carbohydr Polym. 2010;82(3):942-951.
74. Zhang Y, Niu Y, Luo Y, Ge M, Yang T, Yu LL, et al. Fabrication, characterization and antimicrobial activities of thymol-loaded zein nanoparticles stabilized by sodium caseinate–chitosan hydrochloride double layers. Food Chem. 2014;142:269-275.
75. Pascoli M, De Lima R, Fraceto LF. Zein nanoparticles and strategies to improve colloidal stability: A mini-review. Front Chem. 2018;6:6.