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Oral administration of zein-based nanoparticles reduces glycemia and improves glucose tolerance in rats

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ABSTRACT

The aim was to evaluate the effect of zein-based nanoparticles on the glucose homeostasis, following oral administration to Wistar rats. For this purpose, bare nanoparticles (NP, with tropism for the upper intestinal regions) and poly(ethylene glycol)-coated nanoparticles (NP-PEG), with the capability to reach the ileum and cecum of animals, were evaluated. Both formulations were spherical in shape, displaying sizes around 200 nm and a negative surface zeta potential. The oral administration of a single dose of these nanoparticles to animals (50 mg/kg) induced a significant decrease of the glycemia, compared control rats and in animals treated with the free protein ($p < 0.001$). Moreover, these nanoparticles improved the glycemic control against an intraperitoneal glucose tolerance test; particularly NP-PEG. These findings would be due to an increased release of glucagon-like peptide-1 (GLP-1) by L-cells, which are more abundant in distal regions of the intestine. In fact, the GLP-1 blood levels of animals treated with nanoparticles were significantly higher than controls (about 40 % and 60 % for NP and NP-PEG groups, respectively). This higher capability of NP-PEG, with respect to NP, to increase the release of GLP-1 and control glycemia would be related to its ability to reach the distal areas of the small intestine.

1. Introduction

Glucose homeostasis is a complex phenomenon involving a wide variety of hormones whose objective is to keep the blood glucose levels within the normal range (4 – 6 mM), avoiding the conditions of hyper- and hypoglycemia (Röder et al., 2016). Insulin is the principal hypoglycemic hormone, and it is produced and stored in the β cells of the pancreas. These cells that act as glycemic sensors are found forming clusters, the so-called islets of Langerhans (Fu et al., 2012). When the glycemia rises, glucose enters the β cells through the GLUT transporters and triggers a signaling cascade that induces an increase in the intracellular calcium concentration and, in last term, leads to the exocytosis of the insulin vesicles (Henquin et al., 2006; Röder et al., 2016). Through the bloodstream, insulin reaches sensitive tissues (*e.g.*, adipose tissue, muscle and liver) and stimulates glucose uptake by the cells, decreasing the circulating levels of this saccharide (Cheng et al., 2010; Jung and Choi, 2014; Lázár et al., 2018). Although glucose is the main inductor for insulin release, other types of nutrients (i.e., amino acids and di- tripeptides) can also act as insulinogogues (Deeney et al., 2000; Fu et al., 2012). However, the mechanisms by which amino acids induce secretion of insulin are very variable, depending on their nature and physicochemical properties (McClenaghan et al., 1996).

After food ingestion, the rise in the blood insulin levels has been shown to be more potent than during an isoglycemic intravenous glucose infusion. This phenomenon is known as the incretin effect and it happens because the presence of food in the lumen stimulates the production of hormones by the enteroendocrine cells present in the gut (Kazafeos, 2011; Mortensen et al., 2003). The hormones involved in this response are the incretins GLP-1 and GIP, from which GLP-1 appears to play a more important role in glucose homeostasis (Holst, 2019).

GLP-1 is stored in granules within the L cells of the gut, whose abundancy increases from proximal to distal regions of the intestine (Pais et al., 2016a). These cells act as sensors and, in response to the presence of nutrients in the lumen, release their GLP-1-containing granules (Müller et al., 2019). The release of GLP-1 starts a few minutes after the ingestion of the meal and lasts for several hours (Pais et al., 2016a). Carbohydrates, fats, and proteins present in the lumen act as GLP-1 release inductors. Regarding the secretagogue effect of proteins and their metabolites, several peptones and amino-acids (e.g. glutamine, glycine, alanine, phenylalanine and arginine) have shown the capability of directly induce GLP-1 release (Pais et al., 2016b), although oligo- or large peptides seem to be more potent than free amino acids (Ishikawa

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et al., 2015). Independently of the insulinogogue effect of GLP-1, which is always glucose-dependent (Clemmensen et al., 2013), it also induces glucose uptake by muscle, adipose tissue and liver (Müller et al., 2019; Rowlands et al., 2018). Moreover, in the liver, GLP-1 also induces hepatic glucose clearance and glycogen synthesis while reducing the hepatic glucose production through the inhibition of glucagon release (Jin and Weng, 2016).

On the other hand, GIP is produced and secreted by the enteroendocrine K cells localized in the gastrointestinal tract. In contrast to the distribution of L cells, K cells are mainly located in proximal regions of the gut, decreasing in number in further sections (Pais et al., 2016a). Although K cells are sensitive to all type of nutrients (sugars, fats and proteins), it has been observed that proteins are the most potent inductors of GIP release (Seino et al., 2010). GIP exerts its function mainly over the pancreas, the bone, the adipose tissue, and the brain. However, in contrast to the effect exerted by GLP-1 in the pancreas, GIP can stimulate both the production of insulin and glucagon (Seino et al., 2010). When glycemia is low (around 4 mM, as in fasted state), GIP induces glucagon release by targeting α cells. However, when blood glucose levels rise (postprandial state), it stimulates insulin release by targeting the β cells (Kimberley and Campbell, 2020).

Zein is an alcohol-soluble protein, with a GRAS regulatory status, that has been extensively proposed as raw material for the preparation of nanoparticles for the oral delivery of biologically active compounds (Luo and Wang, 2014; Reboredo et al., 2022; Weissmueller et al., 2016). This popularity would be related with a high loading capability for lipophilic compounds, as well as with an important facility to decorate the surface with hydrophilic compounds and, thus, modify their biodistribution in vivo (Martínez-López et al., 2020). On the other hand, zein would induce some beneficial biological effects related to glucose homeostasis. Thus, it has been shown that the ileal administration of zein hydrolysates would decrease glycemia levels in rats, improving the glucose tolerance, by stimulating the secretion of GLP-1 (Higuchi et al., 2013; Mochida et al., 2010). Based on these previous reports, orally administered zein-based nanoparticles could, potentially, induce the secretion of incretins and, hence, improve the glucose homeostasis. If so, these kinds of nanoparticles could be used alone or in combination with other hypoglycemic drugs to improve the glucose management in diabetic patients.

In this context, the aim of this work was to evaluate the in vivo effect of two different types of zein nanoparticles on the glucose homeostasis, following oral administration. For this purpose, bare nanoparticles (NP), which possess mucoadhesive properties (Inchaurraga et al., 2019), and poly(ethylene glycol)-coated nanoparticles (NP-PEG), with a mucus-diffusive demeanor (Reboredo et al., 2021), were evaluated in healthy Wistar rats.

2. Material and methods

2.1. Materials

Zein, L-lysine, poly(ethylene glycol) 35,000 Da (PEG35), Orlistat®, Nile red, isopropanol, glucose, sodium azide, agarose, and Triton X-100 were purchased from Sigma-Aldrich (Steinheim, Germany). Ethanol absolute was obtained from Scharlab (Sentmenat, Spain). Dipeptidyl peptidase-4 (DPP-4) inhibitor, aprotinin, insulin ELISA kit (EZRMI-13 K) and GIP ELISA kit (EZRMGIP-55 K) were obtained from Merck (Darmstadt, Germany). GLP-1 ELISA kit (YK160 GLP-1 EIA) was purchased from Yanaihara Institute Inc. (Awakura, Japan). Rat somatostatin ELISA kit (E-EL-R0914) was purchased from Elabscience (Houston, USA).

2.2. Preparation of nanoparticles

2.2.1. Preparation of bare nanoparticles (NP)

Zein nanoparticles were prepared by desolvation as described previously (Penalva et al., 2015), with minor modifications. In brief, the protein (200 mg) and L-lysine (30 mg) were dissolved in 20 mL ethanol 55 % before adding 20 mL purified water. The ethanol was eliminated in a rotatory evaporator (Büchi Labortechnik AG, Switzerland) and the suspension of nanoparticles was purified by tangential flow filtration. Finally, the aqueous suspension of nanoparticles was dried in a Büchi Mini Spray Dryer B-290 apparatus (Büchi Labortechnik AG, Switzerland).

2.2.2. Preparation of PEG-coated nanoparticles (NP-PEG)

The coating of nanoparticles with PEG was performed by the addition of PEG 35,000 to the aqueous suspension of nanoparticles (after the elimination of ethanol) at a PEG35-to-zein ratio of 0.5. After incubation under agitation for 30 min at RT, the nanoparticles were purified and dried as described above.

2.3. Physico-chemical characterization of nanoparticles

Particle size, zeta potential and polydispersity index (PDI) were measured after dispersing the dried formulations in ultrapure water, at 25 °C in a Zetasizer analyzer system (Brookhaven Instruments Corporation, Holtsville, NY, USA).

The shape and surface morphology of nanoparticles were examined by scanning electron microscopy (SEM). For this purpose, 1.5 mg of the dried powder were dispersed in 1 mL purified water. The aqueous suspension was mounted on SEM grids, dried, and coated with a gold layer using a Quorum Technologies Q150R S sputter-coated (Ontario, Canada). Images from were taken using a ZEISS Sigma 500 VP FE-SEM (Oberkochen, Germany) apparatus.

2.4. In vivo evaluation of nanoparticles in healthy rats

2.4.1. Strain and housing conditions

Male Wistar rats weighing 180–220 g were purchased from Envigo (Indianapolis, IN, USA). Animals were housed with free access to normal chow and water. After seven days of acclimation, animals were fasted overnight but with free access to water. All the procedures were performed following a protocol previously approved by the "Ethical and Biosafety Committee for Research on Animals" at the University of Navarra in line with the European legislation on animal experiments (protocol 071–19).

2.4.2. Evaluation of the hypoglycemic effect of nanoparticles

For this experiment, healthy rats were divided in different groups of at least 6 animals. Each group received orally-one of the following treatments: (i) free zein in water (50 mg/kg), (ii) bare zein nanoparticles in water (NP; 50 mg/kg), (iii) PEG-coated zein nanoparticles (NP-PEG; 50 mg/kg). All the treatments were dispersed in 1 mL water prior administration to animals through oral gavage, using a stainless-steel cannula.

As controls, one group received orally 1 mL water. At different times, 300 μL of blood samples were collected, from the tail vein, into K3EDTA tubes (Sarstedt, Nümbrecht, Germany). Protease inhibitors (DPP-4 inhibitor 50 μM, and aprotinin 500 KIU) were added to the tubes just after the collection of the blood. At each extraction, glucose levels in blood were quantified using an Accu-Check® Aviva glucometer (Roche Diagnostics, Basel, Switzerland). The blood-containing tubes were centrifuged at 2500 g for 10 min at 4 °C. The plasma was withdrawn, placed into new microtubes and stored at −80 °C until use. Finally, insulin, GIP, GLP-1 and somatostatin levels in the blood of rats were quantified by ELISA, following the indications of the kit's manufacturers.

2.4.3. Intraperitoneal glucose tolerance test (ipGTT) in healthy rats

The ipGTT was performed to evaluate the change in the glycemic control of rats receiving the different treatments (50 mg nanoparticles per kg) when facing an intraperitoneal injection of glucose (2 g/kg). Animals were fasted overnight prior to any procedure and treatments

were administered by oral gavage 2 h before the intraperitoneal injection of glucose. For this purpose, rats were divided into 4 different groups ($n \geq 6$): (i) untreated control (1 mL of purified water), (ii) free zein dispersed in 1 mL water; (iii) 1 mL of a suspension of bare nanoparticles in purified water (NP), (iv) 1 mL of a suspension of PEG-coated nanoparticles (NP-PEG). Blood glucose levels were measured at several times, before and after the intraperitoneal injection of glucose: −120 min (just before oral administration of nanoparticles), T_0 (prior to the intraperitoneal injection of glucose) and 15, 30, 60 and 120 min after the glucose overload. Blood samples (50 μL) were collected from the tail vein and the glycemia was measured using an Accu-Check® Aviva glucometer (Roche Diagnostics, Basel, Switzerland). The total exposure to glucose, measured as the are under the curve (AUC) obtained from the plots of the blood glucose levels during time, was calculated using the PKsolver software (Zhang et al., 2010). The ratios between AUCs of the different treatments were calculated as the quotient between the AUCs for the treatment and the control group.

2.5. Statistical analysis

Means and standard errors were calculated for every data set. All the group comparisons and statistical analyses were performed using a one-way ANOVA test followed by a Tukey-Kramer multiple comparisons test, except for the data obtained from the efficacy studies in rats, where the statistical analysis performed was a two-way ANOVA. All calculations were performed using GraphPad Prism v6 (GraphPad Software, San Diego, CA, USA) and the curves were plotted with the Origin 8 software (OriginLab Corp, Northampton, MA, USA).

3. Results

3.1. Nanoparticles characterization

The main physico-chemical properties of both formulations are summarized in Table 1. Both formulations displayed an average size of around 200 nm, with very low PDI indexes (<0.1). Regarding the zeta potential, both formulations showed a negative zeta potential, without differences between bare and PEG-coated nanoparticles. The images obtained by SEM showed that the resulting nanoparticles consisted of homogenous populations of around 200 nm, confirming the results found by DLS. Moreover, the nanoparticles display a spherical shape and a smooth surface, without apparent differences between bare and PEG-coated nanoparticles (Fig. 1A and B, respectively).

3.2. Evaluation of the hypoglycemic effect of nanoparticles

The effect of the oral administration of the different treatments on the glycemia of rats is shown in Fig. 2. The administration of an aqueous suspension of free zein at a dose of 50 mg/kg did not induce any relevant effect in the glycemia of rats. However, the same dose of NP or NP-PEG induced a significant decrease in the blood glucose levels of the animals, that lasted for at least 6 h. The hypoglycemic effect of zein nanoparticles was clearly observed three hours post-administration. At this time point, for both formulations, the decrease in the initial levels of glucose in blood was about 25 %. Nevertheless, NP-PEG appeared to offer a more intense and lasted hypoglycemic effect than NP. Thus, 6 h after administration, the hypoglycemic effect induced by the oral

Table 1
Physico-chemical characteristics of bare (NP) and PEG-coated nanoparticles (NP-PEG). Data expressed as mean ± SD, $n \geq 3$.

Formulation	Size (nm)	PDI	Zeta potential (mv)
NP	199 ± 28	0.04 ± 0.03	−52 ± 4
NP-PEG	203 ± 13	0.08 ± 0.01	−50 ± 1

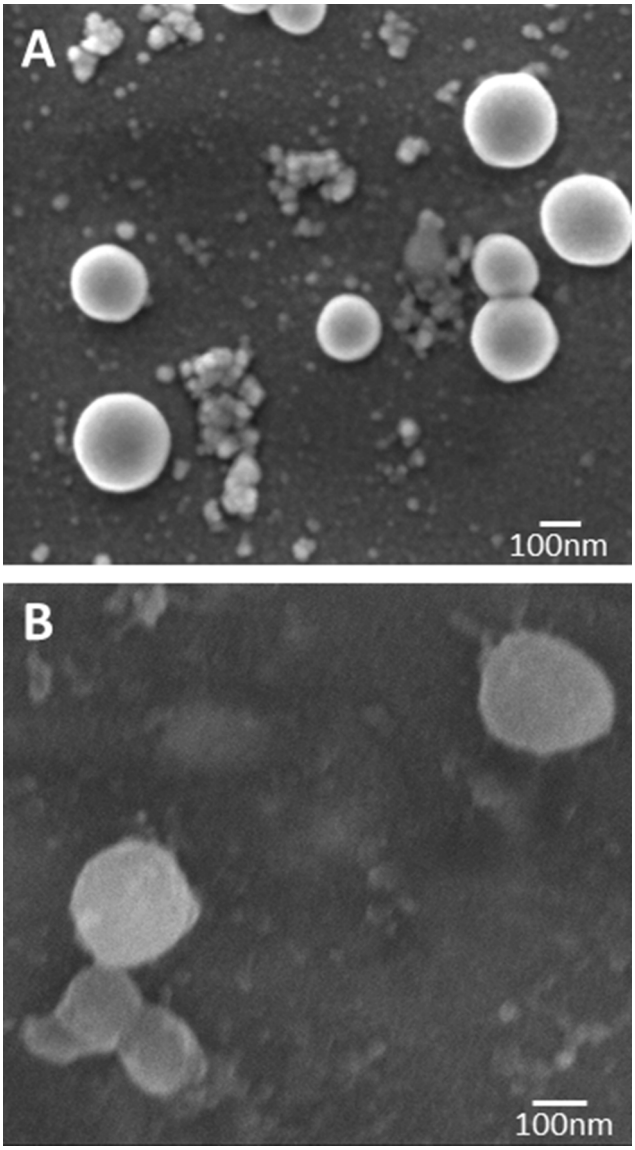

Fig. 1. SEM microphotograph of NP (A) and NP-PEG (B).

administration of NP-PEG was significantly higher than for bare nanoparticles ($p < 0.01$).

The effect of the oral administration of the treatments on different hormones involved in the glucose homeostasis was also assessed. At the same extraction points at which blood glucose levels were measured, the following hormones were quantified: insulin, GLP-1, and GIP. Fig. 3 shows the changes in the blood levels of insulin. In fasted control animals, insulinemia drastically and rapidly decreased to values below 10 % of the basal levels. On the contrary, animals receiving a suspension of the free protein showed a fast increase in the insulinemic values, increasing more than 50 % of the basal levels 3 h post-administration. On the other hand, animals treated with either NP or NP-PEG displayed an insulinemic profile different to that observed for animals receiving the free protein. Thus, during the first 3 h after the oral administration of nanoparticles, no apparent modification of insulin levels in blood were observed. However, after these first 3 h, the levels of insulin clearly increased, reaching those observed for animals treated with the aqueous suspension of the protein.

The effect of the treatments on the blood levels of GLP-1 in healthy rats is shown in Fig. 4. Control animals and animals treated with the suspension of free zein displayed similar GLP-1 values, without significant differences, during the 6 h post-administration. However, animals

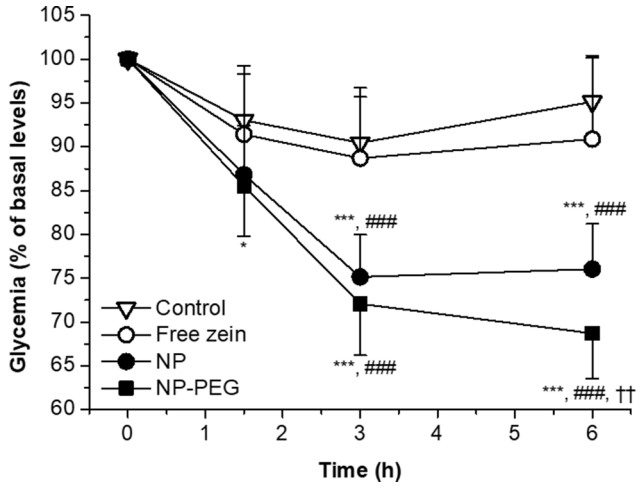

Fig. 2. Blood glucose levels of animals treated orally with zein nanoparticles (NP; NP-PEG) or an aqueous suspension of free zein. All the treatments were administered at a dose of 50 mg/kg. Data expressed as mean ± SD (n ≥ 6). *: p < 0.05; ***: p < 0.001 compared to control. ###: p < 0.001 compared to free zein. ††: p < 0.01 compared to NP.

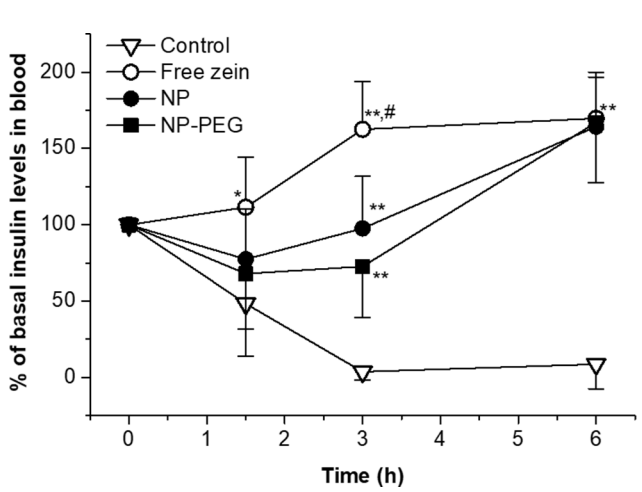

Fig. 3. Blood insulin levels of healthy rats treated orally with zein nanoparticles (NP; NP-PEG) or an aqueous suspension of free zein. All the treatments were administered at a dose of 50 mg/kg. Data expressed as mean ± SD (n ≥ 6). *: p < 0.05; **: p < 0.01 compared to control. #: p < 0.05 compared to NP-PEG.

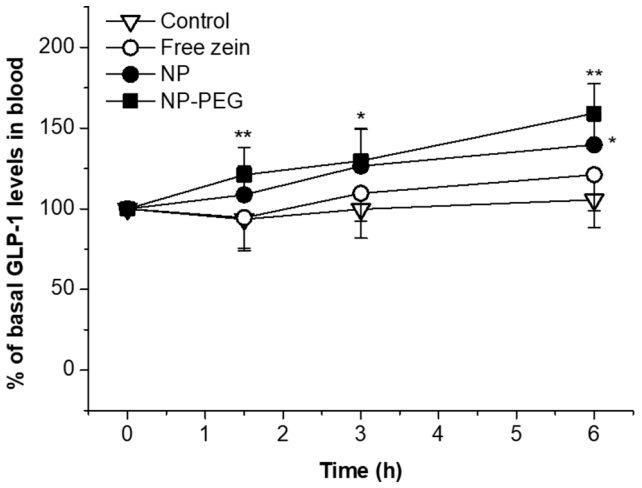

Fig. 4. Blood GLP-1 levels of healthy rats treated orally with zein nanoparticles (NP; NP-PEG) or an aqueous suspension of free zein. All the treatments were administered at a dose of 50 mg/kg. Data expressed as mean ± SD (n ≥ 6). *: p < 0.05; **: p < 0.01 compared to control.

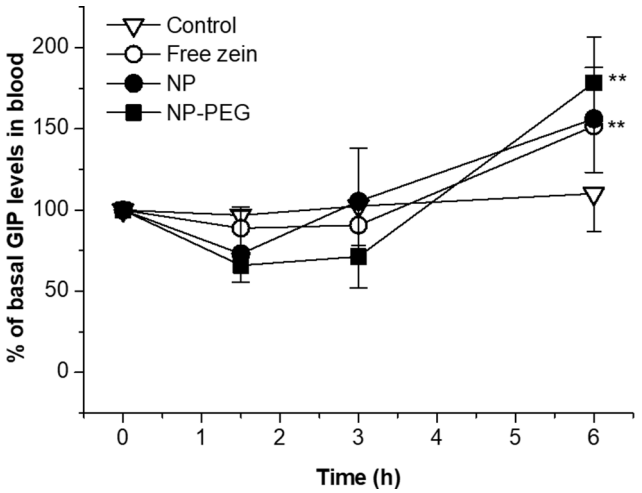

Fig. 5. Blood GIP levels of healthy rats treated orally with zein nanoparticles (NP; NP-PEG) or an aqueous suspension of free zein. All the treatments were administered at a dose of 50 mg/kg. Data expressed as mean ± SD (n ≥ 6). **: p < 0.01 compared to control.

receiving either NP or NP-PEG presented a significant increase in the levels of GLP-1. This increase, which started 1.5 h after the beginning of the experiment, reached its maximum at the last extraction point, 6 h post-administration. At this extraction point, NP induced a 39 % increase (over the initial levels) while NP-PEG led to a 59 % rise of the GLP-1 levels, compared to basal values.

The effect of the treatments on the blood levels of GIP are shown in Fig. 5. For animals treated with either zein nanoparticles or the aqueous suspension of the protein, during the first 3 h post-administration, a slight decrease in the blood levels of this hormone was observed. At 6 h post-administration, for all the zein treatments, the levels of GIP were significantly higher than for control animals (p < 0.01).

Fig. 6 illustrates the somatostatin levels during the first 3 h post-administration for animals treated with either NP or NP-PEG. Both types of treatment induced an important secretion of this hormone, particularly in animals treated with NP-PEG. Thus, 3 h post-administration, the levels of somatostatin in blood of control animals were reduced by half while, for NP-PEG treated animals, their blood levels were increased by approximately 45 %.

3.3. Intraperitoneal glucose tolerance test (ipGTT) in healthy rats

The response to a glucose overload in rats was evaluated through an intraperitoneal glucose tolerance test. Fig. 7 shows the changes in the glycemia of rats during the 2 h after the intraperitoneal challenge with a glucose injection. All the treatments significantly reduced the glycemic increase 15 min after the challenge, compared to the controls (p < 0.001). However, this reduction in the glycemic increase was significantly higher in the group of animals treated with NP-PEG than in animals receiving either free zein or NP (p < 0.05). Thirty min post-challenge, the glycemia levels were significantly lower in animals treated with NP-PEG than those in animals treated with either NP (p < 0.05) or the free protein (p < 0.001).

The main pharmacodynamic parameters of the ipGTT are summarized in Table 2. For control animals, the highest glycemic value (C_{max}) was reached in only 30 min (T_{max}) after the intraperitoneal injection of glucose. The oral administration of nanoparticles 2 h before the challenge, slowed down the rapid increase in the blood glucose levels of animal which were moderately lower than for control animals. Likely,

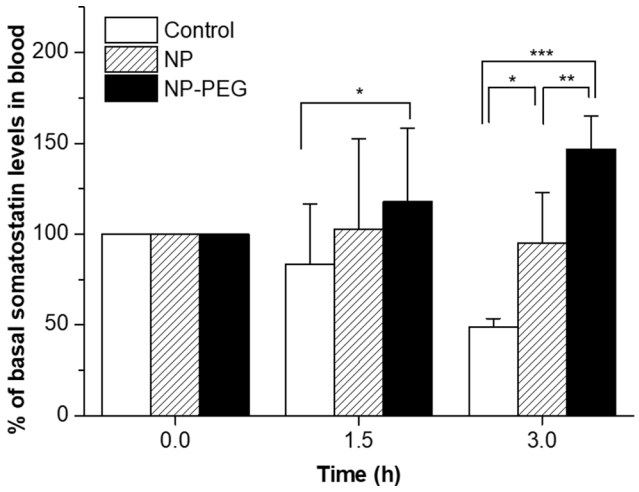

Fig. 6. Somatostatin blood levels of healthy rats receiving water (control), NP and NP-PEG. All the treatments were administered at a dose of 50 mg/kg. Data expressed as mean ± SD (n ≥ 6). *: p < 0.05; **: p < 0.01 compared to control.

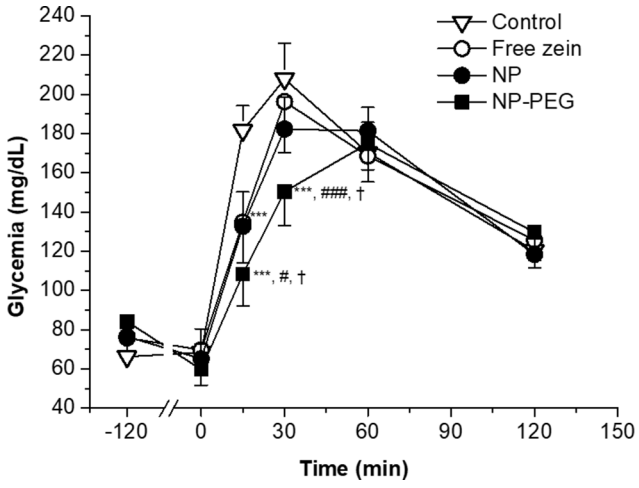

Fig. 7. Effect of the different treatments over the blood glucose levels of healthy male Wistar rats after an intraperitoneal injection of glucose (2 g/kg). All the treatments were orally administered 2 h prior to the glucose injection, at a dose of 50 mg/kg. Control: animals receiving water; Free zein: zein in purified water; NP: bare zein nanoparticles dispersed in 1 mL purified water; NP-PEG: PEG-coated zein nanoparticles dispersed in 1 mL purified water. Data expressed as mean ± SD (n ≥ 6). *: p < 0.05; **: p < 0.01; ***: p < 0.001 compared to control. #: p < 0.05; ###: p < 0.001 compared to free zein. †: p < 0.05 compared to NP.

Table 2
Main pharmacodynamic parameters of rats receiving an oral administration of water (control); free zein; bare nanoparticles (NP); or PEG-coated nanoparticles (NP-PEG). All the treatments were administered at a 50 mg/kg dose and 2 h prior to the ipGTT (2 g/kg). Data expressed as mean ± SD (n ≥ 6). *: p < 0.05 compared to control.

	C_{max} (mg/dL)	T_{max} (h)	AUC (mg/dL/min)	AUC ratio
Control	207 ± 18	0.50 ± 0.00	18952 ± 1528	1.00
Free zein	196 ± 26	0.57 ± 0.17	18319 ± 821	0.96 ± 0.04
NP	182 ± 16	0.71 ± 0.24	18225 ± 815	0.96 ± 0.04
NP-PEG	174 ± 13*	0.93 ± 0.17*	16885 ± 868*	0.89 ± 0.04*

animals treated with NP-PEG displayed a statistically relevant decrease in the total exposure to glucose (AUC; p < 0.05).

4. Discussion

The interaction of nutrients, and the products generated during their digestion process, with the cells of the intestine stimulates the release of hormones involved in the glycemic response. This phenomenon, known as the incretin effect, is mainly attributed to the role of GIP and GLP-1. In healthy subjects, the incretin effect accounts for up to 70 % of the total amount of insulin released in response to an oral glucose load (Nauck et al., 1986), demonstrating the importance of the incretin effect for the maintenance of glucose homoeostasis (Nauck and Meier, 2018). In this context, incretin-based therapies (particularly based on GLP-1 receptor agonists and DPP-4 inhibitors) have been recently developed and represent important options for the management of patients with type 2 diabetes mellitus (Cobble, 2012; Nauck et al., 2021).

Among others, zein hydrolysates have been shown an interesting capability to stimulate GLP-1 secretion by a direct action on ileal L-cells and, as a consequence, a reduction of the glycemia in rodents (Hira et al., 2009). In the present work, the possibility of reproducing similar biological effects with nanoparticles based on the whole protein and orally administered after dispersion in water was evaluated. For this purpose, bare and PEG-coated nanoparticles were selected. Conventional zein nanoparticles display mucoadhesive properties and their localization within the gut appear to be restricted to the mucus layer of the upper regions of the gastrointestinal tract (Inchaurraga et al., 2015). On the contrary, the "decoration" of the nanoparticles with PEG 35,000 at a PEG-to-zein ratio of 0.5 (NP-PEG) yields nanocarriers with mucus-permeating properties capable of targeting the epithelium of the distal region of the small intestine and the cecum (Reboredo et al., 2021).

The efficacy of empty nanoparticles was carried out in healthy rats, instead of diabetic rats. In type 1 diabetes rat model (i.e, high dose streptozotocin), characterized by a destruction of the pancreatic beta cells, the production and secretion of insulin is absent (King, 2012). On the other hand, subjects with type 2 diabetes usually show a decreased response to incretins (Boer and Holst, 2020).

The oral administration of zein nanoparticles to healthy animals induced a significant decrease of blood glucose levels (Fig. 2), compared to that observed in control animals and in those treated with the free protein (dispersed in water). In control animals, the glycemia remained at very similar levels throughout the 6 h monitored. However, insulin levels fell to very low levels, practically testimonial, in three hours. This phenomenon may be associated to the effect of inhaled anesthesia (isoflurane), which has been demonstrated to diminish the insulinemic values (Zardooz et al., 2010), combined with the fasting state, in which the circulating levels of the hormone are also diminished (Jørgensen et al., 2021).

On the other hand, oral administration of zein, either in its free form or as nanoparticles, induced an insulinotropic effect that countered the effect of the anesthesia. While free zein induced an insulinemic increase from the beginning of the experiment, the animals treated with nanoparticles displayed an initial decrease in their insulinemia, followed by a significant increase in the blood insulin levels from 3 h after administration (Fig. 3). This different behavior would be due to a different distribution between the two forms of zein (as free protein or in the form of nanoparticles). Thus, the hydrophobic character of zein hampers its dispersion in aqueous media and facilitates the generation of aggregates that results in a sticky mass (Zhang et al., 2022) that would be retained in the stomach, where proteolysis by hydrochloric acid and proteases would occur, releasing peptones and amino acids (some of which with insulinogogue activity) that would be, then, absorbed. The action of these peptides and amino acids would lead to the increased levels of insulin in blood (Fig. 3). Moreover, zein is particularly rich in leucine (around 19 % of the total amino acid residues) (Shukla and Cheryan, 2001) and this amino acid acutely stimulates insulin secretion (Yang

et al., 2010). Nevertheless, when the blood levels of these amino acid rise, the glucose uptake by peripheral tissues is inhibited (even in conditions of hyperinsulinemia (Pisters et al., 1991)) and the protein anabolism is potentiated (James et al., 2017). These would lead to the apparently contradictory results found in animals receiving the oral suspension of zein: highest values of insulin in blood without a reduction in the blood glucose levels.

On the contrary, the aqueous dispersion of zein nanoparticles would be rapidly emptied from the stomach to the small intestine, minimizing their residence and digestion in the stomach of animals. In fact, in previous studies, we have demonstrated that NP show a tropism for the upper regions of the small intestine, whereas oral NP-PEG move faster than NP and rapidly reach the distal region of the small intestine and cecum (Reboredo et al., 2021). Another important difference between both formulations is that NP (possessing mucoadhesive properties) remain trapped in the protective mucus layer, whereas NP-PEG can diffuse through the mucus and reach the surface of the intestinal epithelium. These differences between both nanoparticle formulations, as well as the distribution of the L cells (which are more abundant in distal regions of the gut), would be responsible for the higher capability to induce the secretion of GLP-1 by mucus-permeating (NP-PEG) than by mucoadhesive nanoparticles (NP) (Fig. 4).

The induction of the secretion of GLP-1 by L cells would be the reason for a better glycemic control when facing to an ipGTT (Fig. 7). Thus, animals treated with NP-PEG showed significantly lower glycemic values during the first 30 min after the glucose injection. Moreover, the T_{max} was delayed from 30 to 60 min and the total exposure to glucose (measured as the AUC) was significantly lower ($p < 0.05$) than for the other treatments. Another important point to highlight is that this better control of glycemia was obtained with a single dose of 50 mg/kg zein nanoparticles. In previous studies, using zein hydrolysates was 2 g/kg, 40-times higher than in the present study (Higuchi et al., 2013).

GIP is secreted in response to food intake and, particularly, in response to the rate of nutrient absorption rather than the mere presence of nutrients in the intestine (Baggio and Drucker, 2007; Pederson and Mcintosh, 2016). In this study, during the first 3 h after the administration of the zein treatments, no increase in the GIP levels in blood was observed (Fig. 5). Interestingly, although the differences were not statistically significant, NP and NP-PEG administration induced a slight decrease on the GIP blood levels. One reason for this finding would be the secretion of somatostatin from the D cells of the gut (Kumar and Singh, 2020), which has a potent inhibitory effect over the GIP synthesis and release (Kumar and Singh, 2020; Verrillo et al., 1988).

In summary, herein we demonstrated that zein in a nanoparticulated form can induce a hypoglycemic response and improve the glycemic control against an ipGTT in healthy animals. Moreover, we found that the mechanisms underlying these findings would be incretin-mediated, particularly the increased secretion of GLP-1 induced by the oral administration of zein nanoparticles. Nevertheless, the main differences observed for NP and NP-PEG would be consequence from their different distribution within the gastrointestinal tract. In any case, these nanoparticles might constitute an alternative to develop effective strategies for the prevention and/or treatment of hyperglycaemic conditions. Finally, due to the capability of zein nanoparticles to accommodate both hydrophilic and hydrophobic drugs (Reboredo et al., 2021), they could be used either as monotherapy or in a combinatory strategy via their loading with other antidiabetic drugs.

CRediT authorship contribution statement

Cristian Reboredo: Conceptualization, Methodology, Data curation, Investigation, Software, Visualization, Formal analysis, Writing – original draft. **Carlos J. González-Navarro:** Conceptualization, Writing – review & editing, Supervision. **Ana L. Martínez-López:** Conceptualization, Methodology, Data curation, Investigation, Formal analysis, Writing – review & editing. **Juan M. Irache:** Conceptualization, Resources, Writing – review & editing, Funding acquisition, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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