



Research Article

Hypoglycemic Effect of Orally Delivered Insulin Nanoparticles Compared to Subcutaneous Recombinant Human Soluble Insulin in Hyperglycemic Male Rats

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Abstract

Background: Insulin is available as an injectable drug and remains the mainstay of therapy. Researchers have attempted to develop an oral formulation of insulin, particularly utilizing nanoparticles (NPs). **Objective:** To assess the efficacy and safety of insulin-loaded D- α -Tocopherol polyethylene glycol succinate (TPGS)-emulsified PEG-capped PLGA NP in comparison to insulin-loaded PLGA NP and subcutaneous (SC) insulin in an in vitro and in vivo diabetic rat model. **Methods:** Two biocompatible and biodegradable NPs were used, in which 20 IU/kg of recombinant human soluble insulin was incorporated. NP1 was PLGA-loaded with human insulin, while NP2 was PLGA-PEG-TPGS-loaded with human insulin. The physical characteristics of the NPs were examined in an in vitro and in vivo study on a hyperglycemic rat model for a 24-hour duration. **Results:** For the first 3 hours, SC insulin showed a better reduction in serum glucose levels (SG) compared to both NP1 and NP2. A smooth, sustained reduction in SG was observed and maintained till the end of 24 hours with both NPs. NP1 maintained SG reduction for 6 hours before experiencing an increase, while NP2 demonstrated superior sustained reduction in SG beyond the 12-hour evaluation period. **Conclusions:** PLGA-PEG-TPGS NP can act as a potential carrier for oral insulin delivery and maintain good glycemic control for up to 24 hours.

Keywords: Drug delivery system, Hypoglycemic rats, Insulin nanoparticles, Polylactic acid-polyglycolic acid copolymer, Polyethylene glycol-poly lactide-polyethylene glycol.

تأثير جسيمات الأنسولين النانوية التي يتم توصيلها عن طريق الفم على سكر الدم مقارنة بالأنسولين البشري القابل للذوبان تحت الجلد في ذكور الفئران المصابة بارتفاع سكر الدم

الخلاصة

الخلفية: يتوفر الأنسولين كدواء قابل للحقن ويظل الدعامة الأساسية للعلاج. حاول الباحثون تطوير تركيبة فموية من الأنسولين، لا سيما باستخدام الجسيمات النانوية NPs. **الهدف:** تقييم فعالية وسلامة PLGA NP المحمل بالأنسولين البشري في المختبر وفي الجسم الحي. **الطريقة:** تم استخدام اثنين من NPs المتوافقة حيويًا والقابلة للتحلل، حيث تم دمج 20 وحدة دولية/كجم من الأنسولين القابل للذوبان البشري المؤتلف. كان NP1 محملاً بـ PLGA بالأنسولين البشري، بينما كان NP2 محملاً بـ PLGA-PEG-TPGS بالأنسولين البشري. تم فحص الخصائص الفيزيائية لـ NPs في المختبر وفي الجسم الحي على نموذج الفئران المصابة بارتفاع السكر في الدم لمدة 24 ساعة. **النتائج:** خلال أول 3 ساعات، أظهر الأنسولين SC انخفاضاً أفضل في مستويات الجلوكوز في الدم مقارنة بكل من NP1 و NP2. ولوحظ انخفاض سلس ومستدام في SG واستمر حتى نهاية 24 ساعة مع كل من النموذجين. حافظ NP1 على انخفاض SG لمدة 6 ساعات قبل أن يشهد زيادة، بينما أظهر NP2 انخفاضاً مستداماً متفوقاً في SG بعد فترة التقييم البالغة 12 ساعة. **الاستنتاجات:** يمكن أن يعمل PLGA-PEG-TPGS NP كناقل محتمل لتوصيل الأنسولين عن طريق الفم والحفاظ على التحكم الجيد في نسبة السكر في الدم لمدة تصل إلى 24 ساعة.

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INTRODUCTION

Diabetes mellitus (DM) is a serious global health concern. The reported prevalence of DM is on the rise globally, according to recent research [1,2]. Approximately 1.4 million Iraqis suffer from DM [3], and the prevalence of type 2 (T2DM) in Iraq ranges from 9% to 14% [3]. A study conducted in the city of Basra, located in southern Iraq, with a sample size of over 5400 individuals revealed a 19.7% age-adjusted prevalence of diabetes among participants aged 19 to 94 years. [4], because of the increased burden of DM. There is growing interest in examining the impact of diabetes and its association with other conditions in DM Iraq patients [5–8]. Current therapies for diabetes are directed towards the substitution of insulin, delivered either by non-oral mode like IV or SC, or using anti-hyperglycemic drugs (orally [9, 10], IV, and SC [11,12]). People with insulin-dependent DM (T1DM) use insulin as the preferred pharmacological therapy to control blood glucose levels [13]. Insulin delivered by a controlled-release system has faced significant challenges because of its agglomeration and precipitation. With a plasma half-life between 3 and 10 minutes for endogenous insulin and after-meal basal insulin returning to normal after 2–4 hours [14], insulin exhibits poor physicochemical characteristics. The principal obstacles to unchanged insulin intake and transportation into the circulatory system are lower pH and protease degradation, which are further exacerbated by its high molecular mass and hydrophilic character [15,16]. Patients undergoing ongoing diabetes treatment also prefer to administer insulin orally because it is easier and more convenient. In this interest, polymeric NPs, liposomes, and lipids have been developed and studied as insulin transporters [17]. For controllable and oral administration of protein and peptide medicines, biodegradable polymer microspheres or NPs, have shown promising results. In comparison to microspheres, nanoparticles (NPs) offer the advantage of being capable of traversing the gastrointestinal (GI) tract and being absorbed by the M cells located in Peyer's patches, which serve as the primary gateway for NP absorption [18]. Due to their capacity to contain and transport pharmaceuticals in a regulated manner, PLGA NPs were employed in nano-delivery systems in several applications [19]. Nevertheless, it is crucial to acknowledge that these NPs possess significant limitations, including their selectiveness in dealing with mucous surfaces [20]. On the other hand, the utilization of poly(ethylene glycol) (PEG) is of significant importance in the enhancement of colloidal system stability inside biofluids and enabling them to transmit across diverse mucosal membranes [21]. The observed enhancement has been associated with the increased stability of NPs and their enhanced ability to traverse mucus barriers [22]. The current study aimed to assess the insulin-loaded TPGS-emulsified PEG-capped

PLGA NP in comparison to insulin-loaded PLGA NP and SC Insulin in an in vitro and in vivo rat animal model of diabetes and assess the physical characteristics of synthesized NPs.

METHODS

Study design and settings

We randomly assigned a total of 40 male rats (adult albino Wistar) weighing between 180 and 250 g to five groups, with each group consisting of eight animals (40 rats in total). We recognized the rodents by the markings on various body parts. The range in mean body weight did not surpass 20%. Before the investigation, we kept the rat in a temperature-controlled setting (22 ± 2 °C) with a reversed diurnal period (12/12 hours) and habituated it for 14 days. Throughout the research, potable water and conventional food were readily accessible. To exclude the possibility of spontaneous diabetes, we measured the SG level of the rodents before administering STZ obtained from Sigma Chemicals Co. in St. Louis, MO, USA, for induction purposes. For the investigation, we chose rats with a normal SBG between 50 and 70 mg/dl [23]. Group I served as the negative control and received distilled water (DW) orally. Group II acted as diabetic control rats without treatment for DM (induced by IP STZ injection). We administered human insulin SC at a dosage of 1 IU/kg body weight to Group III (positive control) DM rats [24]. Orally administering 20 IU/kg of body weight of human insulin coated with PLGA to Group IV DM rats and Group V DM rats treated with PLGA-PEG-TPGS coated with 20 IU/kg of human insulin; all drugs and interventions for all groups were administered once after 4 days of induction by STZ, and evaluation was done for all groups in the 24-hour interval following the administration. For groups IV and V, the appropriate dried polymeric NPs were suspended in a sodium carboxymethyl cellulose (NaCMC) solution containing 1 percent (w/v) NaCMC to create an oral preparation, as this compound is considered a non-toxic, non-irritant and biocompatible material, making it a suitable medium to produce stable colloidal solutions with bioadhesive properties.

Induction of diabetes in rats

The researchers induced diabetes in the rats by acclimating them to their surroundings, subjecting them to an overnight period of fasting, and administering a single intraperitoneal (IP) injection of a newly formulated solution containing STZ (60 mg/kg of body weight) [25] in 0.1 M cold citrate buffer (pH 4.5). To mitigate the risk of severe hypoglycemia resulting from the excessive release of insulin generated by STZ destruction of pancreatic cells, we provided the rats with

a 5% glucose solution overnight. If the rodents' blood glucose levels surpassed 250 mg/dl 72 hours after the administration of STZ, they were classified as diabetic. The administration of therapy for the experiment commenced four days after the STZ injection, hence designating this day as the day of the experiment [26]. Non-diabetic rats completed the identical procedures, receiving distilled water (DW) orally. At the end of this stage, animals that were not subjected to induction with SBG levels ranging from 50 to 70 mg/dl were included in the study.

Preparation of Polymeric NPs

Human insulin, PLGA–NP1

PLGA was purchased from Sigma-Aldrich® [Resomer® RG 503 H, Poly(D, L-lactide-co-glycolide), acid terminated, lactide: glycolide 50:50, MW 24,000–38,000]. We employed the double emulsion method mentioned in previous studies to prepare the designated insulin-loaded PLGA NPs [27]. We combined 2 mL of Huminsulin® (100 IU/mL, Novo Nordisk) and 3 mL of PLGA (30 mg/mL) in dichloromethane to form a water-in-oil (W/O) emulsion using a sonicator system (Misonix Sonicator S-4000, Newtown, USA [35W]) in an ice bath (about 0 degrees Celsius) for 3–5 minutes. The researchers incorporated the resulting emulsion within a PVA aqueous solution with a concentration of 0.5 mg/l, serving as the secondary water phase, to create a water-in-oil-in-water (W/O/W) double emulsion. We evaporated the organic mixture while agitating it beneath a fume hood at air pressure. We isolated the concentrated NPs by subjecting the mixture to centrifugation with a force of 20,424 g. Meticulous washing with distilled water and collection of the resulting supernatant came afterward [27].

Human insulin, PLGA, PEG, and TPGS – NP2

As explained by Malathi *et al.* [26], we made a solution by mixing 50 mg of TPGS and about 100 mg of PLGA copolymer, which has the same molar ratio as NP1. We did this in 5 ml of acetone. The solution was sonicated with a probe sonicator for 60 seconds, and it swirled constantly at a temperature of 4°C. Subsequently, a volume of 2 mL of Huminsulin® was introduced into the solution with continuous agitation, followed by sonication for an additional 60 seconds. The resulting solution was maintained in an ice bath to create a water-in-oil (W/O) emulsion. Further sonicating for 180 seconds was then done with 10 mL of an aqueous PEG 2000 (0.1 percent w/v) solution of a double emulsion (W/O/W) and was constantly agitated for a 6-hour duration for organic solvent elimination. The insulin poly(lactic co-glycolic acid) TBGS was subsequently

produced after 15 minutes of centrifugation at 12000 rpm and 4°C.

Nanoparticle characterization

After the synthesis of the NPs, the characterization to determine various properties was done, and the size and particle distribution of the NPs were determined by utilizing dynamic light scattering (DLS) and zeta potential (ζ) using a zeta potential analyzer measured by electrophoretic light scattering (ELS).

Determination of particle size

Particle size and distribution (mean diameter and polydispersity index) were determined using a DLS device, a NanoBrook 90Plus (Brookhaven Instruments Corporation, USA). The measurements of mean particle size and polydispersion index (PDI) were documented, and all of these measurements were conducted in triplicate.

Zeta potential

The ζ was assessed using a zeta potential analyzer (Brookhaven NanoBrook ZetaPlus®), which is measured by ELS [28].

Determination of Encapsulation Efficiency (EE)

The indirect technique was utilized to evaluate EE% [29]. Shortly, NPs were centrifuged at 20 krpm for 10 minutes, and the insulin concentration in the supernatant was quantified utilizing a well-established high-performance liquid chromatography (HPLC) method. We measured the duration of insulin retention to be 7.9 ± 0.05 minutes with an injection volume of 30 μ l. Insulin had a maximum wavelength of detection (λ_{max}) of 210 nm. A quantity of approximately 5 mg of NPs was solubilized in 5 mL of acetone, utilizing vortex mixing for 15 minutes. Subsequently, the mixture was subjected to centrifugation (12 krpm at 4°C) for 10 minutes. We conducted the spectrophotometric determination of the concentration of insulin in the supernatant at a wavelength of 210 nm using a Perkin-Elmer spectrophotometer (model λ 35, Waltham, MA, USA) [26].

In-vitro drug release from NPs

The in vitro release profile of the NPs was observed at a temperature of 37°C in a medium consisting of phosphate-buffered saline. At 210 nm, the amount of insulin released was monitored spectrophotometrically, and 24 hours of insulin release were seen with both NPs [26].

High-performance liquid chromatography (HPLC)

As described previously by Zhang *et al.* [30], the NPs were isolated from the aqueous suspension medium using ultracentrifugation at a speed of 40,000g and a temperature of 10 °C for 30 minutes. Researchers measured the quantity of free insulin in the clear supernatant remaining after centrifugation using high-performance liquid chromatography (HPLC) with an HP1100 system manufactured by Agilent in the United States. The experimental setup involved the utilization of an HPLC system consisting of a pump and UV-V detector. Insulin elution was performed using a Hypersil ODS C18 column with dimensions of 200 x 4.6 mm, 5 µm. The mobile phase consisted of a combination of water, acetonitrile, and trifluoroacetic acid in a ratio of 82:18:0.3. The flow rate of 1.0 ml/min was utilized in the experiment, whereas the detection process was done at a wavelength of 210 nm. We introduced 20 µl of the transparent supernatant into the High-Performance Liquid Chromatography (HPLC) apparatus. We then recorded the insulin peak area and used a standard curve to calculate the concentration of insulin.

Sample size and randomization

For sample size computation, the program G Power was utilized [31], based on Cohen's principles [32]. We used a table of random integers to construct the groupings at random. We minimized misunderstanding by placing the animals in labeled containers and giving them tail tags [33].

Outcome measures

During the research, we drew blood samples at regular intervals (baseline, after 30 minutes, after 3 hours, after 12 hours, and after 24 hours) to study SBG levels (ACCU-CHEK® performa) and serum insulin (ELISA technique). We assessed the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), TG (triacyl glycerol, TG), urea, and creatinine after the experiment. After completing therapy, we starved all animals for 12 hours and administered intraperitoneal anesthesia using 80 mg/kg of ketamine and 10 mg/kg of xylazine. After complete anesthesia, all rats were euthanized using exsanguination (cardiac puncture), a method appropriate for tissue collection and preservation [34,35].

Biochemical analysis

The analysis of the biochemical parameters was done employing specialized kits provided by BIOLABO S.A.S.®: cholesterol (CHOD PAP), triglycerides (TRIGLYCERIDES GPO Method), ALT (ALT-TGP Colorimetric Method), AST (AST-TGP Colorimetric

Method), urea (UREA Colorimetric Method), and creatinine (CREATININE Colorimetric Method). While insulin levels (Rat Insulin, INS ELISA Kit, Sunlong®, China) were measured based on the sandwich-ELISA method,

Scanning Electron Microscopy (SEM)

The morphology of the surface was imaged by TESCAN mira3, Czech Republic with a high resolution showing a scale bar of 200-500 nm [26].

Ethical approval

The animal study protocol received approval from the ethical committee of the College of Medicine at Baghdad University (Approval code: PHA 03-5 on 2022-02-21).

Statistical analysis

All calculations were performed using "GraphPad Prism version 10.0.0 for Windows, GraphPad Software, Boston, Massachusetts, USA." One-way ANOVA was used to compare distinct continuous groups (all variables adhere to the normal distribution as determined by the Anderson Darling test), a post hoc Tukey test was performed to compare pairwise comparisons, and an independent t-test was utilized for the in vitro release research. P-values were two-tailed and considered significant if they were less than 0.05.

RESULTS

Table 1 illustrates the physical characteristics of both NPs; the particle size (PS) and PDI were significantly higher in NP1 compared to NP2, and NP2 had significantly higher zeta potential compared to NP1.

Table 1: Assessment of NP characteristics

Polymer name	Particle size (nm)	Zeta potential (mV)	EE (%)	PDI
NP1	197.8±3.3	-26.0±5.9	56.9± 2.5	0.37±0.02
NP2	123.0±4.3	-6.8±0.4	62.4±5.3	0.26±0.02
<i>p</i> -value	<0.001	0.005	0.179	0.003

Figure 1 illustrates SEM images for the synthesized NPs. Throughout the experiment, the cumulative insulin release (CIR) was significantly higher in NP2 compared to NP1. NP2 showed a steady rate of insulin release, while for NP1, it was accelerated in the first 6 hours and started to decrease afterward, as demonstrated by Table 2. The SBG after 3 hours was not statistically significant compared to group I, while for NP1, the maximum reduction of SBG was achieved after 6 hours.

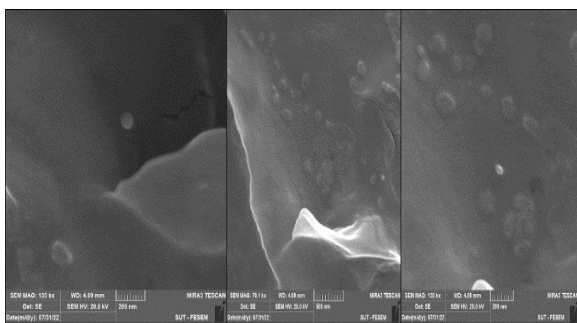


Figure 1: SEM images of different NPs, (A) left image NP2, (B) middle image NP2, (C) Right image NP1.

Table 2: Cumulative insulin release from different NPs.

Polymer	0.5 hr	3 hrs	6 hrs	12 hrs	24 hrs
Group IV	18.65±2.91	28.93±2.64	41.35±2.00	43.73±2.48	47.45±2.40
Group V	11.36±2.90	17.20±3.08	25.23±2.51	38.46±1.71	43.64±1.14
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001

NP2 achieved the maximum increase in serum insulin after 12–24 hours, and it was not significantly different compared to non-diabetic rats, as illustrated by Table 4. Table 5 gives details of changes in biochemical parameters after the end of the experiment.

DISCUSSION

Researchers frequently use PLGA copolymers as an insulin delivery system for oral administration [36]. The absorption of polymer NPs is significantly influenced by particle size. When compared to particles with a diameter of >500 nm, NPs with a size of 100 nm have a hundred times more intestinal absorption [37]. In the present study, the PS for NP1 was 197.8 ± 3.3 nm, and for NP2, it was 123.0 ± 4.3 nm, and this difference was statistically significant. This size reduction will enhance the GIT absorption and delay the release, as shown in previous studies [24, 30, 37], which was reflected by enhanced systemic absorption for NPs practically for NP2. Insulin release in vitro for NP1 was more rapid compared to NP2, and as the experiment progressed, the CIR for NP1 reduced. On the other hand, NP2 showed steady insulin release since some of the insulin will be localized on the surface of the NP and molecules with a more negative charge (which is measured by zeta potential) will adhere more to the surface of the NP. Since the charge was more negative for NP1 compared to NP2, as seen in Table 1, this explains the higher and earlier burst of insulin released from NP1. The extent of the decrease in insulin burst release [30] suggests that a significant portion of insulin was enclosed inside the formulation matrix. Table 1 shows that NP2 has a higher EE% than NP1 in this regard. GIT is composed of myocytes with a surface charge of around -50 mV; the greater the negative surface charge of the NP, the more difficult it is for the NP to traverse the GIT membranes. PEG has a hydrophobic interaction with insulin, but it forms many h-bonds. The peculiar structure of PEG increases the protein's durability and maintains an

aqueous coating that sustains the presence of water molecules necessary for the protein to carry out its biological function. TPGS exhibits strong emulsifying properties, leading to increased solubility and bioavailability. We tuned the analytical settings, including sonication duration, sonication rate, centrifugal time, and centrifugal speed, to yield NPs of almost the same size in the series. The particle diameter should be less than 500 nm to maximize interaction with the intestinal mucosa and enhance insulin absorption via the digestive system [38]. For the current investigation, we administered two oral NP formulations to rats with induced diabetes. It was observed that both NP formulations exhibited efficacy in decreasing levels of blood glucose. The NP1 hypoglycemic effect was maintained for 6 hours, and then an increase in blood glucose was seen, while the NP2 hypoglycemic effect was maintained for 24 hours. In terms of initial blood glucose reduction, for NP1, the maximum reduction was around 40% after 6 hours, and after 24 hours, it reached around 90% of the initial blood glucose, while for NP2, after 12 hours, the blood glucose was reduced to 30% of the initial blood glucose and slightly increased to 40% of the initial blood glucose level. This indicates that NP2 has a longer duration of activity than both NP1 and SC-soluble insulin (a maximum reduction of 20% after 3 hours and a 90% initial insulin level after 24 hours). Several studies examined the effect of oral insulin NP on blood glucose. In the Malathi *et al.* study, the authors examined a molecule labeled with the name ISTPPLG, which is much similar to the NP2 examined in the current study except for the use of two concentrations of PLGA (80/20 and 70/30), while in the current study it was 50/50 PLGA and 20 IU/kg dose of insulin used. Both NPs showed a significant reduction in blood glucose levels, which peaked around 6 hours for 80/20 PLGA NP and 12 hours for 70/30 PLGA NP [26]. Whenever delivered orally, insulin degrades in the stomach in the presence of proteolytic enzymes [39]. Enclosing insulin in a matrix-like structure can protect it

from digestive enzymes. This would become possible by coating insulin molecules in a matrix similar to the one provided in NP2. Insulin coated with polyethylene glycol (PEG) exhibits resistance to degradation by digestive enzymes. The formulation exhibited prolonged concentration in the small intestine and extended availability in the circulation due to PEG characteristics, including its mucoadhesive capacities and soluble properties, as well as its capacity to prevent protein aggregation. This led to delayed absorption. PEG serves as a stabilizing agent for nanospheres, hence enhancing the half-life and intestinal residence duration

of insulin. Tobio *et al.* demonstrated that adding PEG as a coating to 160 nm polylactic acid NPs (PLA NPs) makes them even more resistant to the clumping and breaking down processes caused by enzymes when they are exposed to simulated gastrointestinal (GI) fluids [40]. The observed reduction in glucose levels following insulin oral delivery with several PLGA-based NPs [41,42] is consistent with the efficiency of NP2 in lowering the level of blood glucose in rats observed in the present investigation [43]. Table 3 shows that NP2 effectively reduced blood glucose levels at a dosage of 20 IU/kg.

Table 3: Assessment of fasting serum glucose

Groups	Baseline	0.5 hr	3 hrs	6 hrs	12 hrs	24 hr
Group I	93.11±5.9 ^a	97.89±8.53 ^c	100.34±3.11 ^d	96.01±7.16 ^c	98.54±6.19 ^c	100.33±8.17 ^d
Group II	568.4± 77.9 ^b	538.91±40.24 ^a	575.43±42.77 ^a	577.80±40.41 ^a	569.43±60.99 ^a	529.11±66.5 ^a
Group III	562.4± 57.2 ^b	207.53±19.91 ^d	122.85±13.07 ^d	234.85±29.09 ^b	477.10±42.02 ^b	63.18 ^a ±525.86
Group IV	519.8± 66.0 ^b	326.94±39.64 ^c	242.67±32.99 ^c	201.43±50.35 ^b	330.68±44.61 ^c	436.03±62.58 ^b
Group V	515.8± 61.3 ^b	450.21±69.51 ^b	333.66±59.43 ^b	245.29±49.53 ^b	162.24±31.99 ^d	208.35±29.94 ^c
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values with identical superscripts are not significant difference ($p>0.05$) using One-way ANOVA and *post hoc* Tukey test.

The glucose-lowering impact of NP1 seemed to peak in the sixth hour, whereas NP2 exhibited a regulated maximum level between 12 and 24 hours. NP2 had a greater hypoglycemic impact than NP1 in the present experiment. The present study in both *in vitro* and *in vivo* insulin release profiles revealed that in NP1, insulin release was much accelerated during the first 6 hours, while in NP2, it was at a steady state (*in vitro* study). This was reflected in the *in vivo* insulin release with

NP2, which increased serum insulin to a comparable level to that of normal non-diabetic rats after 12–24 hours of administration, a state that was not achieved by NP1 and only after 3 hours of SC insulin administration (Table 4). NP2 effectively and long-lastingly lowers blood sugar because they protect insulin from being broken down by enzymes in the GI tract and have a PS (123 4.3 nm) that makes it easier for insulin to be absorbed in the gut.

Table 4: Assessment of fasting serum insulin

Groups	Baseline	0.5 hr	3 hrs	6 hrs	12 hrs	24 hr
Group I	3.19±0.12 ^a	3.26±0.18 ^a	3.11±0.11 ^a	3.31±0.118 ^a	3.19±0.20 ^a	3.20±0.19 ^a
Group II	0.51±0.21 ^b	0.41±0.13 ^d	0.35±0.11 ^d	0.45±0.12 ^d	0.43±0.14 ^d	0.36±0.10 ^d
Group III	0.36±0.17 ^b	1.76±0.18 ^b	3.12±0.29 ^a	0.52±0.15 ^d	0.47±0.19 ^d	0.58±0.11 ^{cd}
Group IV	0.59±0.14 ^b	1.27±0.35 ^c	2.17±0.14 ^b	1.48±0.46 ^c	1.34±0.16 ^c	0.71±0.20 ^c
Group V	0.43±0.13 ^b	0.95±0.19 ^c	1.49±0.22 ^c	2.21±0.50 ^b	3.04±0.27 ^a	2.99±0.23 ^a
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values with identical superscripts are not significant difference ($p>0.05$) using One-way ANOVA and *post hoc* Tukey test.

Due to its amphiphilic nature, the addition of PEG to the surface of NP2 might give a high permeability characteristic to the intestinal cell membrane [26]. It was shown that the utilization of Hp55-coated capsules, which contained insulin-loaded PLGA/RS (Eudragit® RS, Evonik Industries, Essen, Germany) NPs, resulted in a prolonged reduction in glucose levels in a rat model with diabetes induced by STZ, according to Wu *et al.* [44]. Comparably, Kumar *et al.* conducted a study examining oral insulin delivery for the treatment of DM employing PLGA-based NPs. The researchers observed that the formulation in question had effectively reduced the levels of blood glucose in diabetic rats induced by STZ [45]. NP2 showed the most substantial decrease in

triglycerides (TG) and TC levels upon completion of the experiment, according to the lipid panel analysis (Table 5). While NP1 showed a similar effect on TG comparable to that of SC insulin but a significantly higher reduction on TC compared to SC insulin, diabetic rats' serum TG and TC were significantly higher than normal non-diabetic rats, much like earlier reports [26,46]. In terms of liver and kidney effects, both NP1 and NP2 showed a non-significant difference in ALT and AST, but NP1 showed a slightly higher level of ALT and AST compared to a normal non-diabetic rate, while NP2 showed a significant increase. For urea and creatinine, both NP1 and NP2 showed higher levels compared to normal non-diabetic rats.

Table 5: Changes in the studied biochemical parameters

Groups	Triglyceride	Total cholesterol	ALT	AST	Urea	Creatinine
Group I	131.65±16.69 ^b	68.20±1.83 ^d	49.59±8.04 ^d	29.18±5.60 ^d	33.33±5.22 ^c	0.51±0.08 ^d
Group II	530.90±19.96 ^a	119.53±2.32 ^a	124.28±21.70 ^a	86.48±18.99 ^a	100.39±16.76 ^a	2.23±0.43 ^a
Group III	481.34±15.60 ^c	109.50±2.90 ^b	106.41±19.24 ^{ab}	67.0±18.99 ^{ab}	77.34±16.83 ^b	1.78±0.28 ^b
Group IV	453.18±34.24 ^c	89.65±3.10 ^c	70.24±14.37 ^{cd}	41.85±3.14 ^{cd}	63.9±15.84 ^b	1.62±0.18 ^b
Group V	304.33±30.62 ^d	74.16±3.88 ^c	87.99±16.25 ^{bc}	58.62±10.72 ^{bc}	58.10±14.00 ^b	0.95±0.13 ^c
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values with identical superscripts are not significant difference ($p>0.05$) using One-way ANOVA and *post hoc* Tukey test.

However, NP2 showed lower levels compared to NP1, and both NP1 and NP2 levels of ALT, AST, urea, and creatinine were lower than diabetic rats that were either treated with SC insulin or without treatment. The decrease in plasma proteins, the increase in circulating amino acids, and hepatic deamination collectively contribute to the subsequent elevation in urea levels, leading to the observed increase in blood urea concentration in diabetic rats. Furthermore, renal insufficiency may be the underlying cause of this condition [47]. Myocytes break down creatine phosphate nonenzymatically to produce creatinine. Fluctuations in creatinine levels indicate compromised glomerular function and are linked to the nephrotoxic effects of diabetes [48]. All these consequences were reduced by both NP2 and, to a lesser extent, by NP1. Aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), represent endogenous enzymes mostly located within cells. However, under some circumstances, these enzymes can escape into the bloodstream, making them useful as diagnostic biomarkers for assessing tissue damage, especially in the liver. During diabetes, aminotransferase activities are heightened [26, 49], and as shown in the current study, NP1 and NP2 offer protection against STZ-induced diabetic liver damage.

Conclusions

PLGA-PEG-TPGS NP can act as a potential carrier for oral insulin delivery and maintain good glycemic control for up to 24 hours. In terms of safety, NP1 and especially NP2 offer a reduction in ALT, AST, urea, and creatinine compared to non-treated diabetic rats and a reduction of both TC and TGs. Thus, we conclude that PLGA-PEG-TPGS NPs are a good candidate for oral insulin delivery.

Conflict of interests

No conflict of interest was declared by the authors

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

- Mikhael EM, Hassali MA, Hussain SA, Shawky N. Self-management knowledge and practice of type 2 diabetes mellitus patients in Baghdad, Iraq: a qualitative study. *Diabetes Metab Syndr Obes.* 2018;12:1-17. doi: 10.2147/DMSO.S183776.
- Al Rashed AM. Pattern of presentation in type 1 diabetic patients at the diabetes center of a university hospital. *Ann Saudi Med.* 2011; 31: 243-249. doi: 10.4103/0256-4947.81529.
- Abusaib M, Ahmed M, Nwayyir HA, Alidrisi HA, Al-Abbood M, Al-Bayati A, et al. Iraqi experts consensus on the management of type 2 diabetes/prediabetes in adults. *Clin Med Insights Endocrinol Diabetes.* 2020;13:1179551420942232. doi: 10.1177/1179551420942232.
- Mansour AA, Al-Maliky AA, Kasem B, Jabar A, Mosbeh KA. Prevalence of diagnosed and undiagnosed diabetes mellitus in adults aged 19 years and older in Basrah, Iraq. *Diabetes Metab Syndr Obes.* 2014;7:139-144. doi:10.2147/DMSO.S59652.
- Gorial FI, Sayyid OS, Al Obaidi SA. Prevalence of sarcopenia in sample of Iraqi patients with type 2 diabetes mellitus: A hospital based study. *Diabetes Metab Syndr.* 2020;14:413-416. doi: 10.1016/j.dsx.2020.04.021.
- Al-Azzawi OFN, Alobaidy MW, Saham MM. Nephrotic range proteinuria; does it predict lung involvement in patients with type 2 diabetes. *Diabetes Metab Syndr.* 2019;13(1):622-625. doi: 10.1016/j.dsx.2018.11.031.
- Hami A, M. Association between HbA1c and Serum Lipid Profile among a sample of Iraqi Patients with Type2 Diabetes Mellitus. *J Fac Med Bagdad.* 2021;63:100-105. doi: 10.32007/jfacmedbagdad.6331864.
- Zainab SA, Mohammed QA, Ahmed AA-M, Hussein KI, Saad AH. Influence of metformin dose and treatment adherence on glycemic control, adiposity, and cardiovascular risk markers in Iraqi patients with T2DM. *J Fac Med Bagdad.* 2022;4:218-226. doi: 10.32007/jfacmedbagdad.6441939.
- Kader S, Al-Shamma KJ, Majeed IA, Al-Ani AT. Comparative study between oral hypoglycemic drugs repaglinide, glibenclamide and rosiglitazone on some biochemical parameters in type 2 diabetic patients. *Iraqi J Pharm Sci.* 2009;18:32-38. doi: 10.31351/vol18iss2pp32-38.
- Yonus SS, Ahmed MA. Mitochondrial copies number and some renal function biomarkers in type 2 diabetes mellitus on metformin. *Iraqi J Pharm Sc.* 2022;31:33-38. doi: 10.31351/vol31iss2pp33-38.
- Abdulrahman AJ, Jabar MA, Najjar SA. Effects of liraglutide on weight control and blood pressure in type 2 diabetes mellitus Iraqi patients. *J Fac Med Bagdad.* 2022;4:227-232. doi: 10.32007/jfacmedbagdad.6441971.
- Hamid OA, Al-Humairi AK. Assessment of the level of adherence to insulin therapy and diet recommendations among diabetes patient. *IJDDT.* 2021;6:205-208.
- Mukhtar Y, Galalain A, Yunusa U. A modern overview on diabetes mellitus: a chronic endocrine disorder. *EJB.* 2020;5:1-14. doi: 10.47672/ejb.409.
- Arnolds S, Kuglin B, Kapitza C, Heise T. How pharmacokinetic and pharmacodynamic principles pave the way for optimal basal insulin therapy in type 2 diabetes. *Int J Clin Pract.* 2010;64(10):1415-1424. doi: 10.1111/j.1742-1241.2010.02470.x.
- Owens DR, Zinman B, Bolli G. Alternative routes of insulin delivery. *Diabet Med.* 2003; 20:886-898. doi: 10.1046/j.1464-5491.2003.01076.x.

16. Khafagy el-S, Morishita M, Onuki Y, Takayama K. Current challenges in non-invasive insulin delivery systems: a comparative review. *Adv Drug Deliv Rev.* 2007;59(15):1521-1546. doi: 10.1016/j.addr.2007.08.019.
17. Jain S, Rathi VV, Jain AK, Das M, Godugu C. Folate-decorated PLGA nanoparticles as a rationally designed vehicle for the oral delivery of insulin. *Nanomedicine (Lond).* 2012;7(9):1311-1337. doi:10.2217/nmm.12.31.
18. Florence AT. The oral absorption of micro- and nanoparticulates: neither exceptional nor unusual. *Pharm Res.* 1997;14(3):259-266. doi: 10.1023/a:1012029517394.
19. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids Surf B Biointerfaces.* 2010;75(1):1-18. doi: 10.1016/j.colsurfb.2009.09.001.
20. Reix N, Parat A, Seyfritz E, Van der Werf R, Epure V, Ebel N, et al. In vitro uptake evaluation in Caco-2 cells and in vivo results in diabetic rats of insulin-loaded PLGA nanoparticles. *Int J Pharm.* 2012;437(1-2):213-220. doi: 10.1016/j.ijpharm.2012.08.024.
21. Al-hadadee LT, AL-Sadoun TH and Jasim BA. The Effect of polyethylene glycol-coated gold nanoparticle on mice's renal function. *IJDDT.* 2021;11:664-668.
22. Ensign LM, Cone R, Hanes J. Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. *Adv Drug Deliv Rev.* 2012;64(6):557-570. doi: 10.1016/j.addr.2011.12.009.
23. Zhu K, Meng Z, Tian Y, Gu R, Xu Z, Fang H, et al. Hypoglycemic and hypolipidemic effects of total glycosides of Cistanche tubulosa in diet/streptozotocin-induced diabetic rats. *J Ethnopharmacol.* 2021;276:113991. doi: 10.1016/j.jep.2021.113991.
24. Cui F, Shi K, Zhang L, Tao A, Kawashima Y. Biodegradable nanoparticles loaded with insulin-phospholipid complex for oral delivery: preparation, in vitro characterization and in vivo evaluation. *J Control Release.* 2006;114(2):242-250. doi: 10.1016/j.jconrel.2006.05.013.
25. Akbarzadeh A, Norouzi D, Mehrabi MR, Jamshidi Sh, Farhangi A, Verdi AA, et al. Induction of diabetes by Streptozotocin in rats. *Indian J Clin Biochem.* 2007;22(2):60-64. doi: 10.1007/BF02913315.
26. Malathi S, Nandhakumar P, Pandiyan V, Webster TJ, Balasubramanian S. Novel PLGA-based nanoparticles for the oral delivery of insulin. *Int J Nanomedicine.* 2015;10:2207-2218. doi: 10.2147/IJN.S67947.
27. Yang J, Sun H, Song C. Preparation, characterization and in vivo evaluation of pH-sensitive oral insulin-loaded poly(lactic-co-glycolic acid) nanoparticles. *Diabetes Obes Metab.* 2012;14(4):358-364. doi: 10.1111/j.1463-1326.2011.01546.x.
28. McNeil-Watson F, Tscharnuter W and Miller J. A new instrument for the measurement of very small electrophoretic mobilities using phase analysis light scattering (PALS). *Colloid Surf A Physicochem Eng Asp.* 1998;140:53-57. doi: 10.1016/S0927-7757(97)00267-7.
29. Sonaje K, Lin YH, Juang JH, Wey SP, Chen CT, Sung HW. In vivo evaluation of safety and efficacy of self-assembled nanoparticles for oral insulin delivery. *Biomaterials.* 2009;30(12):2329-2339. doi: 10.1016/j.biomaterials.2008.12.066.
30. Zhang X, Sun M, Zheng A, Cao D, Bi Y, Sun J. Preparation and characterization of insulin-loaded bioadhesive PLGA nanoparticles for oral administration. *Eur J Pharm Sci.* 2012;45(5):632-638. doi: 10.1016/j.ejps.2012.01.002.
31. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods.* 2007;39(2):175-191. doi: 10.3758/bf03193146.
32. Charan J, Kantharia ND. How to calculate sample size in animal studies? *J Pharmacol Pharmacother.* 2013;4(4):303-306. doi: 10.4103/0976-500X.119726.
33. Festing MF. Design and statistical methods in studies using animal models of development. *ILAR J.* 2006;47(1):5-14. doi: 10.1093/ilar.47.1.5.
34. Underwood W and Anthony R. AVMA guidelines for the euthanasia of animals: 2020 edition. Retrieved on March 2020; 2013:2020-2021.
35. Pierozan P, Jernerén F, Ransome Y, Karlsson O. The Choice of Euthanasia Method Affects Metabolic Serum Biomarkers. *Basic Clin Pharmacol Toxicol.* 2017;121(2):113-118. doi: 10.1111/bcpt.12774.
36. Santander-Ortega MJ, Bastos-González D, Ortega-Vinuesa JL, Alonso MJ. Insulin-loaded PLGA nanoparticles for oral administration: an in vitro physico-chemical characterization. *J Biomed Nanotechnol.* 2009;5(1):45-53. doi: 10.1166/jbn.2009.022.
37. Desai MP, Labhasetwar V, Amidon GL, Levy RJ. Gastrointestinal uptake of biodegradable microparticles: effect of particle size. *Pharm Res.* 1996;13(12):1838-1845. doi: 10.1023/a:1016085108889.
38. van de Weert M, Hoehstetter J, Hennink WE, Crommelin DJ. The effect of a water/organic solvent interface on the structural stability of lysozyme. *J Control Release.* 2000;68(3):351-359. doi: 10.1016/s0168-3659(00)00277-7.
39. Morishita M, Morishita I, Takayama K, Machida Y, Nagai T. Novel oral microspheres of insulin with protease inhibitor protecting from enzymatic degradation. *Int J Pharm.* 1992;78:1-7. doi: 10.1016/0378-5173(92)90348-6.
40. Tobío M, Sánchez A, Vila A, Soriano I I, Evora C, Vila-Jato JL, et al. The role of PEG on the stability in digestive fluids and in vivo fate of PEG-PLA nanoparticles following oral administration. *Colloids Surf B Biointerfaces.* 2000 Oct 1;18(3-4):315-323. doi: 10.1016/s0927-7765(99)00157-5.
41. Cui FD, Tao AJ, Cun DM, Zhang LQ, Shi K. Preparation of insulin loaded PLGA-Hp55 nanoparticles for oral delivery. *J Pharm Sci.* 2007;96(2):421-427. doi: 10.1002/jps.20750.
42. Pan Y, Li YJ, Gao P, Ding PT, Xu H, Zheng JM. *Yao Xue Xue Bao.* 2003;38(6):467-470.
43. Sun S, Liang N, Kawashima Y, Xia D, Cui F. Hydrophobic ion pairing of an insulin-sodium deoxycholate complex for oral delivery of insulin. *Int J Nanomed.* 2011;6:3049-3056. doi: 10.2147/IJN.S26450.
44. Wu ZM, Zhou L, Guo XD, Jiang W, Ling L, Qian Y, et al. HP55-coated capsule containing PLGA/RS nanoparticles for oral delivery of insulin. *Int J Pharm.* 2012;425(1-2):1-8. doi: 10.1016/j.ijpharm.2011.12.055.
45. Kumar PS, Ramakrishna S, Saini TR, Diwan PV. Influence of microencapsulation method and peptide loading on formulation of poly(lactide-co-glycolide) insulin nanoparticles. *Pharmazie.* 2006;61(7):613-617. PMID: 16889069.
46. Asad M, Aslam M, Munir TA, Nadeem A. Effect of Acacia nilotica leaves extract on hyperglycaemia, lipid profile and platelet aggregation in streptozotocin induced diabetic rats. *J Ayub Med Coll Abbottabad.* 2011;23(2):3-7. PMID: 24800330.
47. Mwafy S, Yassin M. Physiological changes associated with streptozotocin induced experimental diabetic rats. *ICBAS.* 2010;12:31-35.
48. Ghazanfar K, Ganai BA, Akbar S, et al. Antidiabetic activity of *Artemisia amygdalina* Decne in streptozotocin induced diabetic rats. *Biomed Res Int.* 2014;2014:185676. doi: 10.1155/2014/185676.
49. Adaramoye OA. Antidiabetic effect of kolaviron, a biflavonoid complex isolated from *Garcinia kola* seeds, in Wistar rats. *Afr Health Sci.* 2012;12(4):498-506. doi: 10.4314/ahs.v12i4.16.