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Orally Administered Prosochit®-Based Nanoparticles of Insulin Ameliorates Alloxan-Induced Diabetes in Rats

Emmanuel O. Olorunsola [1,*], Koofreh G. Davies [2], Enomfon B. Essien [1], Mfonobong F. Alozie [3], Musiliu O. Adedokun [4] and Fakhrul Ahsan [5]

[1] Department of Pharmaceutics and Pharmaceutical Technology, University of Uyo, Uyo 520003, Nigeria
[2] Department of Human Physiology, University of Uyo, Uyo 520003, Nigeria
[3] Department of Pharmaceutical Microbiology and Biotechnology, University of Uyo, Uyo 520003, Nigeria
[4] Department of Pharmaceutics and Pharmaceutical Technology, Federal University Oye-Ekiti, Ekiti 371104, Nigeria
[5] Department of Pharmaceutical and Biomedical Sciences, California Northstate University, Elk Grove, CA 95757, USA
* Correspondence: emmanuelolorunsola@uniuyo.edu.ng or olorunsolaeo@yahoo.com; Tel.: +234-8035067306

Abstract: This work was aimed to assess the antidiabetic effect of orally administered Prosochit®-based nanoparticles of insulin in an animal model. Five batches of insulin-loaded nanoparticles were prepared as dry water-in-oil-in-water emulsions using different emulsifiers (prosopis gum, Prosochit® 201, Prosochit® 101, Prosochit® 102, and chitosan) for the outer emulsion. Unloaded Prosochit® 101-based nanoparticles were also formulated. The morphology and size distribution of the nanoparticles were studied using a scanning electron microscope and Zetasizer. Forty alloxan-induced diabetic Wistar rats were divided into eight groups. The different groups were administered daily with different formulations (unloaded nanoparticles, the 5 loaded nanoparticles equivalent to 50 IU insulin per kg, purified water, and Actrapid) for 14 days. Blood glucose level was monitored and determined over 24 h. Fasting blood sugar was also taken on days 3, 5, 7, and 14. A graph of the percent blood glucose level relative to time 0 h was plotted against time. The particles showed a water-in-oil-in-water constitution. Both the drug-loaded and the unloaded Prosochit®-based nanoparticles were of nano dimension. There was a significant difference ($p < 0.0001$) in the antidiabetic effects of all insulin-loaded nanoparticles compared with the negative control. There was no significant difference across the insulin-loaded nanoparticles of prosopis gum, Prosochit® 201, Prosochit® 102, and chitosan while the insulin-loaded Prosochit® 101 nanoparticles showed the best activity, which is comparable to subcutaneous insulin, reducing blood glucose levels to 32.20 ± 3.79%. All the oral Prosochit®-based insulin nanoparticles are characterized by appreciable antidiabetic activity with the activity of Prosochit® 101-based nanoformulation being comparable to that of the subcutaneous insulin.

Keywords: alloxan-induced; diabetes; insulin; nanoparticles; Prosochit®

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1. Introduction

Insulin is the major treatment for type 1 and many type 2 diabetes mellitus. It is usually administered parenterally, but other routes such as the pulmonary and nasal have been investigated, with some levels of positive clinical outcomes [1]. A major issue associated with these two alternative modes of administration is that only a small proportion of insulin can reach the systemic circulation to elicit the desired pharmacological action. Drug retention in the periphery and the development of insulin resistance are also serious challenges in the pulmonary and intranasal administration of insulin [2]. The oral route is another alternative for administering this golden agent for the management of diabetes.

The delivery of insulin through the oral route has several advantages, including the avoidance of pain, discomfort, and risk of infection that characterize continuous injection [3]. Oral delivery also improves the portal level of the drug and curtails peripheral

hyperinsulinemia, which is associated with other routes of administration [4]. Even though the orally administered insulin is more susceptible to metabolism by the liver thereby reducing its overall bioavailability, it can minimize the risk of hypoglycemia and the immune response in peripheral tissues, which occurs in the case of parenteral administration. Oral administration also minimizes the incidence of weight gain by reducing insulin exposure in the peripheral system [4].

Two major limiting factors of oral delivery of insulin are: degradation by proteolytic enzymes and poor intestinal absorption [2]. The susceptibility of insulin to proteolytic enzymes is due to the fact that the drug is a polypeptide hormone while the poor absorption is due to its large molecular mass of 5808. Several works have been carried out to address the biopharmaceutical and pharmacokinetic challenges of insulin. The poor stability of the drug in the gastrointestinal tract due to the activities of proteolytic enzymes has been approached by the incorporation of enzyme inhibitors [5] and formulation of the drug in a lipid system [6] while the poor intestinal absorption has been approached by employing nanotechnology to reduce the particle size of the drug formulation [7] and by incorporating permeation enhancers to alter the tight junctions in the intestinal epithelium [8].

Two or more polymers can be combined by blending or chemical reaction to derive a substance that will serve a desired purpose or purposes [9]. Prosochit® (PC) is a novel co-processed excipient of prosopis gum (PRG) and crab shell chitosan (CTS) with inherent controlled release and absorption-enhancing properties [10]. Prosopis is a commercially important plant genus used for many medical purposes [11]. Various parts of the plant have traditionally been used as anticancer, antidiabetic, anti-inflammatory, and antibacterial agents [12–14]. Chitosan, on the other hand, has been observed to enhance the stability of nanovehicles [15]. It also improves protein stability and lengthens its duration of action in systemic circulation [16]. Hence, it has been widely investigated in nanoformulations for oral delivery of insulin.

Nanoparticles can be prepared with biodegradable polymers, and as such can be investigated for oral, nasal, rectal, pulmonary, and ocular administration of insulin [17]. Of all these routes of administration, the oral is the most convenient. Moreover, orally administered insulin is the closest to endogenous insulin as it is transferred directly to the liver through the portal vein [18]. Furthermore, the desirable characters of the components of Prosochit® could enhance the performance of oral insulin. The present work, therefore, was aimed to evaluate the antidiabetic effect of orally administered Prosochit®-based nanoparticles of insulin using an animal model.

2. Materials and Methods

2.1. Materials

The materials used include: three types of Prosochit® coded Prosochit® 201 (PC201), Prosochit® 101 (PC101), and Prosochit® 102 (PC102) derived from prosopis gum and crab shell chitosan in ratios of 2:1, 1:1, and 1:2 respectively, as described by Olorunsola and Usungurua [10]. Prosopis gum was extracted from *Prosopis africana* seeds while chitosan was extracted from *Callinectes gladiator* as described in the same work of Olorunsola and Usungurua [10].

Other materials used are: Actrapid soluble insulin 100 IU/mL (Novo Nordisk, Germany), soluble insulin powder from bovine pancreas (Lot no. SLB25307, Sigma Aldrich Chemie, GmbH Germany), liquid paraffin (BDH Chemicals, Poole, UK), Span 60 (BDH Chemicals, England), sodium hydroxide (BDH Chemicals), and hydrochloric acid (Fisher Scientific International Company, Saint Louis, MO, USA).

2.2. Experimental Animals

Approval for the experimentation was obtained from the Animal Ethics Committee, University of Uyo, Uyo, Nigeria (protocol number UU/Pharm/2019/14). In total, 60 Wistar rats, each weighing 140–200 g, were obtained and housed in a cross-ventilated room (temperature of 25 ± 2.5 °C) for 7 days for acclimatization before the experiment. The Wistar

rats were maintained and handled according to the internationally accepted laboratory animal use and the guidelines and rules for animal experimentation.

2.3. Preparation of Insulin-Loaded Nanoparticles

Nanoemulsions were formulated as water-in-oil-in-water (*w*/*o*/*w*) double emulsions using a modified form of the method described by Mumuni et al. [19] as used in our previous work [20]. The inner emulsion was formulated as 0.175 g (500 IU) insulin dissolved in 5 mL purified water and emulsified with 20 mL liquid paraffin using 0.2 g Span 60 and homogenization at 1000 rpm for 10 min. The outer emulsion was formulated using 8.575 g of the appropriate polymer (prosopis gum, Prosochit® 201, Prosochit® 101, Prosochit® 102, or chitosan) as emulsifying agent. Each polymer was dispersed in 49.6 mL 0.2 N HCl and the dispersion neutralized using a sufficient amount of 0.1 N NaOH. The first (inner) emulsion was poured into the dispersion of the second emulsifier and the system emulsified by homogenization at 1000 rpm for 10 min. The batches were named based on the excipient used for the second emulsification.

A Prosochit® control batch (unloaded nanoparticles) was also formulated. In this case, water (without insulin being incorporated) was used as the inner phase of the first emulsion, and Prosochit® 101 was used as the emulsifier for the outer emulsion.

The weight of the nanoemulsion of each batch was determined followed by freeze-drying for 3 days using a Clifton freeze-dryer to obtain the appropriate nanoparticles: F3, F4, F5, F6, F7, and F8, which are unloaded PC101-based nanoparticles, PRG-based insulin nanoparticles, PC201-based insulin nanoparticles, PC101-based insulin nanoparticles, PC102-based insulin nanoparticles, and CTS-based insulin nanoparticles, respectively, as indicated in Table 1. The final weight of each batch was also determined.

Table 1. Formulations administered to the different groups of rats.

Group	Formulation Administered
1	Purified water (F1): 10 mL per kg weight of rat (negative control)
2	Actrapid soluble insulin (F2): 50 IU per kg weight of rat (positive control)
3	Unloaded PC101-based nanoparticles (F3): 1.81 g per kg weight of rat
4	PRG-based insulin nanoparticles (F4): 1.80 g per kg weight of rat
5	PC201-based insulin nanoparticles (F5): 1.89 g per kg weight of rat
6	PC101-based insulin nanoparticles (F6): 1.82 g per kg weight of rat
7	PC102-based insulin nanoparticles (F7): 1.93 g per kg weight of rat
8	CTS-based insulin nanoparticles (F8): 1.87 g per kg weight of rat

PRG = prosopis gum; PC201 = Prosochit® 201; PC101 = Prosochit® 101; PC102 = Prosochit® 102; CTS = chitosan.

The degree of drying of the nanoparticles was calculated using the formula:

$$\text{Degree of drying} = \frac{\text{weight of nanoemulsion} - \text{final weight of nanoparticles}}{\text{weight of nanoemulsion}} \times 100 \quad (1)$$

The percent drug loading of each batch of the nanoparticles was calculated using Equation (2):

$$\text{Percent drug loading} = \frac{\textbf{amount of insulin used for the formulation}}{\textbf{final weight of the nanoparticles}} \times 100 \quad (2)$$

2.4. Scanning Electron Microscopy (SEM)

A small sample of nanoparticles was mounted on the carbon tape and placed in the sample holder, which was subsequently loaded into a PhenomProx electron microscope (Phenom World, Eindhoven, The Netherlands). Light was made to fall on the sample producing an image as the sample was being scanned in the microscope. The image was taken at the best resolution.

2.5. Examination of the Particle Size Distribution of Loaded and Unloaded Nanoparticles

Particle size distribution of the loaded and the unloaded PC101-based nanoparticles was determined using a Zetasizer Nano ZS (Malvern Panalytical, Malvern, UK).

2.6. Induction of Diabetes

The rats were fasted for 12 h, after which diabetes was induced by single intraperitoneal injection of alloxan monohydrate dissolved in normal saline and given at a dose of 150 mg/kg body weight of the rat. Using a Fine test glucometer (Osang Healthcare, Dongan, Korea), fasting blood sugar was taken after 72 h to confirm the induction of diabetes.

2.7. Study Setting

Forty rats showing sign of diabetes (fasting blood sugar > 160 mg/dL) were selected from the rat population. The alloxan-induced diabetic rats were divided into 8 equal groups, $n = 5$.

2.8. Evaluation of the Antidiabetic Effect of the Formulations

Wistar rats of each group were administered appropriate formulations as stated in Table 1. Members of group 1 were administered purified water 10 mL/kg (negative control), group 2 animals were administered Actrapid soluble insulin 50 IU per kg weight of rat (positive control), and group 3 animals were administered unloaded nanoparticles of Prosochit® 101 (Prosochit® control). Groups 4 to 8 were administered insulin-loaded nanoparticles of different polymers in amounts equivalent to 50 IU insulin per kg weight of rat.

The purified water was administered using oral canula; for the nanoparticles, a 1 g quantity was dispersed in 10 mL purified water and the amounts of the dispersions containing the required nanoparticles of insulin were administered orally through oral canula while the Actrapid soluble insulin was administered subcutaneously.

Blood samples were taken from the tail vein at 0, 1, 2, 3, 6, 12, and 24 h and the glucose level was determined using a Fine test glucometer (Osang Healthcare, South Korea). Administration of the formulations was continued for 14 days, and fasting blood sugar was determined using the glucometer on days 3, 5, 7, and 14. Graphs of the percent blood glucose level (taking the value obtained before drug administration as the base line) were plotted against time for the 24-h period and 14-day period.

2.9. Statistical Analysis

Data were analyzed using one-way analysis of variance followed by Tukey–Kramer multiple comparison test using the GraphPad InStat package. Significance of difference was set at $p < 0.05$, where K is greater than 4.726.

3. Results

3.1. Degree of Drying and Percent Drug Loading

The weight of the nanoemulsions before drying, final weight of the nanoparticles, degree of drying, and percent drug loading are presented in Table 2. The values of the degree of drying ranged from 89.08% to 89.57% while the percent drug loading for the loaded nanoparticles ranged from 0.90% to 0.98%.

Table 2. Parameters related to drug loading and drying of the nanoemulsions.

S/N	Batch	Weight before Drying (g)	Weight after Drying (g)	% Drying	Percent Drug Loading (%)
1	Unloaded	166.50	18.12	89.12	Nil
2	PRG	169.34	18.02	89.36	0.97
3	PC201	181.80	18.96	89.57	0.93
4	PC101	166.44	18.18	89.08	0.96

Table 2. *Cont.*

S/N	Batch	Weight before Drying (g)	Weight after Drying (g)	% Drying	Percent Drug Loading (%)
5	PC102	180.52	19.34	89.29	0.90
6	CTS	176.16	18.74	89.36	0.98

Unloaded = unloaded nanoparticles; PRG = prosopis gum-based insulin nanoparticles; PC201 = Prosochit® 201-based insulin nanoparticles, PC101 = Prosochit® 101-based insulin nanoparticles, PC102 = Prosochit® 102-based insulin nanoparticles, CTS = chitosan-based insulin nanoparticles.

3.2. Scanning Electron Micrographs

The scanning electron micrographs of the six batches of nanoparticles are shown in Figure 1. Each micrograph showed inner white color, middle black color, and outer grayish-white color representing the three phases of the nanoemulsions. The inner white color, middle black color, and outer grayish-white color, for clarity, have the markings X, Y, and Z, respectively, in Figure 1b.

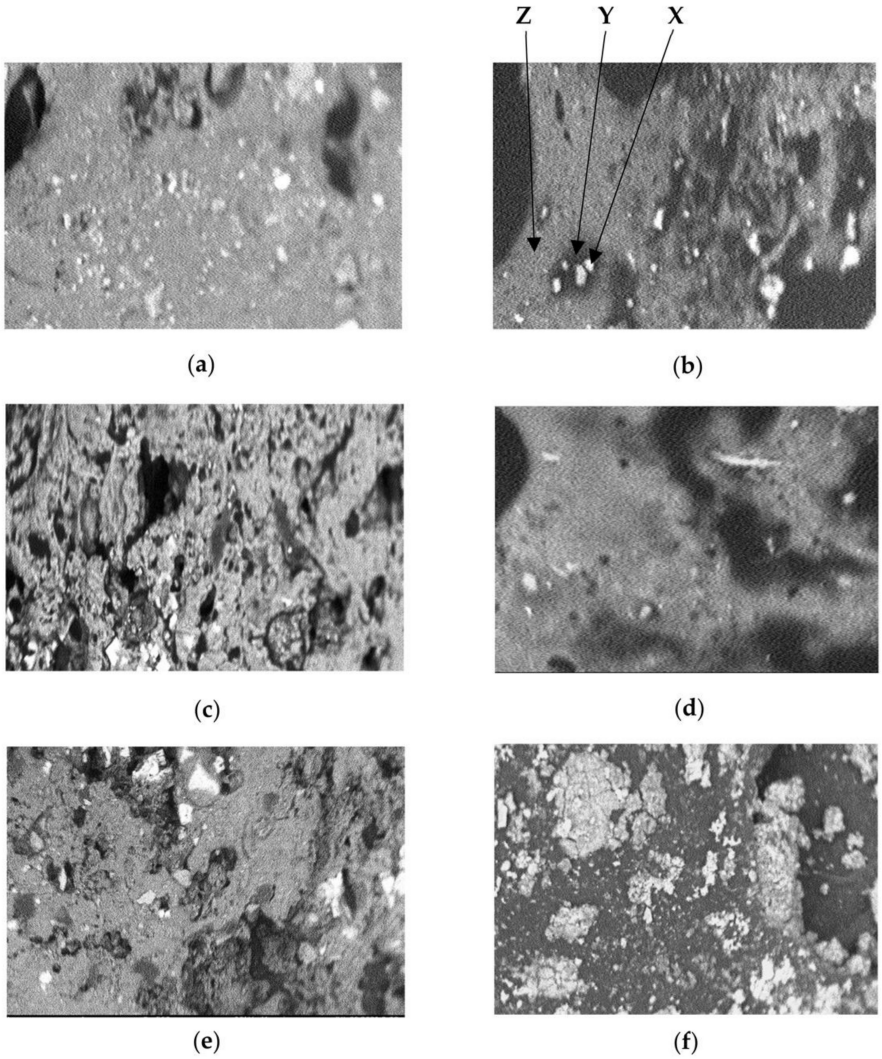


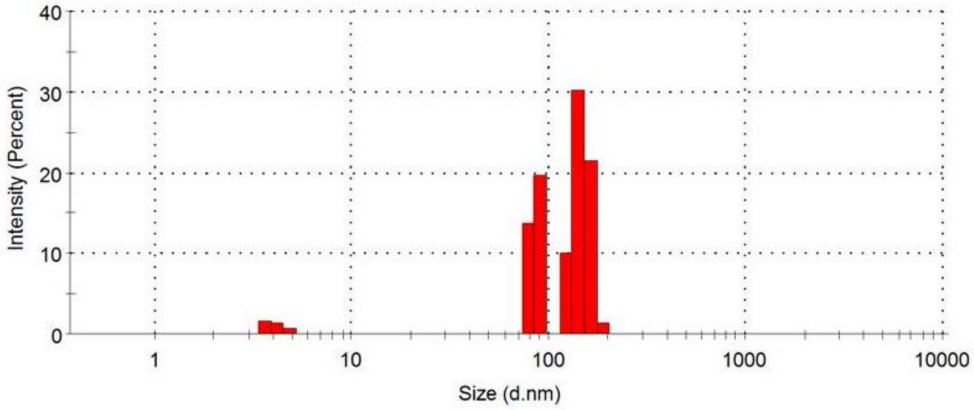
Figure 1. SEM of (**a**) unloaded nanoparticles; (**b**) PRG-based insulin nanoparticles; (**c**) PC201-based insulin nanoparticles; (**d**) PC101-based insulin nanoparticles; (**e**) PC102-based insulin nanoparticles; (**f**) CTS-based insulin nanoparticles (1000× magnification).

The inner water globules of the nanoparticles without insulin (unloaded nanoparticles) are near spherical and are well dispersed (Figure 1a). The particles of the inner emulsion of the PRG-based insulin nanoparticles (Figure 1b) are fewer and larger than those of

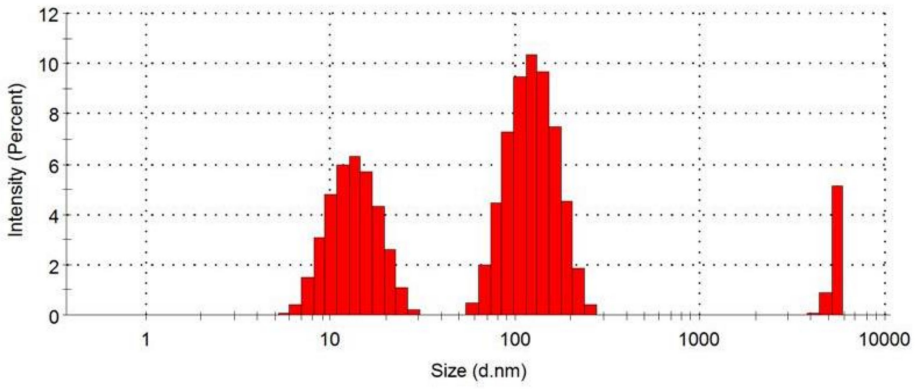
the unloaded nanoparticles apart from those that coalesced. The PC101-based insulin nanoparticles had a similar morphology (Figure 1d) to the unloaded nanoparticles. The dispersions of PC201 and PC102 were very fine (Figure 1c,e). However, there were some large particles in the dispersion of PC102, which are similar in appearance to those of the CTS-based nanoparticles (Figure 1f).

3.3. Particle Size Distribution

The particle size distribution of the unloaded PC101-based nanoparticles and that of the loaded nanoparticles are shown in Figure 2. The size distribution of the two formulations were characterized by three peaks each. All the peaks, apart from the last peak of the loaded nanoparticles were on the nanoscale; and the last peak had a low intensity and represented less than 7% of the total volume of the formulation. The first peak of the loaded nanoparticles was characterized by a higher intensity and higher average diameter compared with the first peak of the unloaded nanoparticles.



(**a**)



(**b**)

Figure 2. Particle size distribution of (**a**) unloaded PC101-based nanoparticles; (**b**) PC101-based insulin nanoparticles.

3.4. Antidiabetic Activity over a 24-h Period

The absolute values of the blood glucose level and variations in the blood glucose level (% change) over a period of 24 h (following a single administration of the various formulations) are shown in Table 3 and Figure 3 respectively.

Table 3. Absolute values of the blood glucose level over a period of 24 h following a single-dose administration of the various formulations.

	Absolute Values of Blood Glucose Level (mg/dL)						
Batch	**Time 0 h**	**Time 1 h**	**Time 2 h**	**Time 3 h**	**Time 6 h**	**Time 12 h**	**Time 24 h**
NTC	316.19 ± 19.25	316.40 ± 23.92	321.60 ± 23.86	326.20 ± 22.27	314.00 ± 19.52	307.40 ± 19.03	306.40 ± 19.74
PTC	376.75 ± 60.89	373.75 ± 51.97	347.50 ± 45.95	330.00 ± 45.36	281.25 ± 33.10	242.25 ± 24.03	233.75 ± 22.54 *
PCC	207.00 ± 11.79	184.00 ± 4.36	156.33 ± 8.25	149.30 ± 3.18	117.33 ± 15.51 *	114.33 ± 13.53 *	206.00 ± 36.47
PRG	318.75 ± 71.22	261.69 ± 98.01	204.31 ± 97.79	206.55 ± 94.88	193.48 ± 94.90 *	193.16 ± 93.83 *	284.00 ± 63.13
PC201	288.75 ± 73.92	300.00 ± 75.54	272.00 ± 79.70	260.66 ± 82.17	237.25 ± 88.59	234.75 ± 89.34	247.00 ± 87.69
PC101	372.75 ± 67.25	347.00 ± 59.32	314.00 ± 67.69	292.50 ± 76.99	280.00 ± 79.53	269.00 ± 73.19	272.03 ± 25.96
PC102	324.00 ± 68.82	281.85 ± 82.00	245.00 ± 91.16	267.25 ± 91.81	257.50 ± 88.91	255.50 ± 89.37	236.00 ± 84.79
CTS	318.00 ± 89.18	262.00 ± 95.15	260.33 ± 97.48	249.66 ± 99.29	199.00 ± 74.51	196.66 ± 72.65	191.00 ± 69.01 *

NTC = negative control, PTC = positive control, PCC = unloaded nanoparticles, PRG = prosopis gum-based insulin nanoparticles, PC201 = Prosochit® 201-based insulin nanoparticles, PC101 = Prosochit® 101-based insulin nanoparticles, PC102 = Prosochit® 102-based insulin nanoparticles, CTS = chitosan-based insulin nanoparticles. Data presented as mean ± S.E.M.; and significance of difference with respect to the glucose level at time 0 h represented by the number of asterisks.

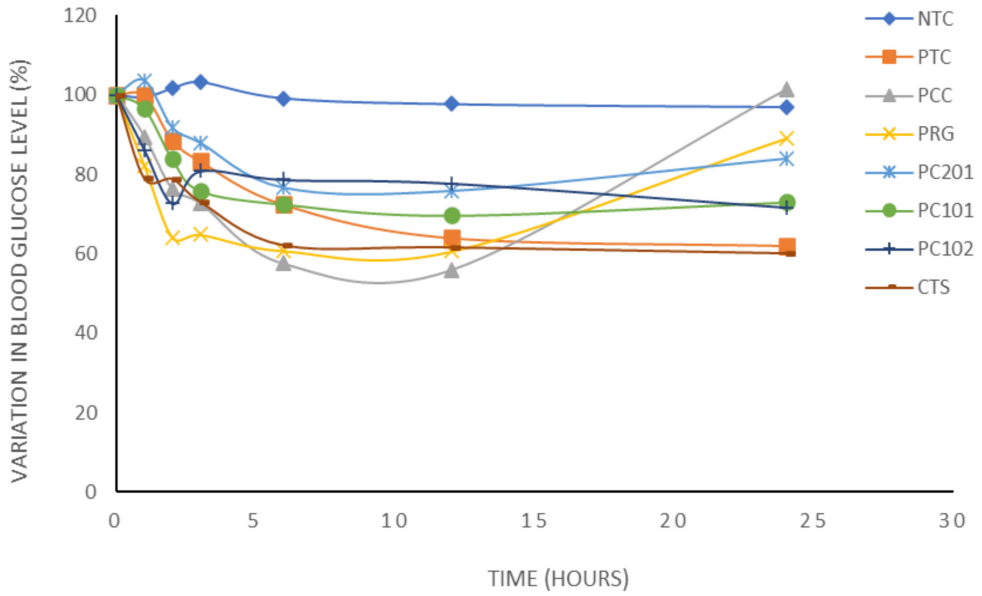


Figure 3. Antidiabetic effect over a 24-h period after single-dose administration of the various formulations (NTC = negative control, PTC = positive control, PCC = unloaded nanoparticles, PRG = prosopis gum-based insulin nanoparticles, PC201 = Prosochit® 201-based insulin nanoparticles, PC101 = Prosochit® 101-based insulin nanoparticles, PC102 = Prosochit® 102-based insulin nanoparticles, CTS = chitosan-based insulin nanoparticles).

The negative control formulation (NTC) had no significant effect on the blood glucose level of rats, and the level did not fall below 96.90 ± 0.85% of the baseline over a period of 24 h after the first dose. On the other hand, there was a marked reduction in the blood glucose level upon administration of the positive control formulation (PTC) as the glucose level was reduced to 62.05 ± 7.70%.

There was a significant difference ($p < 0.05$) in the percent blood glucose reduction by the unloaded nanoparticles (PCC) and formulation PRG compared with the negative control at 6 and 12 h. However, there was no significant difference between the percent blood glucose reduction by these formulations and the negative control at 24 h. Additionally, there was no significant difference in the percent blood glucose reduction by the formulations containing different types of Prosochit® at 24 h. The chitosan-based formulation (CTS) showed a similar effect to the positive control in reducing the blood glucose level over the 24-h period.

3.5. Antidiabetic Activity over Fourteen Days

The absolute values of the blood glucose level and the variations in the blood glucose level (% change) over a period of 14 days (with daily administration of the various formulations) are shown in Table 4 and Figure 4 respectively. A continuous reduction in the blood glucose level was observed with the positive control while the level remained relatively constant and high with daily administration of purified water (the negative control). The unloaded nanoparticles, which are the Prosochit® control (PCC), showed a greater reduction in the blood glucose level compared with the negative control.

Table 4. Absolute values of the blood glucose level over a period of 14 days with daily administration of the various formulations.

	Absolute Values of Blood Glucose Level (mg/dL)					
Batch	**Time 0 h**	**After 1 Day**	**After 3 Days**	**After 5 Days**	**After 7 Days**	**After 14 Days**
NTC	316.19 ± 19.25	306.40 ± 19.74	302.40 ± 19.87	301.60 ± 19.97	300.60 ± 19.51	281.80 ± 17.73
PTC	376.75 ± 60.89	233.75 ± 22.54 *	202.00 ± 8.39 *	173.25 ± 4.83 ***	159.50 ± 6.06 ***	121.75 ± 5.51 ***
PCC	207.00 ± 11.79	206.00 ± 36.47	196.00 ± 42.53	185.33 ± 30.28	174.33 ± 25.77	136.66 ± 23.21
PRG	318.75 ± 71.22	284.00 ± 63.13	251.02 ± 23.98 *	225.99 ± 6.57 *	217.22 ± 4.09 *	122.00 ± 3.72 **
PC201	288.75 ± 73.92	247.00 ± 87.69	220.75 ± 70.73 *	180.00 ± 43.13 *	155.75 ± 26.54 *	118.24 ± 9.99 **
PC101	372.75 ± 67.25	272.03 ± 25.96	224.34 ± 28.80 *	161.75 ± 21.85 ***	158.75 ± 20.37 ***	120.02 ± 17.43 ***
PC102	324.00 ± 68.82	236.00 ± 84.79	185.25 ± 43.52 *	127.75 ± 21.77 **	146.75 ± 23.59 **	122.25 ± 8.14 **
CTS	318.00 ± 89.18	191.00 ± 69.01 *	176.00 ± 62.79 *	139.00 ± 28.59 **	132.33 ± 27.09 **	126.01 ± 32.00 **

NTC = negative control, PTC = positive control, PCC = unloaded nanoparticles, PRG = prosopis gum-based insulin nanoparticles, PC201 = Prosochit® 201-based insulin nanoparticles, PC101 = Prosochit® 101-based insulin nanoparticles, PC102 = Prosochit® 102-based insulin nanoparticles, CTS = chitosan-based insulin nanoparticles. Data presented as mean ± S.E.M.; and significance of difference with respect to the glucose level at time 0 h represented by the number of asterisks.

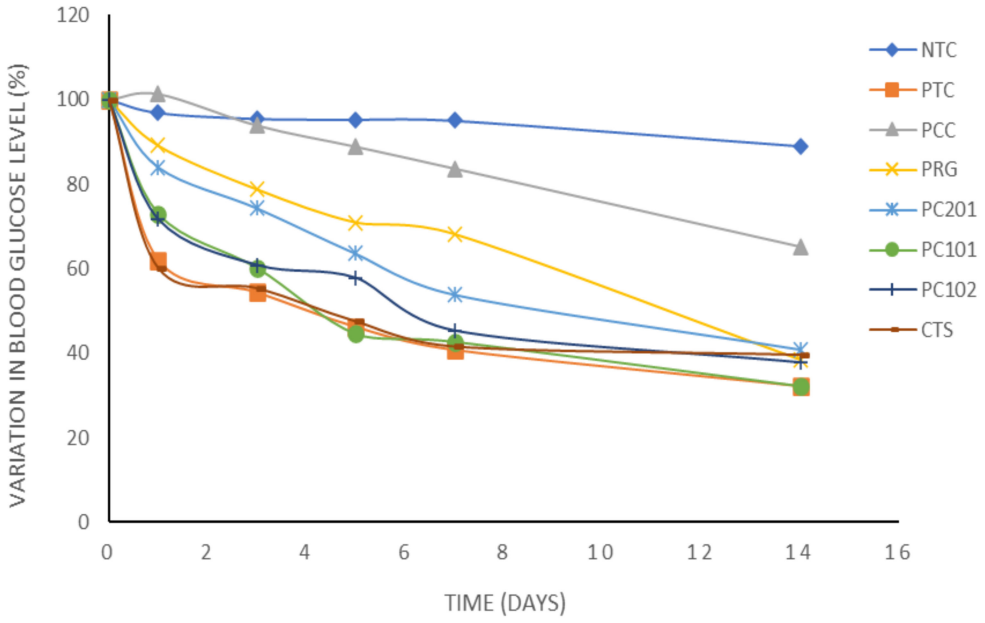


Figure 4. Antidiabetic effect over a 14-day period with daily administration of the various formulations (NTC = negative control, PTC = positive control, PCC = unloaded nanoparticles, PRG = prosopis gum-based insulin nanoparticles, PC201 = Prosochit® 201-based insulin nanoparticles, PC101 = Prosochit® 101-based insulin nanoparticles, PC102 = Prosochit® 102-based insulin nanoparticles, CTS = chitosan-based insulin nanoparticles).

Unlike the 24-h period, the PRG (prosopis gum-based nanoparticles of insulin) formulation did not show any significant difference in reducing the blood glucose level compared with the other test formulations. There was a significant difference ($p < 0.0001$) in the per-

cent glucose level reduction by all insulin-loaded nanoparticles compared with the negative control for the 14-day treatment. The PC101 (Prosochit® 101-based nanoparticles of insulin) formulation showed the best activity among the test formulations and its activity (reducing blood glucose level to 32.20 ± 3.79%) was not significantly different from the positive control (the subcutaneous insulin which reduced the blood glucose level to 32.30 ± 3.89%).

4. Discussion

Unlike many research works, where there are wide variations in the drug loading capacities, for instance, the work of Mumuni et al. [19], which showed a drug loading capacity ranging from 0.7% to 1.7%, a narrow range of 0.90% to 0.98% was targeted in this research. This was achieved by ensuring a relatively close degree of drying.

The inner white color of the scanning electron micrographs reflects the inner water phase of the water-in-oil-in-water emulsions, the black color reflects the oil globules, and the outer grayish-white color reflects the outer water phase. The three colorations are indicative of the water-in-oil-in-water constitution of the nanoparticles [21].

The micrographs in Figure 1a,d are similar because they contain the same emulsifier (Prosochit® 101) in the outer emulsion, with the only difference between the two formulations being the inclusion of insulin in the dispersion in Figure 1d. The large particles in the dispersion containing Prosochit® 102 can be attributed to the presence of chitosan as such particles were found in the dispersion containing only chitosan as the emulsifier of the outer emulsion (Figure 1f).

The first peak in the size distribution of the unloaded nanoparticles can be attributed to the inner water globules with a mean particle size of 4 nm while the second and third peaks can be attributed to the oil phase with mean particle sizes of 90 and 150 nm, showing the nanodimension of the unloaded particles. The two peak values being attributed to the oil phase can be explained using the emulsification by the two constituents of Prosochit®. Our previous work on the design, formulation, and characterization of a Prosochit®-based nanoparticulate system of insulin for oral delivery had already shown that the particle sizes of the three types of insulin-loaded Prosochit®-based particles are of a nanodimension [20].

Figure 2 provides a comparison of the particle size distribution of the loaded and unloaded nanoparticles. Drug loading caused an increase in the particle size of the inner water globules. This inference is based on the position of the first peak of the loaded nanoparticles relative to the position of the first peak of the unloaded nanoparticles. The drug loading also caused an increase in the particle size of the oil globules represented by the positions of the second and third peaks in both Figure 2a,b. The third peak of very low intensity observed in the size distribution of the loaded nanoparticles (Figure 2b) is indicative of very few particles of sizes >1000 nm. As the intensity is very small, the effect of the large particles on the activity of the formulation might not be significant.

Ordinarily, the size distribution of every batch is supposed to be characterized by two peaks: the first peak for the particle size of the inner emulsion and the second for the outer emulsion. However, since Prosochit® is a composite of two polymers, three peaks could be obtained in some cases. This is the reason for the three peaks observed with the loaded and the unloaded PC101-based nanoparticles. However, both formulations are still of a nanodimension.

Characterization of a formulation is necessary to ascertain the production of a stable product that is of suitable quality. Effective delivery of the orally administered formulation can be guaranteed once the integrity of the drug is secured by emulsification and by the components of Prosochit® since cellular uptake of particles depends on the particle size [22]. The stability of the three types of Prosochit®-based nanoparticles of insulin at the gastric pH was demonstrated in our previous work as none of the nanoparticles showed drug release above 10.1% in dissolution medium pH 1.2 over 2 h. The three formulations were also comparable in terms of drug release and permeation as > 60% drug release and 49.1–54.9% drug permeation were reported [20].

The good performance of the chitosan-based formulation within the 24-h period can be partly attributed to the stabilizing effect of chitosan [16]. The excipient is also known to enhance intestinal permeation by temporarily widening the tight junctions of epithelial cells, thus promoting drug delivery [23,24]. Moreover, the work of Mesiha et al. [25] has long shown that the presence of a permeation enhancer in an emulsion incorporating insulin can promote the absorption of the drug.

Under 12 h, the activities of the PRG and PCC formulations corresponded to better antidiabetic effects compared with the positive control. Prosopis gum, which is the polymer used as the emulsifier for the outer emulsion of the PRG formulation has been shown to possess antidiabetic activity [12,14]. The observed enhanced activity of the formulation can be attributed to this polymer. The same explanation can be extended to the PCC (Prosochit® control formulation) formulation, which of course does not contain insulin but contains prosopis gum as one of the constituents.

The insignificant difference in the degree of blood glucose reduction at 24 h by the three formulations containing the different types of Prosochit® shows that there is no significant difference in the antidiabetic effects of a single-dose administration of the three different Prosochit®-based nanoparticles of insulin. They are also less effective compared with the positive control on single-dose administration.

For the 14-day observation, the continuous reduction in the blood glucose level by the positive control formulation shows the effectiveness and reliability of the product. The antidiabetic activity of the unloaded nanoparticles (PCC) is an indication of the level of antidiabetic activity of the excipient itself as already explained. Unlike the observation during the 24-h period, the PRG formulation was not observed to be significantly different in reducing the blood glucose level because the measurement was no longer taken until 24 h after drug administration. This further confirms the short-term antidiabetic activity of prosopis gum.

Since all the insulin-loaded nanoparticles exhibited appreciable antidiabetic activity, this is indicative of nanotechnology enhancing the performance of orally administered insulin. It is also in agreement with the report of Erel et al. [26], whereby the activity of orally administered insulin was enhanced using an emulsion-based nanoformulation. The antidiabetic effects of the PRG, PC201, PC102, and CTS formulations are comparable while the PC101 formulation is comparable to the subcutaneous administration of insulin.

The high activity of the test formulations containing Prosochit® can be linked to the various desirable properties built into the nanoparticles, which include the protective property and antidiabetic effect of prosopis gum [12,14]; the protein-stabilizing effect and permeation-enhancing activity of chitosan [16,23]; formulation as nanoparticles to reduce particle size and enhance absorption [25]; and the shielding of insulin from proteolytic enzyme by the oil component of the water-in-oil-in-water formulation [27]. It has also been shown that intact protein can be easily absorbed [28].

Previous work of Olorunsola et al. [29] showed the ability of Prosochit® to enhance drug delivery. A chitosan-based delivery system has also been shown to be specifically good for the delivery of peptides and proteins [26,30]. It is, therefore, not unexplainable for Prosochit® to effectively facilitate a good antidiabetic effect from orally administered insulin.

Prosochit® 101 is a co-processed excipient from equal proportions of prosopis gum and chitosan, optimizing the effect of the two excipients. This fact, in addition to the enumerated desired properties of the Prosochit®-based nanoparticulate formulation, is responsible for the high antidiabetic activity of the oral nanoparticulate formulation PC101 (Prosochit® 101-based nanoparticles of insulin), which is not significantly different from the reference subcutaneous insulin formulation.

The limitations of this work include the wide variation of the absolute values of the blood glucose level after the induction of diabetes (time 0 h) and the high values of the standard error of mean of the variation in the blood glucose level of the groups that received the prosopis gum nanoformulation, Prosochit® 102 nanoformulation, and chitosan nanoformulation of insulin. The death of one rat each from the positive control group and

the groups that received Prosochit® 101 nanoformulation and chitosan nanoformulation of insulin was recorded in the course of the laboratory work, thereby affecting the n value of those groups.

5. Conclusions

Prosochit® alone is characterized by a level of antidiabetic activity, which is attributable to prosopis gum. Antidiabetic activities of the Prosochit® 201-based and the Prosochit® 102-based nanoparticles of insulin are similar to those of the prosopis gum-based and the chitosan-based formulations. Prosochit® 101-based nanoparticulate formulation of insulin is capable of reducing the blood glucose level to 32.20 ± 3.79% of the initial value after a 14-day administration to diabetic rats; this effect is comparable to that of the subcutaneous insulin administration.

6. Patent

Prosochit®, NG Patent 2016/00355, which was used to generate the insulin nanoparticles, is an innovation of one of the authors.

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