



Research Article

Nanoparticle Tactics for the Oral Delivery of Insulin in Alloxan-Induced Hyperglycemia in Rat (*Rattus norvegicus domestica*) Model

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Abstract | Administration of peptide drugs like insulin through the oral avenue specially the gastro-intestinal tract represents the great challenges. Polymeric nanoparticles are suitable colloidal carriers for insulin delivery. The current study was carried out to evaluate nanoparticle tactics for the oral delivery of insulin in the alloxan-induced hyperglycemia in rat model. Twenty male rats of age four months were randomly allocated into four different groups (T_0 , T_1 , T_2 , and T_3). After acclimatization diabetes was induced in three groups of rats (T_1 ; positive control, T_2 ; insulin only insulin, and T_3 ; insulin with nano particles) by administered alloxan injection at dose of 120mg/kg body weight intraperitoneally. Group T_0 was kept for negative control while T_1 was kept positive control. T_2 were considered as insulin treated group at 21IU/kg and T_3 as insulin with nano particles of same dose as insulin. Over the sequence of the experimental trials, observations were noted for blood glucose level, glycosylated hemoglobin, and body weight. Blood glucose levels were increased significantly ($P<0.05$) in all treated groups compared to the negative control group. Similarly, bodyweight was decreased significantly ($P<0.05$) in all alloxan treated groups compared to negative control. There was significant falling in blood glucose level in insulin with nanoparticle treated group (T_3) compared to only insulin treated group (T_2) and (T_1) that was received alloxan. The present study shows that insulin administration of oral route with nanoparticles was significantly decrease blood glucose levels and increase body weight. Hence, it can be concluded that oral insulin supplementation with nano particles may have significant effect on hyperglycemic controls.

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Introduction

Diabetes is a very serious disease that has affected the health of every society, family, and individuals around the globe. It is one among the top ten causes of death globally and 1.6 million people

died in 2016 by diabetics (WHO, 2018). In 2019, global health expenditure on diabetes was \$760 billion and expected to rise up to \$845 billion in 2045 (Elfein, 2019). Diabetes are of many types but type 1 diabetes, type 2 diabetes, and gestational diabetes are considered as the most common (NIDDK, 2016).

The international diabetes federation has stated the global, national, and regional occurrence of diabetes from the year 2000 (Saeedi *et al.*, 2019). The insulin hormone is used to manage diabetes normally by an administered subcutaneous route that controls blood glucose level but around 60% of patients fail to maintain long term hyperglycemic control (Saaddine *et al.*, 2006). Resistance to injectable insulin has been identified as a major reason for clinical inertia and lack of achievement of target glycemic goals (Balasubramanyam *et al.*, 2013). The patients fear the harden of insulin therapy, associated hypoglycemia, and increases weight gain. Patients expect the development of oral insulin as it will be easy administration, more convenient, and lead to better glycemic control (Mukhopadhyay *et al.*, 2012). Taken of therapeutic peptide drugs such as insulin via the oral route such as the gastrointestinal tract enzymes are one of the main challenges. Colloidal drug carriers have been advanced for controlled drug release and epitomize an exciting method to increase the acceptance and passage of peptide drugs such as insulin via oral route (Cui *et al.*, 2006). Polymeric nanoparticles (NP) are appropriate colloidal carriers for insulin delivery and several examinations have been implemented to determine appropriate route of administrations (Barenholz, 2001). Polymeric NPs are solid colloidal nanocarriers that provide controlled release of peptides depending on the surface modifications by biodegradable polymers.

Chitosan (CS), a hydrophilic natural polymer, has been used in protein and peptide encapsulated NP formulation for its exceptional appearances including biocompatibility, biodegradability and mucoadhesivity (Pan *et al.*, 2002; Ding *et al.*, 2006). Therefore, the current study was carried out to evaluate the nanoparticle tactics for the oral delivery of insulin in alloxan-induced hyperglycemic rat model, to determine the efficacy of nanoparticle with insulin in the alloxan induced diabetic rat on live and blood glucose level and glycated hemoglobin, and to investigate the effect of nanoparticle with insulin on live body weight in alloxan-induced diabetic rats.

Materials and Methods

Preparation of house

The experimental shed was swept and washed with tap water followed by disinfection and air drying. All utensils required for the experiment such as feeder,

water bottle, micro tube, syringe, needle, etc., were collected and the shed was properly designed with adequate ventilation.

Experimental animal and design

Twenty male rats (*Rattus norvegicus domestica*) of 30 days were purchased from International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B), Mohakhali, Dhaka and the rats were brought to the shed prepared at the laboratory of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh, and divided into four different groups having 5 rats in each group randomly. The divided groups were designated as T₀ (Control no diabetes), T₁ (Control of diabetes), T₂ (Diabetes with the insulin-treated group), and T₃ (Diabetes treated of insulin with nanoparticles). In each group, feed and water were provided *ad-libitum*.

In T₀ group, rats were administered with saline water orally. Bodyweight and blood glucose were recorded at day 0 (pre-treatment) before and after meal in the interval of seven days within 21 days at the same intervals with other groups. In T₁ group, after acclimatization of body weight, level of blood glucose was measured after 18 hrs. of starvation then alloxan was induced by intraperitoneal injection of 120 mg/kg body weight (Kuetze, 2017). In T₂ group, blood glucose level and body weight were measured; similarly, after of starvation then alloxan monohydrate was injected at a same dose of T₁ group. After 32 hours of alloxan monohydrate injection, diabetes was confirmed. Later, insulin was fed orally at a dose of 21 Unit/kg body weight for 21 days. During the treatment of insulin, body weights and level of blood glucose were recorded in the interval of 7 days for 21 days. In T₃ group, as above mentioned after checking blood glucose level and body weight before and after meal, and starvation then alloxan was injected as in T₁ group. Diabetes was confirmed as done in T₂ group. After that insulin with nanoparticle was fed orally at a dose of 21 Unit/Kg body weight for 21 days. During the treatment of insulin with nanoparticles body weights and level of blood glucose were recorded at an interval of seven days for 21 days.

Collection, preparation and administration of insulin with nanoparticles

Chitosan, sodium tripolyphosphate was purchased from the Sigma-Aldrich in Germany, while distilled water, acetic solution and magnetic stirrer were

available in the laboratory.

Preparation of insulin with nanoparticles

Insulin with nanoparticles was prepared according to ionotropic gelation method (Aktaş *et al.*, 2005) by dissolving 4.375 mg of chitosan in 2.5 mL of the acetic acid aq. solution to attain concentrations of 1.75 mg/mL and subsequently 1 mg of tripolyphosphate was dissolved in 2.5 mL of distilled water to get concentrations of 0.4 mg/ml. The solutions were mixed using magnetic stirring at room temperature. 5 mL of insulin was added to the tripolyphosphate solution under slowly stirring at room temperature to obtain concentrations of 7.5 ml. Insulin with nanoparticles was prepared by adding premixed solutions of TPP-insulin drop wise to 2.5 mL of chitosan solution at room temperature until a stable colloidal suspension was formed spontaneously under gentle magnetic stirring.

Administration of insulin with nanoparticle

Insulin with nanoparticle was given to the rats through the oral route by using syringes at a dose of 21IU for 21 days.

Collection, storage, and administration of insulin

Insulin human USP (rDNA) was purchased from the local market in Dinajpur and it was stored at 4°C in a refrigerator for a day and it was given it orally to the rats at a dose of 21 IU/kg for 21 days.

Recording of different parameters

Blood glucose level: To measure the level of blood glucose of rats, blood samples were collected at 7 days interval for 21 days on 0, 7th, 14th, and 21st day. The rat was held at the base of the neck, after confirmed that the rat is not able to escape, the tip of the tail was pinched with a needle to bring the blood. After that the blood is coming out of the tail then the blood was put on the strip in sugar check and the blood glucose meter was active to read the level of blood glucose of the rat.

Determination of glycosylated haemoglobin test (HbA1c): To determine the glycosylated haemoglobin of each group of the rat's blood samples were collected at the end of the experiment and were transported to the diagnostic center to determine the level of glycosylated hemoglobin in each rat by using automatic machine analyzer.

Recording of body weight: Bodyweight of all groups were recorded before the treatment at Day 0 and during the treatment period on 7th, 14th, and 21st day using digital electric balance.

Statistical analysis

All results are expressed as mean \pm SD. The groups were compared using SPSS version 22 and the results were considered statistically significant when at $p < 0.05$. Analysis of variance (ANOVA) was done to determine the significance between groups.

Results and Discussion

The present study was carried out to determine the nanoparticle tactics for the oral delivery of insulin in alloxan-induced hyperglycemic rat model. The results and discussions of all experiments were presented below:

Effects of insulin with nanoparticle on blood glucose level in alloxan induced hyperglycemic rat model

Blood glucose level (mmol/L) was decreased in insulin with nanoparticles treated group. The level of blood glucose of different groups of rats is shown in Table 1. The study revealed that blood glucose level was low in group T₃, which was treated using insulin with nanoparticles compared to T₂ group which was treated only using insulin with the same a dose at 21.IU/kg.

The effect of insulin with nanoparticles at a dose of 21 IU/kg body weight in lowering blood sugar showed a statistically significant compared with the T₂ group.

Table 1: Effects of insulin with nanoparticle on blood glucose level in alloxan induced hyperglycemic rat model.

Group	T ₀	T ₁	T ₂	T ₃	Level of significance
Day 0 (mean \pm SD) mmol/L	4.74 \pm 0.95	5.98 \pm 1.02	4.69 \pm 1.25	5.63	NS
Day 7 (mean \pm SD) mmol/L	5.81 \pm 0.75	22.07 \pm 5.08	17.12 \pm 5.75	13.37 \pm 4.38	**
Day 14 (mean \pm SD) mmol/L	5.0 \pm 0.76	22.82 \pm 5.14	17.37 \pm 5.14	12.69 \pm 4.09	**
Day 21 (mean \pm SD) mmol/L	4.56 \pm 0.86	19.63 \pm 6.27	16.55 \pm 3.71	11.56 \pm 4.76	**

**indicates significant at $P \leq 0.01$, and NS: Non-significant.

At a dose of 14 IU insulin/kg, rats exhibited a huge loss in glucose than was gained using a control insulin-chitosan solution. An even greater decline in glucose levels was noticed by increasing the nanoparticle dose to 21 U insulin/kg. Bowman and Leong (2006) theorize that “chitosan nanoparticles may defend insulin from gastrointestinal degradation and also improve uptake through muco-adhesion and/or permeation improvement”. Nanoparticles protect proteolytic enzymes that destroy insulin when administered orally. The level of blood glucose was highly decreased in the treatment of insulin with nanoparticles. The blood sugar level is almost coming to the normal levels. The present results agree with other results too (Lee *et al.*, 2004; Serpe *et al.*, 2004; Colonna *et al.*, 2008). The result of this study also agrees with Grenha *et al.* (2008). Gan and Wang (2007) described that nanoparticles reduced blood glucose level of streptozotocin-induced diabetic rats when it provided orally at insulin doses of 50 U/kg and/or 100 U/kg. The result of this study indicates that a dose of 21 IU/kg of insulin with nanoparticles might be a beneficial adjuvant for oral hyperglycemic agents in an alloxan induced diabetes. Similarly, Sarmiento *et al.* (2007) described that nanoparticles with insulin massively increase intestinal absorption for administration of oral insulin in their study. Streptozotocin induced hyperglycemic rat nanoparticles were decreased serum glucose levels at different doses of insulin of 50 and 100 IU/kg up to 59 and 55%, respectively. Inchaurreaga *et al.* (2020) showed that nanoparticles were lowered the blood glucose level in hyperglycemic rats at a dose of 50 IU/kg up to 20% of their basal glucose levels.

Effects of insulin with nanoparticle on glycosylated hemoglobin in alloxan induced hyperglycemic rat model

The results obtained from the diagnosis of glycosylated hemoglobin (HbA1c) among experimental animals were concluded that negative control T_0 was the lowest in glycosylated hemoglobin while positive control T_1 was the highest in the glycosylated hemoglobin.

In the treatment control groups, the glycosylated hemoglobin was the lowest at the insulin with nanoparticles group T_3 , while insulin-treated group T_2 was the highest in the glycosylated hemoglobin (Figure 1). Affy *et al.* (2019) described that ZnONPs was reduced glycated hemoglobin significantly compared to diabetic group. Alkaladi *et al.* (2014) indicated that decline in sugar level of blood and increased significantly for the level of insulin in

hyperglycemic rats which were treated with ZnONPs.

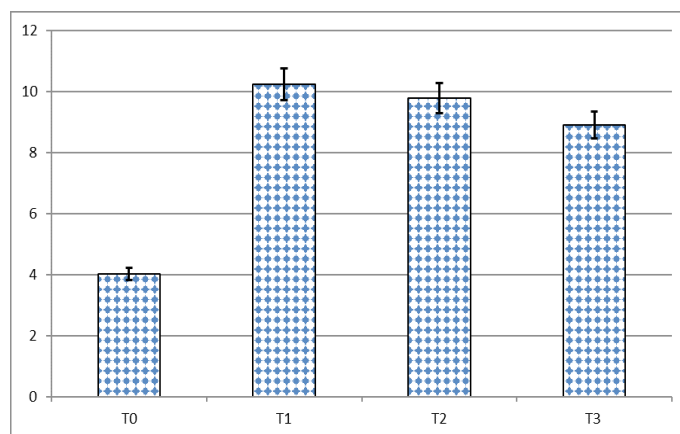


Figure 1: Effects of insulin with nanoparticle on glycosylated hemoglobin in alloxan induced hyperglycemic rat model.

Effects of insulin with nanoparticles on body weight in alloxan induced hyperglycemic rat model

The percentage of body weight gain was increased over 21 days in the group T_3 following oral administration of insulin with nanoparticles at 21 IU/kg while the insulin-treated group T_2 were decreased in the bodyweight at 7th, 14th and 21st day as it was described in (Figure 2). Lenzen (2008) found that diabetic rats that were not treated exhibit massive decrease in their body weight compared to the control rats. Kasetti *et al.* (2010) also showed that increased insulin levels in hyperglycemic rats when they are treated selenium nanoparticles hence protected weight loss. Also, this study indicated that T_1 (positive control/ control with diabetic) were showed remarkable loss in body but T_3 (nanoparticles with insulin treated group) increased body weight after treated nanoparticles with insulin. Therefore, nanoparticles might be better in the control of diabetes.

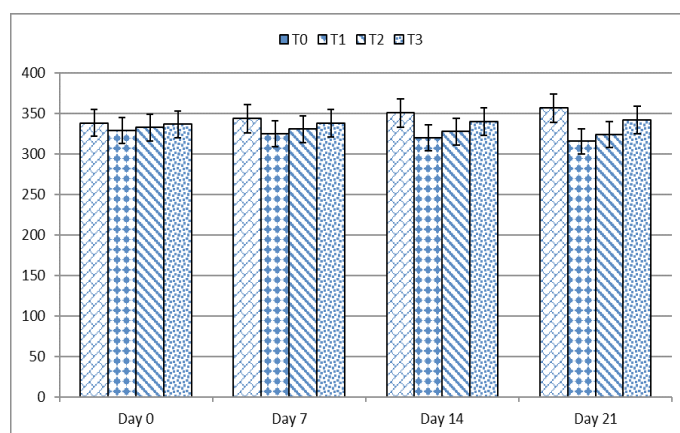


Figure 2: Effects of insulin with nanoparticle on body weight in alloxan induced hyperglycemic rat model.

Conclusions and Recommendations

The present study was concluded that insulin with nanoparticles showed better results in reducing the level of blood glucose in alloxan-induced diabetic rats after administration orally at 21IU/kg. A similar dose of insulin was shown that less effective when administered orally in alloxan-induced diabetics due to insulin degradation by proteolytic enzymes and lack of transport across the intestinal epithelium. Nanoparticles enhance the uptake and transport facility of peptide drugs such as insulin during oral administration. Therefore, it is recommended further research to be done on different types of nanoparticles with different protein hormones.

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Novelty Statement

The study showed the positive effects of insulin administration via oral route with nanoparticles. Insulin with nanoparticle significantly decrease blood glucose levels and increase body weight, which helps in maintaining healthy life.

Author's Contribution

AH performed the research and wrote original draft. RI supervise the study, provide critical comments and review the manuscript. MBR co-supervise the study and provide critical comments and review the manuscript. AIM and PM review the original draft, prepare the final manuscript and provide critical comments. All authors read the manuscript and agree to be responsible for any aspect of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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