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Recombinant oral human insulin tablets preparation

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ABSTRACT

Background: Diabetes mellitus is a chronic overwhelming disease that is treated with insulin. Subcutaneous insulin injection has several drawbacks such as nerve damage, microbial contamination, thermal instability, and pain. Exogenous insulin is essential in the management of type 1 diabetes and plays a complementary role in the management of type 2 diabetes, where oral hypoglycemic agents play a major role in treatment. Pain, injection site lipodystrophy, nerve damage, thermal instability, and microbial contamination during injection are the major side effects of insulin administered via the IV or SC route. **Aim:** Formulation of recombinant oral human insulin tablets by bioinformatics and peptidomimetics. **Methods:** In this study, insulin was produced by recombinant DNA technology using bioinformatics. Protease inhibitors and polymeric adhesives were added to enteric-coated insulin tablets for oral insulin. Test insulin was tested in animal models and compared to standard subcutaneous insulin for efficacy. Human insulin tablets were made by a wet granulation process with varying concentrations of starch, sucrose, talc, and sodium carboxymethylcellulose. DSC and FTIR spectroscopy were used for drug and polymer compatibility studies. A pre-formulation characterization of the active ingredient (API) was performed. Similarly, post-compression parameters such as wetting time, disintegration time, in vivo bioavailability, in vitro drug release, and water absorption studies of optimized formulations were evaluated. **Results:** In this study, we designed and developed insulin that can be formulated as an oral drug delivery system. The efficacy of the experimental insulin was nearly 60% in Phases 1/2 human clinical trials. **Conclusion:** New insulin tablet formulations helped overcome the drawbacks of subcutaneous injections of insulin.

Introduction

Diabetes mellitus is a metabolic irresistible syndrome globally defined by hyperglycemia and erstwhile ketoacidosis due to beta-oxidation of fatty acids to provide energy; it consequences on insulin deficiency and/or insulin resistance[1]. Approximately 10% of diabetics worldwide are due to type 1 diabetes mellitus[2]. It is characterized by an absolute deficiency of insulin caused by an autoimmune attack on the beta cells of Langerhans of the pancreas[3]. Insulin is essential for the treatment of Diabetes mellitus type 1. Type 2 diabetes has a strong genetic component. It results

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from a combination of insulin resistance and dysfunctional beta cells. Obesity is the most common cause of insulin resistance[4]. The endocrine pancreas in the adult human consists of nearly 1 million islets of Langerhans interspersed throughout the pancreatic gland[5]. Within the islets, at least four hormone-producing cells are present[6]. Their hormone products include islet amyloid polypeptide (IAPP, or amylin), which modulates appetite, gastric emptying, and glucagon and insulin secretion; glucagon, the hyperglycemic factor that mobilizes glycogen stores; insulin, the storage and anabolic hormone of the body; somatostatin, a universal inhibitor of secretory cells; and pancreatic peptide, a small protein that facilitates digestive processes by a mechanism not yet clarified[7].

•The endocrine portion of the pancreas islets of Langerhans consists of different cells that secrete different peptide hormones; Insulin from beta cells[8]; Glucagon from alpha cells[9]; Somatostatin from sigma cells which locally regulates insulin and glucagon secretion and pancreatic polypeptide[10]. Insulin is a small protein, with a molecular weight of about 6000 Daltons. It consists of two chains held together by disulfide bonds[11]. Insulin has anabolic effects, increasing glucose uptake and storage by many tissues, and increasing amino acid uptake and protein production by cells through increased ribosomal activity. Insulin regulates blood sugar levels. After eating, carbohydrates are broken down into glucose, the body's main source of energy. Glucose then enters the bloodstream. The pancreas responds by producing insulin, which allows glucose to enter the body's cells to provide energy. It also stores excess glucose for energy. After eating when insulin levels are high, excess glucose is stored in the liver in the form of glycogen.When insulin levels drop between meals, the liver releases glycogen in the form of glucose into the bloodstream. This keeps blood sugar levels within a narrow range. Insulin is important for lowering blood sugar levels in people with type 1 and type 2 diabetes. Also diabetes in pregnancy[12]. In 1921, a young surgeon named Frederick Bunting and his assistant Charles Best found a way to remove insulin from a dog's pancreas. Therapeutic insulin was developed from crude extracts of animal pancreases to recombinant human insulin and insulin analogues. The time-response profiles of insulin and formulations have been intentionally altered to better mimic the endogenous insulin response[13]. The standard model for insulin therapy is subcutaneous injection using a conventional disposable needle and syringe[14]. The disadvantages of insulin injections are: injection site lipodystrophy; nerve injury; thermal instability; microbial contamination during injection[15]. In this study, we aimed to overcome these shortcomings by developing a novel oral delivery system containing recombinant thermostable and acid-stable modified insulins developed by genetic engineering and peptidomimetics. This improved the physicochemical properties of insulin.

Materials and methods:

Materials:

Starch, sucrose, talc , sodium caroxy methylcellulose, Magnesium stearate, Magnesium silicate, talc, sucrose DC and SBBPI.

All chemical and biochemical materials were purchased from Algomhoria pharmaceutical company, Cairo, Egypt, Alnasr pharmaceutical company, Abo zabal Alkhanka, Qalyobia, Egypt and Merck Millipore and Sigma-Aldrich, Germany.

Ethical statement: In the present study, we followed All applicable national, international and/or institutional guidelines for the attention and utilization of humans and animals. All processes carried out in study including humans and animals were authorized by the local authorities, Ethical committee for human and animal handling at Cairo university(ECAHCU), at the faculty of Pharmacy, Cairo University, Egypt in agreement with the recommendations of the weather-all report with approval number P-2-2-2021. All efforts were performed to ablate the number of humans and animals utilized and their suffering during study. The type of the study: Screening experimental study. Source of animal models: They were obtained and legalized by the pharmacology and toxicology department of the faculty of pharmacy, Cairo University, Egypt.

Inclusion criteria for animal models are :

Adult male obese animals(rabbits and /or mice) (ii) Animal models can be induced by hyperglycemia. (iii) Animal blood glucose levels can be easily estimated. (iv) Obese animal models. Adult obese male rabbits weighing about 2kg, and obese male albino mice weighing between 160-190gm were utilized in the existing study. Mice were acclimatized for one week before the experiment. At a humidity(50%±5), light-dark cycle (12/12 h), and a controlled temperature (25±2 0C). Mice were provided with a commercially accessible natural diet of chow(Elnasr pharmaceutical and chemical company).

Exclusion criteria are:

Young animals. (ii) Pregnant female animals. (iii) Animal blood glucose levels can not be easily estimated. (iv) Non-obese animals.

Place and date of the study: This study was done in the faculty of pharmacy, Cairo University, Egypt between February 2020 and October 2021.

Methods:

Biosynthesis of recombinant thermostable insulin:

Synthesis of human insulin was performed by recombinant DNA technology victimizing Saccharomyces cerevisiae BJ1824 as an expression host. The C-terminus was 6x histidine, the promoter was AUG1, the inducer was methanol, and PYES2-DEST52 was the expression vector. The gene from human insulin of interest was cloned using primer for expression of the human insulin through polymerase chain reaction(PCR) process:

Primer for expression of recombinant human insulin:

Forward primer 5--3-:

ACATTGGTGCTACCAGCCTC

Tm=60.04 0C,Ta=55.04 0C

Reverse primer 5--3-:

GCGGGTATCGCTGGTATGAA

Tm=59.97 0C,Ta=54.97 0C. The PCR process was based on three straightforward steps: denaturing the insulin gene template into single strands at 94 0C; annealing the primers to each original strand at 56 0C; and last, extending the new DNA strands from the primers for insulin expression by the Taq polymerase at 72 0C. The number of copies of the insulin DNA gene was increased by repeating these three processes 27 to 30 times. When PCR was done, the quantity and size of the DNA fragments generated during PCR were checked using a process called electrophoresis; then for digestion of the plasmid it was subcloned into PYES2-DEST52 recombinant plasmids exploiting HindIII and EcoRI restriction endonuclease II, followed by ligase enzyme ligation. Recombinant plasmids were first designated and propagated in E. coli Top 10 (Invitrogen, USA) and then transformed into the expression host Saccharomyces cerevisiae BJ1824. For insulin production 2% galactose was utilized as an inducer, yeast transformants were grown at 30 °C exploiting YNBG selective medium (0.67% yeast nitrogen base without amino acids supplemented with appropriate nutrients and 2% galactose); furthermore maintained in YPG-rich medium(2% bacteriopeptone, 1% yeast extract, 2% galactose).

Clarification and purification of recombinant human insulin: A centrifuge at 4000 rpm for 3 minutes was carried out. Clarification of soluble insulin protein precursors from culture supernatants was finished by precipitation(salting out) of 100 ml of supernatant with 53 ml of 4.1 M ammonium sulfate with ammonium sulfate, followed by purification by nickel affinity chromatography. Recombinant fusion insulin proteins with polyhistidine-tagged proteins could be rapidly purified from supernatants on nickel columns employing immobilized metal affinity chromatography

(The metal ligand was a nickel metal ion, but the target biomolecule was a polyhistidine-tagged fusion protein.). Prior to final formulation, the preparation was sterilized via filtration through a 0.22 micron sterile Whatman 1541-042 filter paper (0.22 micron) obtained from USA[16].

Formulation of oral insulin tablets: Preparation of film-coated micronized insulin tablets containing bioadhesive(such as sodium carboxy methyl cellulose(1mg/gm tablet) which is an anionic polymer-forming hydrogen bond with mucin and is characterized by high mucoadhesive and low toxicity) and protease inhibitors such as aprotinin or Soybean Bowman-Birk protease inhibitor(SBBPI)(0.5 mg/g tablet) which inhibits both trypsin and chymotrypsin. Tablets of micro-particles of insulin 10 mg/g tablet were prepared by the wet granulation method. Magnesium aluminum silicate 3mg/g tablet was added as an excipient. It was a binder, glidant, and disintegrant. Starch 15.5mg/g tablet was added as diluent. Magnesium stearate 1mg/g tablet was added as a lubricant agent. All ingredients were passed through 80# mesh sieve. All ingredients were passed through 80# mesh sieve. 80 mesh is a medium size U.S. Mesh size was a0.0075(185μm) with a nominal sieve opening with a typical wire diameter of 0.120mm.the die size ranged from 7-9mm. The film-coated tablets were prepared via the aqueous film coating method (film coating is a single process that involves the deposition of a thin film polymer such as 100-micrometer hydroxypropyl methylcellulose phthalate via spraying coating solution onto the tablet beds in a pan coater followed by immediate drying to form thin, film and enteric coat on the micronized tablets in presence of plasticizer such as polyethylene glycol(200-6000)). The evaluation of biological activity of oral insulin drug delivery systems was done through rabbit animal models induced by hyperglycemia and mouse convulsion method using 2 and 2 dose assay.

Evaluation tests of oral insulin tablets: These tests were carried out as per British pharmacopeal specifications.

Compatibility check: FT-IR spectroscopy (Perkin-Elmer 1600 FTIR spectrophotometer) and DSC (Shimadzu-DSC 50) were used to characterize and determine the compatibility of recombinant insulin and various excipients used in the manufacture of oral tablet formulations. The optimized formulation was mixed with 200 mg KBr and compressed into discs scanned at 5 mm/s with a resolution of 1 cm −1 over the range 4000–200 cm −1 . Thermal analysis experiments were performed using various scanning calorimeters (DSC). Samples of optimized formulations were heated in sealed aluminum pans over a temperature range of 0–4000 °C at a constant rate of 110 °C/min under a nitrogen purge (35 mL/min).

Hardness: A diameter compression test was performed according to British Pharmacopoeia Technique 2.9.8 using a Monsanto hardness tester (USA). According to standard literature, a hardness of 2 kg/cm2 was acceptable for oral insulin tablets. The pressure required to break a diametrically arranged matrix tablet with a coil spring was measured on 20 tablets.

Friability:Thoroughly washed and accurately weighed, a 6.5g sample of the whole tablets was placed in a Roche friability tester drum. After 100 revolutions of the drum, the tablets were accurately weighed, shaken off the powder, and ejected. The upper acceptable limit for weight loss was 1%. Twenty tablets were weighed and placed in the Roche Friabilator testing apparatus. Upon dropping into the device, the tablet rolled and was repeatedly impacted. After 100 processes, the tablet was dedusted. The degree of friability was determined by the percent weight loss of the tablets.

Wetting time:To perform the wetting time test, two layers of rectangular absorbent paper (10 cm x 7.5 cm) were placed in a Petri dish and thoroughly wetted with distilled water. The tablet was then placed in the center of the plastic tray and a stopwatch was used to record the time it took for the water to diffuse through the absorbent paper.

Determination of water absorption ratio: A tissue paper folded in two was placed in a petri dish (inner diameter: 6 cm) containing 7 ml of purified water. The tablets were then placed on tissue paper and thoroughly wetted. The wet tablets were separated and reweighed.

Disintegration test: The examination was conducted in accordance with British Pharmacopoeia 2019 requirements. Using distilled water kept at 37 C, one tablet was put in each of the six tubes. We then watched to see if the tablet would dissolve. At the expiration of the time restriction, the basket containing the fluid was raised, and the pills' total breakdown was witnessed.

Weight variation: From each batch 20 tablets were chosen randomly and their average weights were calculated utilizing digital weighing balance(Essae Teraoka ltd);then percentage weight difference was estimated and checked with British pharmacopoeia 2019 specifications.

Determination of uniformity of drug content: Twenty tablets of each formulation were weighed and powdered. 10 mg of this powder was weighed and dissolved in 100 ml of distilled water. After the mixture was sonicated for 170 seconds and filtered through Whatman #40 filter paper, the filtrate was diluted with distilled water and subjected to UV light irradiation which induced disulfide bond photolysis and insulin-dityrosine covalent dimerization. The absorbance was assessed at 275 nm wavelength[17].

In vitro drug release profile: Distilled water was used as the dissolution medium(300 ml) at 37 0C, PH 7.4 and 50 rpm(paddle) in presence of phosphate buffer 6.8. samples were collected at 3, 6, 8, 11,16,19, 60, 120, 240 minutes intervals according to European pharmacopoeia specifications 2020 and the withdrawn volumes were replaced by equivalent amounts of the plain dissolution medium. The amount of insulin released was measured using UV spectrophotometer at 275 nm wavelength.

Stability study: It was carried out for optimized formulation. The storage conditions utilized for stability studies were accelerated conditions 40 0C and room temperature 30 0C. Optimized formulation tablets were kept, striped and packed in humidity chamber for thirty days on above mention temperature. The parameters that were measured before and after the storage for one month comprised hardness, the percentage friability, disintegration time and drug content.

Screening and bio-assay of insulin: Rabbit blood sugar method for screening and bio-assay:

Principle: Insulin lowers blood glucose levels in rabbits, and the reduction in blood glucose levels is directly proportional to dose. Our study used 100 rabbits. Rabbits weighed approximately 2 kg.

procedure: Rabbits weighing 2 kg were used. A preliminary experiment was performed by injecting graded doses of standard insulin (0.1-0.5 IU/kg)

subcutaneously (s.c.) into each rabbit in the positive control group. On the other hand, oral test insulin tablets containing graded doses of test insulin (0.1-0.5 IU/kg) were administered to the test group via the oral route after 18 hours of fasting. Rabbits that had convulsions within 5 hours were excluded. The rabbits were then randomly divided into four groups, fasted for at least 18 hours, and blood samples were taken from the ear vein to measure initial blood glucose levels (BGL). Each group was then injected with doses of insulin according to double and double dose assays, blood samples were taken hourly for 5 hours, the samples from each rabbit were pooled, and BGL was ascertained. A drop in blood sugar was recorded. A crossover test was performed the next day. The average reduction in her BGL for each dose selected was calculated to determine relative efficacy[18].

Mouse convulsion method for bio-assay only using 2 and 2 dose assay technique: Principle: insulin decreases the blood glucose level in mice.When it reaches a critical level the hypoglycemic convulsion occurs. The percentage of mice showing convulsions is directly proportional to the dose.

Procedure: 2 and 2 dose assay techniques were carried out. 100 Mice weighing 45-50 gm were fasted for 12-24 hours and kept at a constant temperature of 29-35 C. The standard insulin was injected with graded doses of insulin (0.1-0.5 IU /kg) intraperitoneally(IP); while the test insulin tablets containing graded doses of insulin(0.1-0.5 IU /kg) were given via oral route of administration and the animals were observed for 1.5 hours. The percentage of animals that died, showed convulsions, or remained on their back for 2-3 seconds when they were turned on their back in each group was determined and the relative potency was calculated. Cross-over tests could not be carried out because the animals might die[19].

Human evaluation of oral insulin tablets via human clinical trials phases 1/2: 3 groups of adult diabetic type1 patients with hyperglycemia greater than 200 mg/dl were included in our study. Each group consisted of 100 subjects:

Group(1)(negative control group) was administrated graded amounts of the placebo by oral route.Group(2)(positive control group) were administrated graded amounts of the standard insulin(0.2-0.5U/kg) intravenously and subcutaneously.

Group(3)(test group) were administrated graded amounts of the test recombinant oral insulin micronized tablets(0.2-0.3U/kg of insulin injection were equivalent to 50 mg of human insulin tablets).

The activity of insulin was estimated by the reduction in blood glucose level during fasting.

In vivo bio-availability study: Before dosing oral tablets 0.7-0.9ml of samples were withdrawn ,and immediately after dosing at 30,60,120,240 minutes.Blood samples were further refrigerated and centrifuged at 4 C within one hour of sampling.Insulin concentrations were determined using HPLC.HPLC analysis was through a reversed phase column utilizing phosphate buffer(PH 4.4) and acetonitrile(660/340, v/v) as mobile phase with a flow rate 0.9ml/min. The limit of UV estimation of insulin concentration in blood was at 275 NM. Area under the curve(AUC) and the % of relative bio-availability were measured. % of relative bio-availability was determined by the following equation: % Relative bio-availability=(AUC Oral/AUC Intravenous)×(Dose Intravenous/Dose Oral)×100%. The same procedures were performed for the control and the standard groups(groups 1 and 2).

Statistical analysis

All cultures were conducted in triplets. Their presentation was by means and standard deviation. One-way analysis of variance (p value≤.05) was used as means for performing statistical analysis and also, statistical analysis based on excel-spreadsheet-software.

Figure 1. It represents the hypoglycemic effect of recombinant thermostable human insulin via rabbit blood glucose assay.

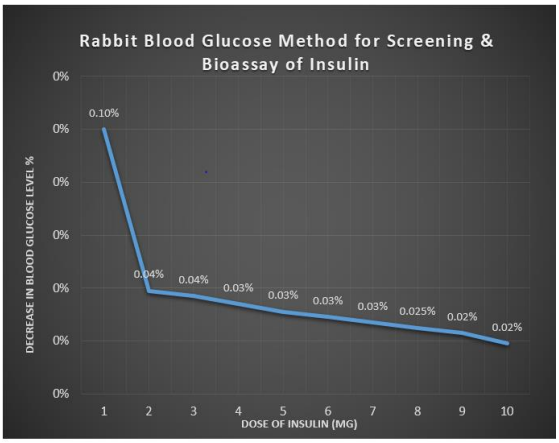


Figure 2. It represents the hypoglycemic effect of recombinant thermostable human insulin via mouse convulsion bio-assay.

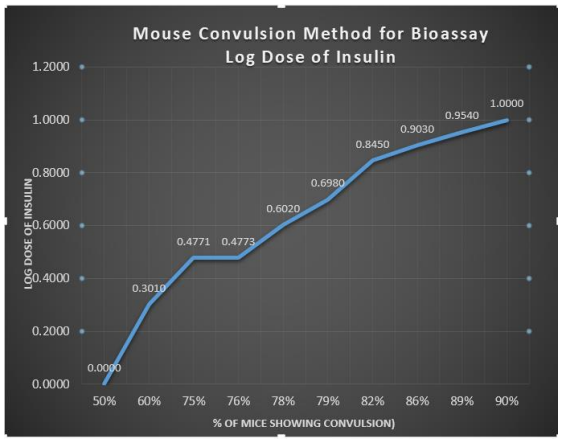


Figure 3. It represents the hypoglycemic effect of standard gliclazide oral hypoglycemic drug via mouse convulsion bio-assay.

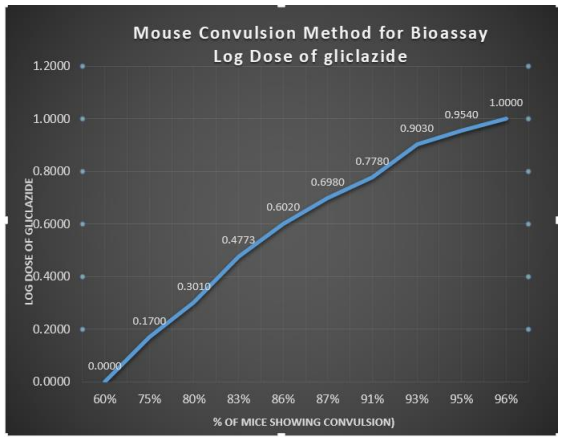


Figure 4. It represents the hypoglycemic effect of oral insulin tablets during human clinical trials phases1/2.

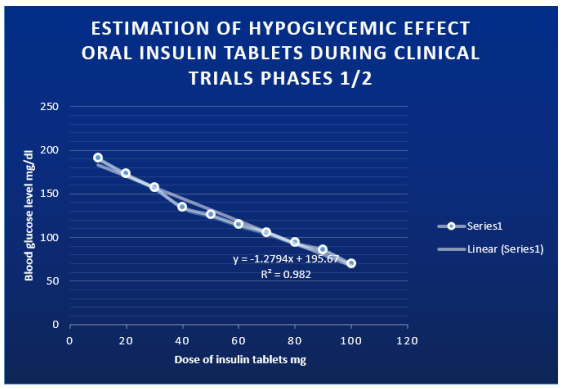


Figure 5. It represents the hypoglycemic effect of standard subcutaneous rapid acting insulin drug delivery system during fasting in human clinical trials phases1/2.

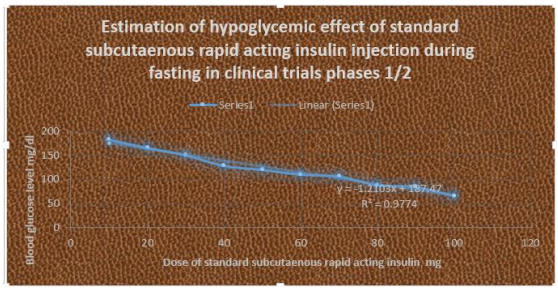


Figure 6. It shows the estimation of area under the curve(AUC) of insulin tablets given during fasting.

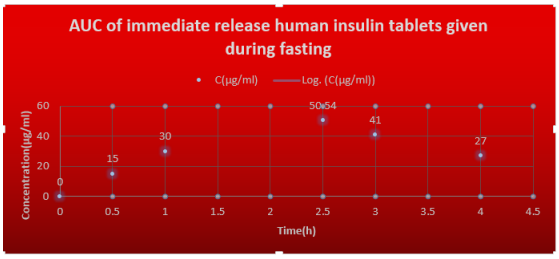


Figure 7. It represents sustained insulin release profile.

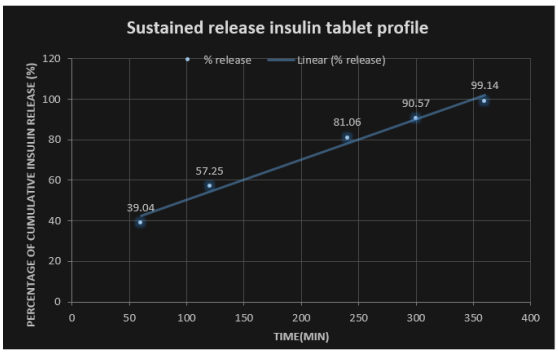


Figure 8. It represents immediate insulin release profile.

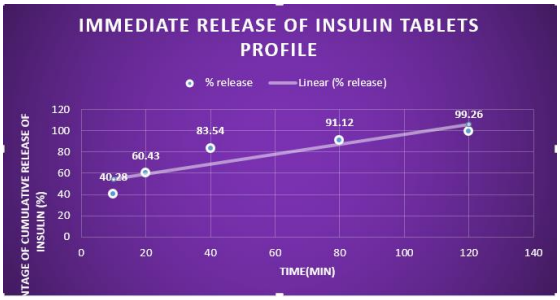


Figure 9. FTIR spectroscopy shows no interaction between recombinant human insulin and excipients.

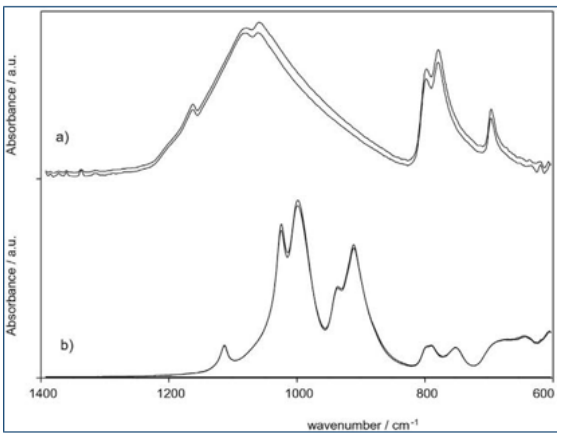
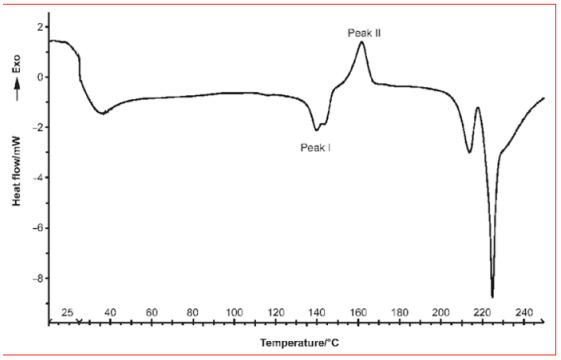


Figure 10. DSC thermal analysis shows no possibility of interaction between recombinant human insulin and excipients.



Results

In the present study, different batches of recombinant human insulin tablets were prepared utilizing various ingredients as starch, sucrose DC, talc, SBBPI etc(Table 2).

Table 2. Batch formulation of Oral tablets of insulin F1-F5 by wet granulation technique:

Ingredients(mg/tablet)	F1	F2	F3	F4	F5
Recombinant insulin	10	10	10	10	10
Starch	15	17	18	16	14
SCMC	1	0.5	1.5	0.5	1.5
SBBPI	1	0.5	1.5	1.5	0.5
Sucrose DC	11	12	12	13	10
Talc	2	3	1	3	3
Mg stearate	3	1	2	2	5
Mg silicate	4	3	1	1	3
Total weight(mg)	47	47	47	47	47

No possible interactions between recombinant human insulin and excipients were indicated by FT-IR and DSC studies. Tablet hardness measurements were performed and observed to be between 1.73 and 1.97 kg/cm2. Weight variation for all formulations was assumed to be within standard British Pharmacopoeia limits. Vulnerability rates ranged from 0.61% to 0.82% and were borderline. Water absorption rates for all formulations were observed between 37.24 and 39.63. Wetting times for all formulations were estimated to be 18-23 seconds. An in vitro disintegration time evaluation of oral tablets was performed. In vitro disintegration times were found to range from 8-10 minutes for formulations F1-F5. A rapid disintegration time of 8 minutes was observed for formulation F2. This is due to the burst effect and rapid absorption of water from the medium. Drug content of recombinant insulin of 98.93% to 99.77% was observed for all formulations, which was in the unexceptionable extent. The release times of the immediate-release insulin tablets ranged from 97.81% to 99.26% after 2 hours at 37°C and 50 rpm, while the controlled-release tablets ranged from 98.35% to 99.14% after 6 hours at 37°C and 50 rpm. Batch F4 showed faster drug release than all other batches. A cumulative drug release of 98.35% at 240 minutes was demonstrated by batch F4 at 37°C and 50 rpm. Batch F2 t50% was observed at 180 minutes. Due to its fast disintegration time and dissolution profile, batch F4 was suitable as an optimized formulation. Batch F4 was formulated with 12 mg sucrose DC and 17 mg starch. The optimal storage temperature for oral insulin tablets (batch F1-F5) has been found to be 2-8°C. An in vivo study was performed by ingesting formulation F2 and the results were compared to intravenous insulin injection. Blood samples were taken at different time intervals and analyzed for drug content using HPLC. The Tmax and Cmax of recombinant human insulin were determined to be 2.5 hours and 50.54 micrograms/mL. The relative bioavailability percentage was estimated by Equation 1 and set at 70%. Bioavailability was enhanced by oral insulin tablets. This could not be denied by the results of in vivo studies. Subcutaneous injections of fast-acting insulin had a Tmax of 1 hour and a Cmax of approximately 480 micrograms/mL at an average dose of 0.2-0.3 U/kg. SC insulin had an onset of action of 10 minutes, a duration of action of approximately 5 hours, and a

bioavailability of approximately 90%. Bioavailability of IV insulin injection was 100%. In the current study, a single oral dose of 40 mg gliclazide, a standard oral hypoglycemic agent, had a biological half-life of approximately 8 hours, a duration of action of 10–24 hours, a CMAX of 2.3 micrograms/mL, and a TMAX of 2 hours. In Phase 1/2 clinical trials in humans, oral insulin tablets had a bioavailability of approximately 70% and an efficacy approaching 60%. The pharmacokinetic profile of micronized insulin tablets during clinical studies showed a rapid onset of action and a biological half-life of 3 hours. The duration of action was approximately 6 hours. This mimicked the physiology of endogenous insulin secreted by Langerhans beta cells of the pancreas. Liver and kidney accounted for the majority of insulin breakdown. Nearly 60% of the insulin released into the portal vein was cleared in the liver via the hepatic insulin protease present in hepatocytes and lysosomes, and about 40% in the kidney. There was no risk of weight gain, hypoglycemia, or hyperinsulinemia. Exogenous injection of standard insulin altered the catabolic profile because insulin was no longer delivered directly to the portal vein. The liver played a minor role (approximately 35%) in insulin breakdown, while the kidney was almost 65% eliminated. Renal injury decreased insulin clearance and prolonged its action. This clearance decrease has been observed with both endogenous oral and exogenous insulin administration. Clinically, declining renal function resulted in a progressive decrease in exogenous and endogenous oral insulin requirements and an increased risk of hypoglycemia.

Rabbit Blood Glucose Method for Screening & Bioassay of Insulin:

Table 3. represents the results of the Rabbit Blood Glucose assay:

Dose of Insulin (mg)	Decrease in Blood Glucose Level %
1	0.10%
2	0.04%
3	0.04%
4	0.03%
5	0.03%
6	0.03%
7	0.03%
8	0.025%
9	0.02%
10	0.02%

Mouse Convulsion Method for Bioassay (Log Dose of Insulin):

Table 4. represents the results of mouse convulsion methods(insulin):

% of Mice Showing Convulsion	Log Dose of Insulin
50%	0.0000
60%	0.3010
75%	0.4771
76%	0.4773
78%	0.6020
79%	0.6980
82%	0.8450
86%	0.9030
89%	0.9540
90%	1.0000

Mouse Convulsion Method for Bioassay (Log Dose of Gliclazide):

Table 5. represents the results of mouse convulsion methods(gliclazide):

% of Mice Showing Convulsion	Log Dose of Gliclazide
60%	0.0000
75%	0.1700
80%	0.3010
83%	0.4773
86%	0.6020
87%	0.6980
91%	0.7780
93%	0.9030
95%	0.9540
96%	1.0000

Estimation of hypoglycemic effect of oral insulin tablets during clinical trials phases 1/2:

Table 6. represents the results of the hypoglycemic effect of oral insulin tablets during human clinical trials phases1/2.

Test insulin dose(mg)	Blood glucose level(mg/dl)
10	191
20	173
30	157
40	135
50	126
60	115
70	106
80	94
90	86
100	70

Table 7. It represents the results of the hypoglycemic effect of standard subcutaneous rapid acting insulin injection during fasting in human clinical trials phases 1/2:

S.C insulin dose(mg)	Blood glucose level(mg/dl)
10	183
20	166
30	151
40	129
50	121
60	111
70	107
80	89
90	85
100	67

Table 8. It shows the estimation of area under the curve(AUC) of immediate release insulin tablets given during fasting:

Time(h)	C(µg/ml)
0	0
0.5	15
1	30
2.5	50.54
3	41
4	27

Table 9. It represents sustained release insulin tablets profile:

Time(min)	% release
60	39.04
120	57.25
240	81.06
300	90.57
360	99.14

Table 10. It represents immediate release insulin tablets profile:

Time(min)	% release
10	40.28
20	60.43
40	83.54
80	91.12
120	99.26

Formulation parameters:

Table 11. Batch formulation F1-F5 hardness,thickness,percentage Fri-ability,diameter and weight variation:

Batch	Hardness(kg/cm2)	%Fri-ability	Diameter(mm)	Thickness(mm)	weight variation(mg)
F1	1.97±0.35	0.61±0.02	6.02±0.01	3.3±0.02	47.08±1.9
F2	1.73±0.35	0.67±0.04	6.07±0.02	3.5±0.01	47.64±1.5
F3	1.75±0.36	0.63±0.01	6.04±0.01	3.4±0.04	46.81±1.7

F 4	1.83±0. 39	0.82 ±0.0 3	6.05± 0.06	3.5±0. 03	47.25 ±1.2
F 5	1.88±0. 46	0.75 ±0.0 1	6.06± 0.03	3.6±0. 07	48.38 ±1.4

Table 12. Drug content uniformity, wetting time, water absorption ratio, disintegration time of batch formulation F1-F5: ratio, disintegration time of batch formulation F1-F5:

Batch	Drug content uniformity	Wetting time(sec)	Water absorption ratio	Disintegration time(min)
F1	99.77 ±2.24	21 ±2.80	38.51 ±1.78	9 ±1.68
F2	98.78 ±1.36	22±1. 91	38.14 ±2.19	8 ±2.71
F3	98.34 ±0.99	18 ±2.99	39.63 ±2.82	9 ±2.10
F4	98.93 ±1.78	23 ±1.87	37.24 ±1.42	10 ±2.08
F5	99.61 ±2.07	19 ±2.04	39.29 ±1.60	10 ±2.54

Table 13. Comparison of different parameters for stability study of batch F2 between its initial production and after the storage for one month:

Evaluation parameter	Initial	After one month
Drug content	98.78± 1.36	98.57 ±1.32
Hardness	1.73 ±0.35	1.89 ±0.25
Disintegration time(min)	8.00±2.71	9.09 ±2.15
Percentage friability	0.67 ±0.04	0.73 ±0.08

Discussion

Screening and bioassay of micronized film-coated insulin tablets containing graded doses of insulin from 1 to 10 mg physically stabilized with protease inhibitors and bioadhesive excipients showed that the lowest effective dose after use reduced normal blood glucose levels in rabbits by 0.1%. Up to 0.039 was 2.5 mg insulin. For the bioassay of only film-coated micronized tablets containing graded doses of insulin, we applied the mouse convulsive method to mice fasted for 24 h using double and double dose assays. We found that 75% of the mice had convulsions due to the hypoglycemic effect of insulin with a dose of 2.8 mg insulin compared to 1.5 mg gliclazide as a standard hypoglycemic agent. In both experiments, hypoglycemia was noted after 2-3 hours and the duration of insulin action was approximately 6 hours. Film-coated micronized insulin tablets using genetic engineering and peptidomimetics could overcome a lot of the disadvantages of insulin injection.

In a comparison with a previous study(Ahmed Gedawy et al, 2017) conducted in Australia, our study showed that the efficacy of oral insulin delivery systems was nearly 60% and bio-availability was approximately 70% during clinical trials phases1/2, while the previous study showed that efficacy of oral insulin delivery systems did not exceed 60% and bio-availability was less than 70% due to difficulties in the absorption of different oral insulin delivery systems. The insulin oral routes of administration showed reduced levels of systemic insulin, therefore less weight gain and hypoglycemic risks than exogenous insulin administrated subcutaneously. As well, it was devoid of pain, risk of infection at the injection site, and lipodystrophy. Oral tablets of insulin manufactured by recombinant DNA technology were successfully prepared to improve its bioavailability,to avoid hepatic first pass metabolism and pre-systemic metabolism in the gastrointestinal tract. There was no possible interactions between the drug and polymers according to FTIR spectroscopy and DSC study. In this work, different batches of recombinant human insulin oral tablets were prepared by wet granulation technique using different ingredients such as starch, sucrose DC. No potential interactions between excipients and insulin were revealed by FT-IR and DSC studies. Starch acts as a disintegrant and diluent. Sucrose DC events as a sweetener. Many excipients exhibited water solubility, which improved patient acceptance. Our research was successful in identifying effective drugs with reduced costs, manufacturing difficulties, and improved patient compliance. There was a positive correlation between disintegration time and wetting time. Lot F4 showed less degradation than all other formulations. Batch F4 was recommended as the optimized recipe. The grind ability and hardness of

Charge F4 were too good. In vivo and stability studies were performed using lot F4. After a month, there was no change, as shown by the stability study. Batch F4 showed a good uniformity in drug content, dissolution profile and disintegration time, promoting good in vivo absorption profile and stability. Insulin bioavailability was enhanced by an oral tablet formulation as demonstrated in in vivo studies. In comparison with the standard hypoglycemic drug gliclazide, insulin tablets showed a shorter biological half-life and duration of action than gliclazide, whereas the insulin drug delivery system was less prone to hypoglycemia and weight gain. Another comparison with glimeprid (a second-generation sulfonylurea for oral hypoglycemia) showed that insulin tablets were less prone to hypoglycemia and weight gain than glimeprid. However, glimeprid showed a longer duration of action (12–24 hours) and a longer half-life (5 hours) than insulin drug delivery systems[20].

Conclusion

Our study was a promising approach to solve many of the side effects of subcutaneous insulin injection routes such as pain, nerve damage, microbial contamination, and lipodystrophy at the injection site.

Conflict of interest

None to be declared.

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References

1. **Parveen Kumar.** Kumar, Clark's clinical medicine; 2020.p.774-811.
2. **Caroline S, Zeind, Michael G.** Applied therapeutics, the clinical use of drugs; 2018; 16:1002-1031.
3. **Anthony T, Bertram K, Marieke K. Trevor K.** Pharmacology examination board review; 2022.p.1142-1257.
4. **Stan B, Jason W, Douglas M.** Applied pharmacology; 2021; 9:276-328.
5. **James O.** Clinical pharmacology made ridiculously simple; 2020; 12:93-109.
6. **Warren L.** Review of medical microbiology and immunology; 2021; 9:211-244.
7. **Swanson Larry N, Souney Paul F, Muntnick Alan H, Shargel Leon.** Comprehensive Pharmacy Review for NAPLEX; 2019.p.678-707.
8. **Fisher Bruce, Champe Pamela, Harvey Richard.** Lippincott illustrated reviews microbiology; 2021; 45:1475-1533.
9. **Dipro Cecily, Schwinghammer Terry, Dipro Joseph, Well Barbara.** pharmacotherapy handbook; 2021; 5:99-114.
10. **Golderg Stephen.** Clinical physiology is made ridiculously simple; 2020; 11:404-4436.
11. **Wilson Golder N.** Biochemistry and genetics; 2019; 23:598-649.
12. **Metting Patricia**. J.Physiology; 2019; 14:437-505.
13. **Ahmed Gedawy, Jorge Martinez, Hani Al-Salami, Crispin R Dass.** Oral insulin delivery: existing barriers and current counter-strategies. J pharmacy pharmacol 2017; 70 (2): 197-213.
14. **NS al-Walili**. Sublingual human insulin for hyperglycemia in type I diabetes.Journal of J Pak Med Assoc 2018; 49 (7): 167.
15. **WC Duckworth et al.** Degradation products of insulin generated by hepatocytes and by insulin protease. J Biol Chem 2017; 4 (2):33.
16. **Nengah et al.** Construction of pY-AF vector for expression of thermostable ∝ -L-Arabinofuranosidase in *Saccharomyces cerevisiae.* J Res Gate 2010; 31 (3): 246-267.

17. **Manuel correia et al.** UV-light exposure of insulin: pharmaceutical implications up on covalent insulin dityrosine dimerization and disulfide bond photolysis. Plos One 2012; 7 (12): e50733.
18. **Sabrina Ruggeberg et al.** The replacement of the rabbit blood sugar bioidentitiy assay by an in vitro test for batch release of insulin glargine drug substance. J Rudiger Hack 2016; 3 (17): 413-427.
19. **K Anuradha et al.** Investigation of central mechanism of insulin induced hypoglycemic convulsions in mice. Journal of India J Exper Biol 2004; 42(4):72-368.
20. **Ananya Sarkar et al**. Pharmaceutical and pharmacological profile of glimepride. J App Pharm sci 2011; 01 (09): 2011: 11-19.

Mohammed Mahmoud Shawky Kassab, Recombinant oral human insulin tablets preparation. IJHS (Egypt) 2023; 1 (1): 18-29.