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Development of Sublingual Recombinant Human Insulin Drug Delivery System by Bioinformatics

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ABSTRACT

Background: Diabetes mellitus is a heterogeneous group of syndromes characterized by elevation of fasting blood glucose that is caused by a relative or absolute deficiency in insulin. Diabetes is the leading cause of amputation and adult blindness and a major cause of nerve damage, renal failure, heart attacks, and stroke. Exogenous insulin is essential for the management of Diabetes mellitus type 1 and has an adjunct role in the management of type 2 diabetes mellitus in which oral hypoglycemic medicines display the leading management role. Pain, Lipodystrophy at the injection site, Nerve damage, Thermolabile, and microbial contamination during injection are the principal adverse effects of insulin administered via IV or SC routes. **Objective:** Production and screening of sublingual recombinant human insulin drug delivery system. **Methods:** Type of study: Screening experimental study. Micronization process like Jet or bead milling was utilized to make micronized particles of recombinant human insulin less than 50 microns which resulted in more drug solubility and better absorption through biological and physiological membranes of the human body. This resulted in better bioavailability of the drug. In our study, micronized Insulin sublingual tablets were designed which were able to avoid the hepatic first-pass effect and gastrointestinal degradation. The sublingual tablets were prepared by direct compression technique utilizing different concentrations of starch 1500 and microcrystalline cellulose. DSC and FTIR spectroscopy were utilized in the drug and the polymer compatibility studies. Evaluation of preformulation properties of active principal ingredient (API) was performed. As well postcompressional parameters such as wetting time, disintegration time, in vivo bio-availability, in vitro drug release, and water absorption ratio study of the optimized formulation were assessed. **Results:** The disintegration time of the optimized formulation was up to 45 seconds. The in vitro release of insulin from optimized sublingual tablets was found to be up to 15 minutes. The percentage relative bioavailability of insulin from optimized sublingual tablets was 81%. The pre-compression parameters were within an acceptable range of pharmacopeia specifications. No possible interactions were noticed between the drug and the polymer via FTIR spectroscopy and DSC study. The sublingual tablet of insulin was tested on animal models, evaluated in human clinical trials phases 1/2, and compared with standard regular soluble insulin injection formulations for efficacy. The sublingual formulation of insulin showed high bioavailability and efficacy. **Conclusion:** The sublingual tablets of recombinant human insulin helped to overcome the disadvantages of subcutaneous injections of insulin.

***Keywords**: Insulin; Diabetes mellitus; Sublingual tablets; Hyperglycemia.*

INTRODUCTION

Diabetes is a prolonged status originating from an absolute or a relative deficiency of insulin.[1] Its characteristic clinical grounds are evidence of glucose intolerance consequent on hyperglycemia and changes in protein and lipid metabolism. Over the long term, these metabolic abnormal conditions bring about the development of complications such as neuropathy, nephropathy, and retinopathy.[2]type 1 diabetics establish about 10% of diabetics worldwide.[3] The disease is defined by an absolute deficiency of insulin crusaded by an autoimmune attack on Langerhans beta cells of the pancreas.[4] The metabolic abnormal conditions of type 1 diabetes mellitus permit hyperglycemia, diabetic acidosis, and hypertriacylglycerolemia.[5] Exogenous insulin is basal for the management of Diabetes mellitus type 1.[6] Type 2 diabetes has a strong inherited factor. It outcomes from a combination of dysfunctional beta cells and insulin resistance. The most common cause of insulin resistance is Obesity.[7]**pancreatic hormones:**
The internal secretion part of the pancreatic Langerhans islets consists of assorted cells that secrete antithetic peptide hormones: Insulin from beta cells, Glucagon from alpha cells, pancreatic polypeptide, and Somatostatin from sigma cells which locally governs glucagon insulin release.[8]**Insulin:** Insulin is a small protein Its molecular weight is approximately 6000 Daltons.[9] It is composed of two chains held together by sulfide chemical bonds.[10] **Pharmacological actions of insulin:** Insulin has constructive-metabolic actions.[11]It enhances glucose intake and retention by numerous tissues.[12] As well as protein manufacture by exploding uptake of amino acids by cells[13], and by flaring ribosomal action.[14] **Insulin Delivery Systems:** The standard mode of insulin therapy is subcutaneous injection utilizing formulaic disposable needles and syringes. **Disadvantages of insulin injection:** Pain, lipodystrophy at the injection site, Nerve damage, Thermolabile and microbial contamination during injection.[15]Our study aimed to overcome these drawbacks by using new sublingual recombinant human insulin drug delivery systems that improved the physicochemical characteristics of insulin.

MATERIAL AND METHODS

Materials

Starch 1500, microcrystalline cellulose, talc, sucralose, sucrose DC, PVP, aerosil, pearlite, and talc. All chemical and biochemical materials were purchased from Algomhoria pharmaceutical company (Cairo, Egypt) and Alnasr pharmaceutical company (Abo zabal Alkhanka, Qalyobia, Egypt).

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:

(i) Adult obese animals (rabbits and /or mice).
(ii) Animals can be induced by hyperglycemia.
(iii) Blood glucose levels can be easily estimated.

Exclusion criteria are: Young thin animal.(ii) Pregnant female animal(iii) Animal blood glucose level which can not be easily estimated.

Adult obese male rabbits weighing about 2kg, and obese male albino mice 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 ^{0}C). Mice were provided with a commercially accessible natural diet of chow (Elnasr pharmaceutical and chemical company).

Type of the study: Screening experimental study

Place and the date of the study: Our study was carried out in the Faculty of Pharmacy, Cairo University between February 2021 and March 2022.

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 the study including humans and animals were authorized by the local authorities, the 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 Weatherall report with approval number P-17-5-2021. All efforts were performed to ablate the number of humans and animals utilized and their suffering during the study.

Methods

Primer for expression of physically stable insulin which was modified using genetic engineering and bioinformatics:
Forward primer:
ACATTGGTGCTACCAGCCTC
Tm=60.04 ^{0}C, Ta=55.04 ^{0}C
Reverse primer:
GCGGGTATCGCTGGTATGAA
Tm=59.97 ^{0}C, Ta=54.97 ^{0}C

Biosynthesis of recombinant human insulin

Design of a new primer for the expression of insulin using genetic engineering and bioinformatics. The synthesis of insulin by recombinant DNA

technology using *Saccharomyces cerevisiae BJ1824* as an expression host. The C-terminal was 6x histidine, the promoter AUG1, the inducer was methanol and PYES2-DEST52 was the expression system vector. Genes of thermostable insulin of interest were cloned using PCR and then sub-cloned into PYES2-DEST52 using Hind III and EcoRI restriction endonucleases II for the digestion of the plasmid, followed by ligation by ligase enzyme. The recombinant plasmid was designated and propagated first in *Escherichia coli* Top 10(Invitrogen, USA), then transformed into *Saccharomyces cerevisiae BJ1824.* For insulin production using galactose as an inducer, YNBG selective medium (0.67% yeast nitrogen base without amino acids supplemented with appropriate nutrients and 2% galactose) was used for the growth of yeast transformants at 30 ^{0}C, followed by maintenance in YPG-rich media (2% bacteriopeptone,1% yeast extract and 2% galactose).

Clarification and purification of recombinant human insulin

Centrifugation was performed for 3 minutes at 4000 rpm followed by clarification of soluble insulin protein precursor from the supernatant of the culture by the precipitation by ammonium sulfate, then purification by Nickel affinity chromatography. Recombinant fused insulin proteins with polyhistidine could be quickly purified from the supernatant via Nickel columns utilizing immobilized metal affinity chromatography(the metal-ligand was a nickel-metal ion; while the target bio-molecule was a polyhistidine tag fusion protein) on Nickle affinity resins after extraction of them by precipitation(salting out) of 100 ml of the supernatant with 53 ml of a 4.1 M ammonium sulfate saturated solution at 25 C following centrifugation at 4000 rpm for 3 minutes. Before the final formulation was yielded the preparations were sterilized by filtration through 0.22-micrometer sterile-grade filters (Whatman-1541-042 filter paper (0.22 micron) purchased from the USA).[16]

Method of the preparation of human insulin sublingual tablets

Table 2 showed the composition of different batches of sublingual tablets prepared by direct compression technique. All ingredients (Insulin, pearlitol, SD200, starch1500, microcrystalline cellulose, sucrose DC, aerosil, sucralose, talc and polyvinyl pyrrolidone polymer (PVP)) were passed through an 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.

Evaluation tests of sublingual insulin tablets:

These tests were carried out as per British pharmacopeia 2019 specifications.

Compatibility study

We characterized recombinant insulin and different excipients utilized in the preparation of sublingual tablet formulations by FT-IR(Perkin-Elmer 1600 FTIR spectrophotometer) spectroscopy and DSC(Shimadzu-DSC 50) to see the compatibility. The optimized formulation was blended with 200 mg KBr; then compressed into discs which were scanned at 5mm/sec with a resolution of 1 cm^{-1} at a range of 4000-200 cm^{-1}. Experiments of thermal analysis were carried out utilizing various scanning calorimeters (DSC). We heated the samples of the optimized formulation in hermetically sealed Aluminium pans at a temperature range of 0-4000 ^{0}C at a constant rate of 110 ^{0}C/minute under a purge of nitrogen (35 ml/min).

Hardness

We performed a diametric compression test according to British pharmacopeial technique 2.9.8 utilizing Monsanto hardness tester (USA). A hardness of 2kg/cm^2 was acceptable in the case of oral or sublingual insulin tablets according to standard literature. For 20 tablets we measured the pressure required to break a diametrically placed matrix tablet, by a coiled spring.

Friability

We dedusted, accurately weighed, and placed a random sample (20 tablets) of the whole tablets corresponding to 6.5 g in the drum of a Roche friability tester. we rotated the drum 100 times and tablets were accurately weighed, dedusted, and removed. 1% was considered acceptable as a maximum weight loss. In the Roche friability test apparatus, 20 tablets were weighed and put in. The tablets were uncovered to the recurrent shocks and rolling consequent of the falls inside the apparatus. The tablets were dedusted after 100 processes. The percentage loss in the weight of the tablets was the determining factor of the friability.

Wetting time

Two layers of rectangular absorbent paper (10cm×7.5 cm) fitted into a petri dish and wetted thoroughly with distilled water; were used for carrying out the test for wetting time. Then we placed the tablet at the center of the plastic dish and recorded the time required for the water to diffuse from the absorbent paper using a stopwatch.

Disintegration test

The test was carried out according to British pharmacopeia 2019 standards. The type of disintegration time tester was DTGi made in Copley, England. We placed one tablet in each of the six tubes and utilized distilled water maintained at 37^0 C; then tablets were observed for disintegration. The basket from the fluid was lifted and observed for the tablets' complete

disintegration at the end of the time limit (two minutes as directed for sublingual tablet).

Weight variation determination

From each batch, 20 tablets were chosen randomly and their average weights were calculated utilizing a digital weighing balance (Mettler Toledo, Switzerland); then percentage weight difference was estimated and checked with British pharmacopeia 2019 specifications.

Determination of water absorption ratio

We kept a piece of tissue paper folded twice in a petri dish (internal diameter 6 cm) incorporating 7 ml of purified water. Then we settled the tablets on the tissue paper and left them to wet wholly. The wetted tablets were separated and reweighed.

Determination of uniformity of drug content

From each formulation twenty tablets were weighed and powdered (tablets were placed inside a bottle then the cap was put back on and was turned clockwise until the tablets were completely crushed and powdered);then10mg of the powder was weighed and dissolved in 100 ml of distilled water. we sonicated the mixture for 170 seconds and filtered it through Whatman filter paper No. 40. Then the filtrate was diluted with distilled water and the absorbance at 275 nm was estimated due to disulfide photolysis and covalent insulin tyrosine dimerization induced by UV light exposure.[17]

In vitro drug release profile

Distilled water was used as the dissolution medium (300 ml) at 37 ^{0}C, PH 7.4, and 50 rpm(paddle) in presence of phosphate buffer 6.8. We collected samples(25ml) at 3,6,8,11,16,19,60,120,240 minutes intervals according to European pharmacopeia specifications 2020 and the withdrawn volumes were replaced by equivalent amounts of the plain dissolution medium. The amount of insulin released was measured using a UV spectrophotometer at 275 nm owing to disulfide photolysis and covalent insulin tyrosine dimerization induced by UV light exposure. The type of dissolution tester was DISi made in Copley, England.

Stability study

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

Screening and bio-assay of biological activity of human insulin utilizing:

Rabbit blood sugar method for screening and bio-assay

Principle: insulin decrease the blood glucose level in rabbits and the decrease in blood glucose level is directly proportional to the dose. In our study we used 10 rabbits, the rabbit weighed approximately 2 kg.

Procedure: 100 rabbits weighing 2 kg are used. A preliminary experiment was carried out by injecting each rabbit of the positive control group with graded doses of standard insulin (0.1-0.5 IU /kg) subcutaneously (S.C); while the test insulin sublingual tablets comprising graded doses of test insulin (0.1-0.5 IU /kg) insulin were given to the test group via sublingual route of administration after fasting for 18 hours for both groups of animal models (psositive control and standard). Any rabbit which showed convulsions within 5 hours was excluded. The rabbits were then randomly distributed into four groups, fasted for 18 at least 18 hours then a blood sample was taken from the ear vein to determine the initial blood glucose level (BGL). Each group was then injected with a dose of insulin according to the 2 and 2-dose assay and blood samples were taken each hour for 5 hours. The samples of each rabbit were pooled and the BGL of the pooled sample was determined. A decrease in the blood glucose level was recorded. Cross over test was carried out the next day. The mean decrease in BGL for each chosen dose was calculated and the relative potency was determined.[18]

Mouse convulsion method for bio-assay only using 2 and 2 dose assay technique

Principle: insulin decrease 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 160-190 gm were fasted for 12-24 hours and kept at a constant temperature of 29-35 ^{0}C. The standard insulin was injected with graded doses of insulin (0.1-0.5 IU /kg) intraperitoneally (IP); while the test insulin sublingual tablets containing graded doses of insulin (0.1-0.5 IU /kg) were given via sublingual 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]

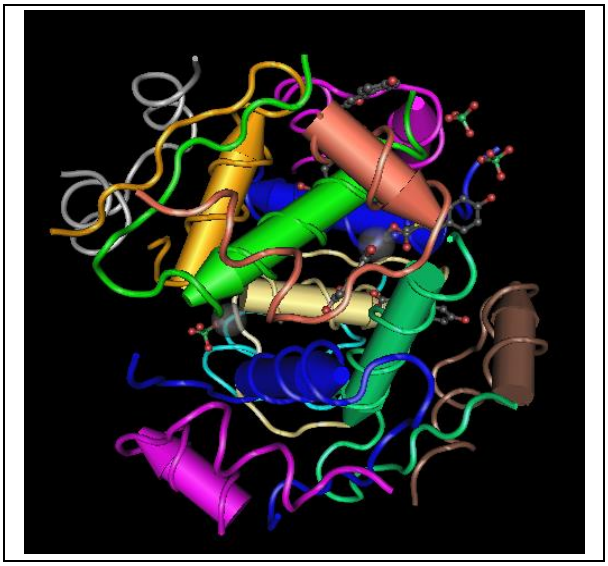


Figure 1. It represents the 3D structure of recombinant human insulin protein manufactured by *Saccharomyces cerevisiae.*

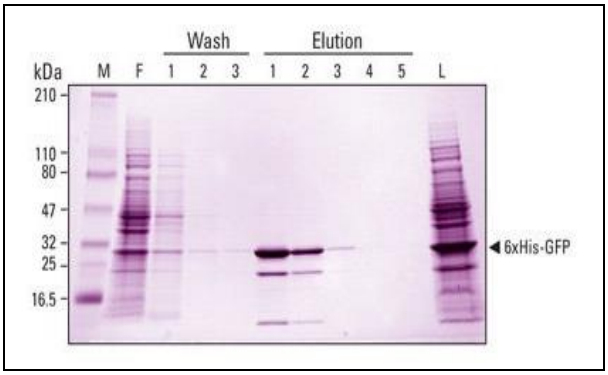


Figure 2. It shows the purification of recombinant human insulin via Nickel columns using immobilized metal affinity chromatography on Nickle affinity resins. The purity of recombinant insulin was about 85%.

Human evaluation of sublingual insulin drug delivery system via human clinical trials phases 1/2

3 groups of adult volunteer diabetic type1 patients with hyperglycemia greater than 200 mg/dl during fasting attending to Al-Qasr Ainy and Zagazig general hospitals were included in our study. Each group consisted of 100 subjects:

Group (1) (negative control group) was administrated with graded amounts of the placebo by the sublingual route. Group (2) (positive control group) was administrated with 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 sublingual human insulin micronized tablets (0.2-0.3U/kg of insulin injection were equivalent to 50 mg of sublingual human insulin tablets). The activity of insulin was estimated by the reduction in blood glucose levels during fasting.

In vivo bio-availability study

Before dosing sublingual tablets 0.7-0.9ml of samples were withdrawn, and immediately after dosing at 30,60,120and240 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 done through a reversed-phase column utilizing phosphate buffer (PH 4.4) and acetonitrile (660/340, v/v) as mobile phase with a flow rate of 0.9ml/min. The limit of UV estimation of insulin concentration in blood was 275 nm. The area under the curve (AUC) and the percentage of relative bio-availability were assessed. The percentage of relative bio-availability was determined by the following equation:

% Relative bio-availability= (AUC Sublingual/AUC Intravenous) × (Dose Intravenous/Dose Sublingual)×100%.

The same procedures were performed for the control and the standard groups (groups 1 and 2).

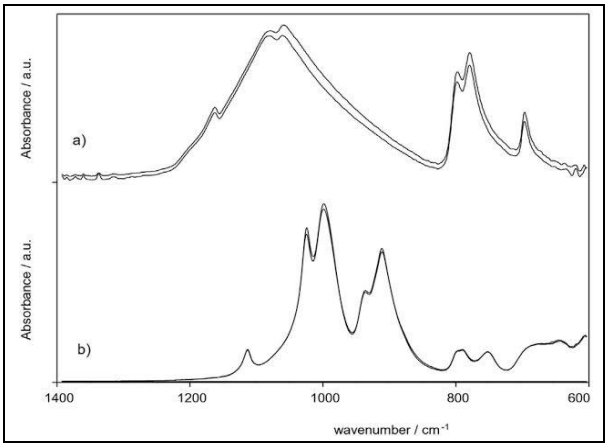
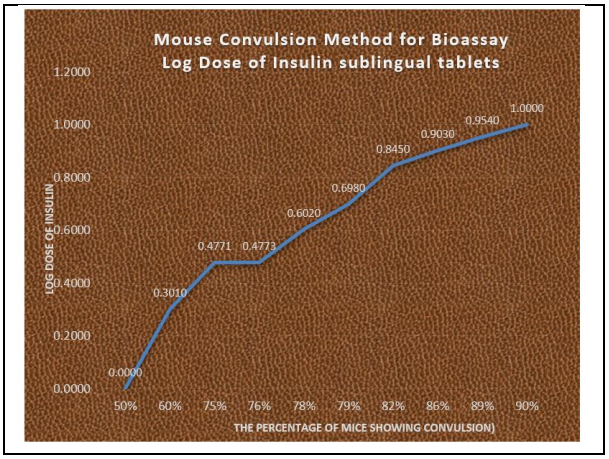


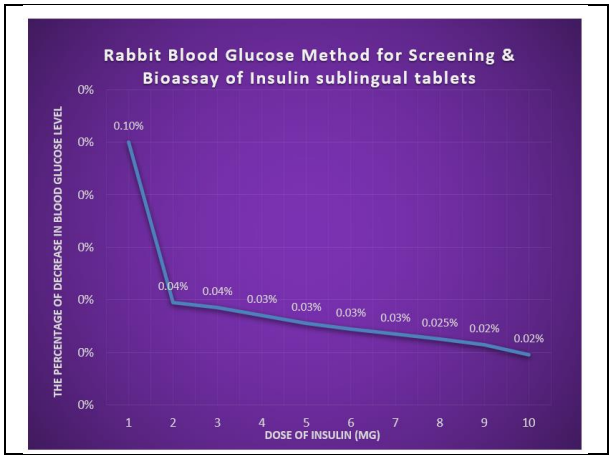
Figure 3. FTIR spectroscopy shows no interaction between recombinant human insulin and excipients.

Statistical analysis

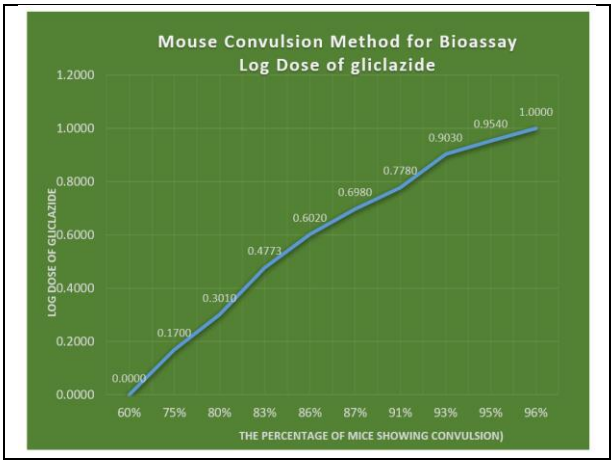
All cultures were conducted in triplets. Their presentation was of means and standard deviation. One-way analysis of variance (p value≤.05) was used as means for performing statistical analysis and also, statistical analysis based on excel-spreadsheet-software.



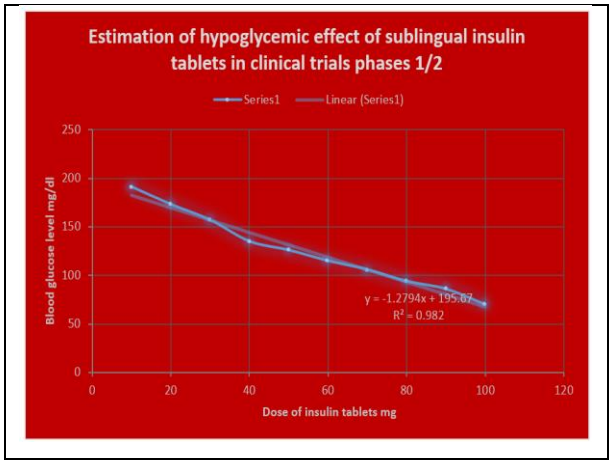
Graph 1. It represents the hypoglycemic effect of sublingual recombinant human insulin via mouse convulsion bio-assay.



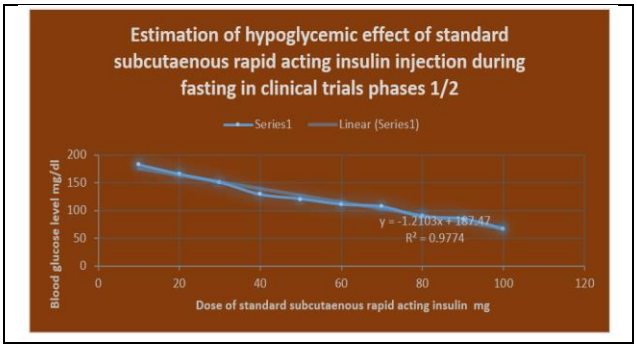
Graph 2. It represents the hypoglycemic effect of sublingual recombinant human insulin via rabbit blood glucose assay.



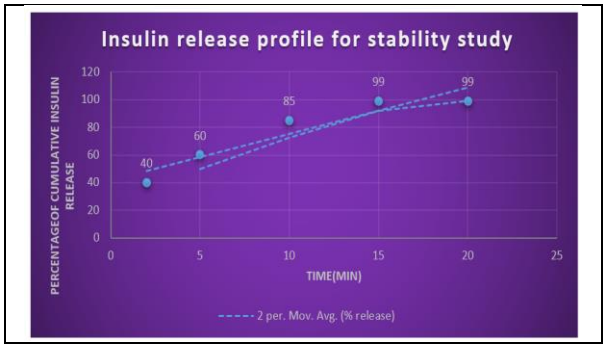
Graph 3. It represents the hypoglycemic effect of standard gliclazide oral hypoglycemic drug via mouse convulsion bio-assay.



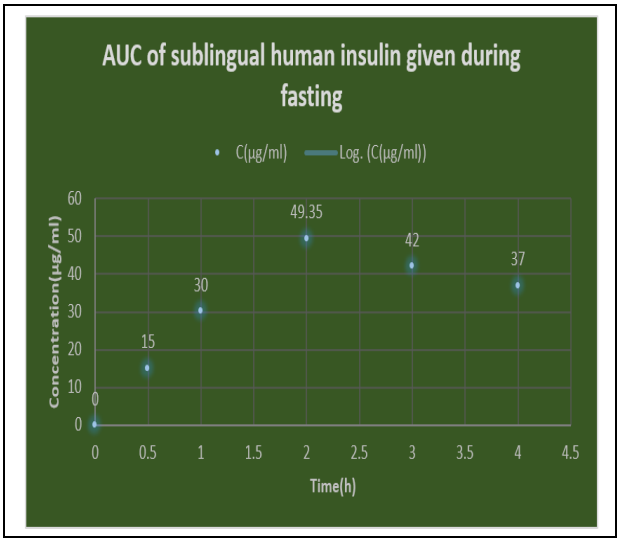
Graph 4. It represents the hypoglycemic effect of sublingual insulin tablets in human clinical trials phases1/2.



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



Graph 6. It represents a comparison of sublingual insulin release profile for stability study between initial release and after one month.



Graph 7. It shows the estimation of the area under the curve (AUC) of insulin sublingual tablets given during fasting.

RESULTS

In our study, we prepared different batches of recombinant human insulin sublingual tablets utilizing various ingredients such as starch 1500, pearlitol SD 200, sucrose DC, sucralose, PVP, etc. (**Table 2**).

No possibility of interaction between recombinant human insulin and excipients was shown by FT-IR and DSC studies. The determination of the hardness of the tablets was done and was observed between 1.74 to 1.99 kg/cm^2. The variation of weight of all formulations was estimated which were within the standard limit as per British pharmacopeia 2019 specifications. We found percentage friability in the range of 0.57 to 0.71% which was within the limit of extent. The ratio of water absorption for all formulations was observed between 38.47 to 42.86. The wetting time for all formulations was estimated between 17 to 24 seconds. We subjected the sublingual tablets for evaluation of in vitro disintegration time. For formulations F1 to F5, in vitro disintegration time was found to be in the range of 45 to 55 seconds. A rapid disintegration time of 45 seconds was observed by formulation F4. This is because of the burst effect and the rapid water uptake from the medium. All formulation's percentage drug content was observed between 97.24% to 98.77% of recombinant insulin which was to an unexceptionable extent. The release time for the sublingual insulin tablets ranged from 97.81% to 99.26% in 15-18 minutes at 37 C and 50 rpm. Batch F4 displayed quicker drug release than all the other batches. 98.26 % cumulative drug release in 15 minutes was demonstrated by batch F4. Batch F4 biological half-life (t_{50} %) of the immediate release insulin sublingual tablets were observed to be 4 minutes; while it was 3 minutes for nitroglycerin (venodilator antianginal drug) sublingual tablets.[20]Owing to the rapid disintegration time and dissolution profile Batch F4 was well-advised as an optimized formulation. Batch F4 was formulated with 15 mg MCC and 5 mg starch 1500. The optimum storage temperature of insulin sublingual tablets (batch F1 to F5) was noticed between 2-8 C. We performed in vivo study by taking formulation F4 and the outcome was compared with intravenous insulin injection. At different time intervals, the blood samples were withdrawn, then analyzed for the drug content utilizing HPLC. Tmax and Cmax of sublingual recombinant human insulin were determined to be 2 hours and 49.35 microgram /ml.% relative bio-availability was estimated by equation 1 and was dictated to be 81%. Bio-availability has been improved by insulin sublingual tablets as was incontestable by results of in vivo study. T max of SC rapid-acting insulin was 1 hour and C max was approximately 480 microgram/ml at an average dose of 0.2-0.3 U/kg. The onset of action of SC insulin was 10 minutes, its duration of action was approximately 5 hours and its bio-availability was about 90%. Bio-availability of IV insulin injection was 100%.

During human clinical trial phases1/2, the bio-availability of insulin sublingual tablets exceeded 80% while the efficacy reached nearly 70%. The pharmacokinetic profile of insulin micronized sublingual tablets during human clinical evaluation displayed rapid onset of action (15-30 minutes), 3 hours biological half-life, and duration of action less than 5 hours; while the onset of nitroglycerin was 1 minute and duration of action was 15 minutes.[20] It mimicked the physiology of endogenous insulin secreted by pancreatic beta cells of Langerhans. The majority of insulin catabolism was accounted for in the liver and the kidneys. Nearly 35% of insulin discharged into the portal vein was debauched by the liver via hepatic insulin protease existing inside the hepatocytes and lysosomes and approximately 65% was degenerated by the kidneys. There were fewer risks of weight gain, hypoglycemia, or hyperinsulinemia. When the standard insulin was injected exogenous, the destructive metabolism profile was changed because insulin was no longer delivered directly to the portal vein. The liver had a secondary role in insulin abjection (approximately 30%), with the kidney debasing about 60%. The renal dysfunction diminished the clearance of insulin and extended its effect. This ablated clearance was detected with both endogenous sublingual insulin and exogenous insulin administration. Health facility, a progressive decline in exogenous and endogenous sublingual insulin requirements, and an enhanced hypoglycemia risk even-ted from a declension in renal utility.

Table 1. Batch formulation of sublingual tablets of insulin F1-F5 by direct compression technique.

Ingredients(mg/tablet)	F1	F2	F3	F4	F5
Recombinant insulin	50	50	50	50	50
Starch 1500	4	3	6	5	8
MCC	16	14	12	15	13
Pearlitol SD 200	60	60	60	60	60
PVP	3	4	5	1	2
Sucrose DC	12	11	10	10	12
Talc	3	2	4	4	3
Sucralose	2	5	3	3	2
Aerosil	2	3	2	4	2
Total weight(mg)	152	152	152	152	152

Table 2. 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 3. 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 4. 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 sublingual insulin tablets during clinical trials phases 1/2:

Table 5. The results of the hypoglycemic effect of sublingual insulin tablets during fasting in human clinical trials phases 1/2.

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 6. 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 7. Batch formulation F1-F5 hardness, thickness, percentage Fri-ability, diameter, and weight variation

Batch	Hardness (kg/cm^2)	% Fri-ability	Diameter (mm)	Thickness (mm)	weight variation (mg)
F1	1.99±0.35	0.57±0.02	6.02±0.01	3.3±0.02	130.21±1.1
F2	1.81±0.35	0.59±0.04	6.07±0.02	3.5±0.01	129.64±1.3
F3	1.74±0.36	0.63±0.01	6.04±0.01	3.4±0.04	132.53±1.4
F4	1.79±0.39	0.71 ±0.03	6.05±0.06	3.5±0.03	131.19±1.2
F5	1.88±0.46	0.68 ±0.01	6.06±0.03	3.6±0.07	132.28±1.7

Table 8. Drug content uniformity, wetting time, water absorption ratio, disintegration time of batch formulation F1-F5.

Batch	Drug content uniformity	Wetting time (sec)	Water absorption ratio	Disintegration time (sec)
F1	99.15 ±2.24	21 ±2.80	38.51 ±1.78	56 ±1.68
F2	97.78 ±1.36	25±1.91	40.14 ±2.19	57 ±2.71
F3	97.24 ±0.99	17 ±2.99	42.86 ±2.82	49 ±2.10
F4	98.77 ±1.78	23 ±1.87	38.47 ±1.42	45 ±2.08
F5	98.61 ±2.07	24 ±2.04	41.29 ±1.60	55 ±2.54

Table 9. Comparison of different parameters for stability study of batch F4 between its initial production and after the storage for one month.

Evaluation parameter	Initial	After one month
Drug content	98.77± 1.78	99.00 ±1.24
Hardness	1.79 ±0.39	1.83 ±0.38
Disintegration time(sec)	45.00±2.08	49.00 ±2.37
Percentage friability	0.71 ±0.03	0.74 ±0.02

Table 10. The sublingual insulin tablets release profile.

Time (min)	% Release
2	40
5	60
10	85
15	99
20	99

Table 11. The estimation of the area under the curve (AUC) of insulin sublingual tablets given during fasting.

Time (h)	C (µg/ml)
0	0
0.5	15
1	30
2	49.35
3	42
4	37

DISCUSSION

For screening and bioassay of of insulin sublingual micronized tablets containing graded doses of insulin from 1 to 10 mg which is physically stable, we found after applying that the lowest effective dose which reduced normal rabbit blood glucose from 0.1% to .039 was 2.5 mg of insulin. For bioassay only of sublingual micronized tablets containing graded doses of insulin, the mouse convulsion method using 2 and the 2-dose assay was applied to fasting mice for 24 hours. It showed that 75% of mice suffered from convulsion due to the hypoglycemic effect of insulin starting from a dose of 2.8 mg of insulin in a comparison with 1.5 mg of gliclazide as a standard hypoglycemic drug. In both experiments hypoglycemia was shown after 2- 3 hours and the duration of the effect of insulin was 4-5 hours. This suggested that the sublingual formulation of insulin improved its physicochemical properties. Sublingual micronized insulin tablets using genetic engineering and peptidomimetics could overcome some disadvantages of insulin injection. In a comparison with a previous study (Ahmed Gedawy et al. 2018)[21]conducted in Australia, our study demonstrated that the efficacy of the sublingual

insulin delivery system was just about 70% and bio-availability was 81% 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 sublingual routes of administration displayed faded levels of systemic insulin, therefore less weight gain and hypoglycemic risks than exogenous insulin administrated subcutaneously. As well as, it was devoid of pain, risk of infection at the injection site, and lipodystrophy.

Sublingual 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 were no possible interactions between the drug and polymers according to FTIR spectroscopy and DSC study. In this work, we embattled different batches of recombinant human insulin sublingual tablets via direct compression technique utilizing different ingredients like micro-crystalline cellulose, starch 1500, sucrose DC, sucralose, PVP, Pearlitol SD 200, etc. NO possibility of interaction between excipients and insulin was unconcealed by the FT-IR and DSC study. Microcrystalline cellulose and starch 1500 event as a disintegration agent. Pearlitol SD 200 events as a sweetener and a diluent. Many excipients showed water solubility and thus had better patient acceptability. Our study was prosperous in terms of decreasing cost, manufacturing difficulties, and stipulating an effective medication with better patient compliance. Direct reciprocity between the disintegration time and wetting time was present. Batch F4 showed less disintegration than all other formulations. Optimized formulation was well advised to be batch F4. Fri-ability and hardness of batch F4 were too good. In vivo and stability studies were carried out on batch F4.No change occurred after one month as was informed by the stability study. Batch F4 demonstrated a good uniformity of the drug content, dissolution profile, and disintegration time and boost a good in vivo absorption profile and stability. Bio-availability of insulin has been improved by sublingual tablet formulation as was indicated via in vivo studies. In a comparison with nitroglycerin sublingual tablets; the sublingual way, which staves off the hepatic first-pass event, is favored for reaching a therapeutic blood level speedily. Glyceryl trinitrate(nitroglycerin) is absorbed with efficiency via this route and achieves therapeutic blood levels within a few minutes.[22]Insulin sublingual tablets have a slower onset of action but longer duration of action than nitroglycerin sublingual tablets. As well they display a higher percentage of relative bio-availability than nitroglycerin.

CONCLUSION

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

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Conflict of interest

The author declares that there isn't any conflict of interest regarding the publication of this paper.

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