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Assessment of Hypoglycemic Effect of an Oral Insulin Formulation in Chickens as an Experimental Animal Model

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ABSTRACT
The study aims to design and evaluate oral insulin for the purpose of achieving the following criteria: effectiveness in controlling blood sugar when taking insulin orally, palatability of the oral dose characteristics in terms of shape, color, smell and taste to be acceptable, especially for children. Stability of oral insulin against intestinal metabolism achieves constant bioavailability as well as oral doses are eligible to all diabetic patients. The oral nano polymer insulin (NPI) was assessed by GC mass, FTIR, electrophoresis, melting point, fluorescence labeling, microscopy, calorimetric, albumin binding assay and hydrophobicity test.
The overall *in vivo* findings showed that NPI had onset of action 35±15 min and 5±1.5 hr duration of action with oral efficacy of 34±4% of that for intraperitoneal (IP) route. The pharmaceutical properties were convenient for oral use.
Keywords: Diabetes, nano polymer oral insulin, chicken hyperglycemia, oral insulin pharmacokinetics
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INTRODUCTION
Diabetes mellitus is not a single disorder, but it is a group of metabolic disorder characterized by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. Recent surveys predict an increase in the prevalence of diabetes in adults from 4% in 1995 to 6.4% by the year 2025 [1]. According to World Health Organization projection, the diabetes population is likely to increase to 300 million or more by the year 2025. The prevalence rate is 8.9% - 12.3% in human population. Sex-related differences in lifestyle may lead to differences in the risk of developing diabetes mellitus, for example women are more likely to be obese or overweight than men and might therefore be expected to have higher prevalence of diabetes mellitus [2]. Based upon the etiology, diabetes mellitus can be divided into two main types, type 1, "Juvenile Diabetes Mellitus" (Insulin Dependent Diabetes Mellitus, IDDM) and type 2, "Adult type" (Non-Insulin Dependent Diabetes Mellitus, NIDDM). Type 1 occurs in childhood, mainly due to destruction of pancreatic β-cell islets through autoimmune-mediated mechanism, resulting in absolute insulin deficiency. Type 2 is more associated with an adulthood and elderly people, which are mainly due to insulin resistance or abnormal insulin secretion [3]. Several pathogenic processes are involved in the development of diabetes. These are ranging from autoimmune destruction of the pancreatic β-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The common symptoms of diabetes mellitus that associated with marked hyperglycemia include increased thirst, increased urinary output, polydipsia, weight loss, sometimes with polyphagia and blurred vision. Impairment of growth and susceptibility to certain infections in addition toketonemia and ketonuria occur due to the abnormalities in carbohydrate, fat, and protein metabolism [4]. Even though insulin therapy and oral hypoglycemic agents are the first line of treatment for diabetes mellitus, however, they have some side effects and fail to significantly alter the course of diabetic complications. Insulin is a hormone secreted from the β cells of the islets of Langerhans, specific groups of cells in the pancreas. It is a protein consisting of two polypeptide chains, one of 21 amino acid residues and the other of 30, joined by two disulfide bridges. It was isolated in 1921 with its first clinical use in 1922 [5]. Insulin undergoes degradation due to pH changes in the gastrointestinal (GI) tract and presence of enzymes like pepsin and pancreatic proteolytic enzymes (such as trypsin and α-chymotrypsin), and absorption of insulin through the GI mucosa is doubtful. Generally speaking, peptides and proteins such as insulin cannot be administered via oral route due to rapid enzymatic degradation in the stomach, inactivation and digestion by proteolytic enzymes in the intestinal lumen, and poor permeability across intestinal epithelium 80% of exerted insulin is normally degraded in the liver and kidneys. The amount of insulin secreted per day in a normal human is about 40 units. The dose of insulin required to control the diabetes varies from patient to patient and from time to time in the same patient [6]. Problems associated with inject able insulin therapy include pain, weight gain and potential hypoglycemia. Weight gain associated with insulin therapy is due to the anabolic effects of insulin, increased appetite, defensive eating from hypoglycemia, and increased caloric retention related to decreased glycosuria [7].Whereas, the problems encountered with subcutaneous insulin injections are pain, allergic reactions, hyperinsulinemia, and insulin lipodystrophy around the injection site. Patients anticipate the early development of an oral insulin as it will be easy to administer, have a lower index of intrusion and more convenient, as well as, more compliance or adherence from the patient, and finally this could lead to better glycemic control and thus prevention of

complications of diabetes. Oral insulin may improve β-cell function by providing β-cell rest and may help in preventing diabetes via induction of 'oral tolerance' or immunomodulating. Oral delivery of insulin would deliver the drug directly into the liver through portal circulation and could mimic the physiological fate of endogenously secreted insulin [8]. Oral insulin may also be able to correct the blunting of first-phase release of insulin, which is difficult with conventional subcutaneous insulins. Challenges to oral insulin delivery include rapid enzymatic degradation in stomach, deactivation and digestion by proteolytic enzyme in the lumen of intestine and poor permeability across intestinal epithelium due to high molecular weight with lack of lipophilicity implicating major hurdle in designing oral insulin formulations [9]. In addition, oral insulin is advantageous since it is delivered directly to the liver, its primary site of action, via the portal circulation, a mechanism very similar to endogenous insulin; subcutaneous insulin treatment however does not replicate the normal dynamics of endogenous insulin release, resulting in a failure to achieve a lasting glycemic control in patients [10]. There are many designs for oral formulations to resist insulin digestion in the GI tract including use of enteric coatings, micro and nano-particulates, mucoadhesive excipients, hydrogel liposomes and permeation enhancers in pharmaceutical industries. Among the most commonly used reactions is amidationreactions. The amide bond is widely prevalent in both naturally occurring and synthetic compounds and it is increasingly important in medicinal chemistry. Amidation reaction is a replacement of C-terminal carboxyl group of a protein with an amide group. This is a typical modification of many bioactive peptides for full activity. This modification prevents ionization of the C-terminus of peptides. It may therefore render it more hydrophobic and better bind to its receptor as well as it may also contribute to peptide stability. The importance of the amidation reaction is clear from the observation that about 50% of mammalian peptide hormones and more than 80% of insect hormones have α-amidated C-termini. The clinical signs of diabetes in birds are similar to those in mammals and include polyuria, polydipsia, polyphagia, and weight loss. Humans and chickens have a single pre-proinsulin gene, indicating similarities in pre-pancreatic expression characteristics. It has been also reported that the organization of the human genome is closer to that of the chicken than that of the mouse. Organ culture studies on glucose, glucagon- and to Ibutamide-stimulation of chick (granivorous bird) endocrine pancreas have suggested similarities in avian and mammalian beta-cell insulin secretary mechanisms and the structure of insulin [11]. Crocetin, the active constituent of saffron was found to possess anti-diabetic activity in fructose-fed rats as it alleviated free fatty acid-induced insulin insensitivity and dysregulated mRNA expression of adiponectin, TNF-a and leptin in primary cultured rat adipocytes. This is suggesting the possibility of crocetin treatment as a preventive strategy of insulin resistance and related diseases. Crocetin increases insulin sensitivity and ameliorates abnormalities related to insulin resistance such as impaired glucose tolerance, hyperinsulinemia, dyslipidemia and hypertension due to high-fructose diet and dexamethasone injection in rats. Crocetin attenuates palmitate-induced insulin insensitivity in rat adipocytes. Additionally, crocetin improved the insulin resistance induced by high-fat diet in rats. The antioxidant effects of crocetin may, at least in part, explain the ability of this compound to attenuate insulin insensitivity [13]. A recent study by Mohajeriand colleagues (2009) reported that treatment by saffron extract is associated with decreased blood glucose and increased serum insulin in diabetic rats. These results may be related to the antioxidant properties of saffron. Saffron, crocin and safranalhave protective effects against oxidation process due to their antioxidant properties [14]. Studies on rodents have shown that fish oil may improve insulin sensitivity or reduce glucose levels [15]. The effects of fish oil on insulin sensitivity and resistance in type 2 diabetic patients are not fully understood.

MATERIAL AND METHODS

Materials

Material	Source
Fluoroisothiocyanate FITC	Sigma, Taufkirchen, Germany
Oleic acid	Panreac, Spain
Stearic acid	Intatrad, Germany
Transcrocetinate	MP Biomedical, Santa Ana, CA, USA
boronic acid	Intatrad, Germany
regular insulin	Humilin, USA
diazepam	Sigma
Na stearate	Intatrad, Germany
human recombinant serum albumin	Sigma-aldrich, Germany
deionized distilled water	Life reagents, Mumbai

Instruments

Instrument	Source
GC mass chromatography	Conquer Scientific, California, USA
Combined electrophoresis	Bio-Rad, Berkeley California. mini sub cell
Computerized fluorescent microscope	Zeits, Germany

Magnetic stirrer	Staurt, UK
Centrifuge	Drucker diagnostic, USA
UV/V spectrophotometer	Cecil, UK
Preparative electrophoresis	Bio-Rad, Berkeley California
FTIR spectroscopy	Alpha Brucker, Germany
ACCU check glucose meter	Roch, Germany
Dissecting set	Somatco, Carolina, USA
Remote thermometer	Seek Thermal, USA
Sonicator	Biologics, USA
Computerized thermo graphic melting point analyzer	Seek-thermal, USA
Calorimeter	Parr, USA
Computerized pH meter	Prime Bioscience, Malaysia
Computerized Laser light microscopy	Zeiss, Germany

Preparation of oral nano polymer insulin NPI

A 200 mg of Transcrocetinate (75%) was added to 20 mg of pent fluoride and 20 ml of oleic acid (100%) at temperature of 60° C using a magnetic carburetor and thermal imaging until be dried for 6 hr, and set as product A. A 1000 mg of Stearic acid (100%) was added to 10 ml of oleic acid and 20 mg of the 5-iodo boronic acid at 60° C using magnetic carburetor and thermal camera until be dried for 6 hr, and set as product B. Product A and B were added to 20 ml of deionized water (DW) and centrifuged for 5 min after that the residue was removed and the same amount of centrifuge was returned to the supernatant. The standard human insulin was dried by lyophilization process to calculate the net weight. An amount of 90 mg of standard insulin was added to product A and the mixture was placed in magnetic carburetor at 60 °C for 6 hr to produce product C. Then 1 ml of product B was added to product C in the magnetic carburetor at 60 °C for 2 hr by observing the thermal imaging to produce product D. This product is placed in the Sonicator 80 watt and 30 KHz frequency at 40 °C for 30 min to form polymers and microscopic networks (product E). The product E was then centrifuged for 5 min at 5000 rpm and the supernatant was isolated and stored at 4 °C for diagnosis, analysis and biological assessment. It is worth mentioning that the insulin substitution by transcrocetinchainis beneficial because in addition to the glucose lowering properties, this chain possesses these properties as confirmed by many studies [16] as well as having the ability to convert insulin molecule to the polymerization nucleus offering more stable structure with oleate and stearate. Moreover, transcrocetinhas properties that make color, smell and taste of the resulting insulin more palatable by patients. Transcrocetinate moiety has many palatable physical properties such as bright yellow color and acceptable flavor. Owing to its bicarboxylic structure, it is able to function as a branching layer that improves the quality of nano-polymer with oleate and stearate [17].

Experimental Animals

Fifteen adult broilers (Gallus gallusdomesticus) of age 6-8 weeks weighing 1.5-2.2 kg, animals were 9 females and 6 males kept in 1 × 1 m unpainted wooden boxes, covered by a mesh on top, in groups of 4-6 individuals, following ethical approval (permit no. M6–12, Swedish Board of Agriculture), the illumination in the housing is supplied an electronic supplementary material. Water was available *ad libitum* but availability of food, commercial chick crumbs (Fågel Start, Svenska Foder AB, Staffanstorp) were divided into two groups. The control group (N=7) was given an oral regular insulin of 10 U/kg. The NPI-treated group (N=8) was treated with oral NPI of 10 U/kg.

Measurement of blood glucose in chicken

Food was removed from cages 6hr before the experiment. Animals had free access to water throughout the study, blood glucose levels were measured by using on call-plus glucose meter. Blood glucose levels were measured every 30 min interval over 120 min. All procedures were approved by the UCSD Animal Subjects Committee and conform to Institute of Laboratory Animal Resources. For induction of hyperglycemia, the high concentration was used because normal avian blood glucose level is approximately double that of healthy mammals.

Pharmaceutical Characterization of the Prepared NPI

GC mass spectrophotometric

Multiple samples were prepared to be characterized by GC mass. Those samples included the test NPI prepared (1 ml liquid form sample), the blank prepared in the same way of NPI but without the lyophilized insulin and the standard regular lyophilized insulin [18].

The combined fluorescent electrophoresis

Different samples were subjected to this procedure for identifying different bands according to their charges, different degrees of carboxylic substitution of the produced insulin and the yield fraction of the modified insulin. FITC was used as described above in accordance to the blank, test NPI and the regular insulin. Electrophoresis set was adjusted to 8 V/cm for 1hr when different samples were added into the standard wells of agarose gel. Combined fluorescent coding electrophoresis provides further confirmation of NPI characteristics such as charge / volume in comparison to normal insulin [19].

The preparative electrophoresis

The whole sample of the modified insulin was separated by agarose-based gel electrophoresis which enabled to separate

the hydrophobic band of the modified insulin for further characterization and *in vivo* assessment [20].

Fourier transforms infrared spectroscopy (FTIR)

This technique employed for the characterization of isolated biological molecules, particularly proteins to determine the structure of biological macromolecules. In FTIR spectroscopy, the light is directed onto the sample of interest, and the intensity is measured using an infrared detector. The intensity of light striking the detector is measured as a function of the mirror position, and this is then Fourier-transformed to produce a plot of intensity versus wave number [21].

The hydrophobicity test

This test was done by using octanol/water mixture into which the test NPI was added and each phase was detected at 280 nm in the UV/V spectrophotometer

NPI hydrophobicity = octanol absorbance /octanol+water absorbance x 100 %

Melting point test

A computerized thermo graphic analyzer was used to estimate melting point of products. Each sample was heated on programmed hot plate. The surface temperatures of the samples and the sample plate were investigated using an infrared thermal camera. All measurements were performed in an ambient atmosphere, and the data obtained were analyzed manufacturer's instructions. The melting temperature of compounds was determined typically within 5 min, and the obtained melting temperature values agreed well with those from differential scanning calorimetry measurements. Since many compounds can be investigated simultaneously in this infrared technology, it should be promising for high-throughput thermal analysis in the pharmaceutical developmental processes [22].

Insulin-albumin binding

Equilibrium dialysis technique and UV/V spectrophotometry can be used to investigate the interaction between insulin and human serum albumin (HSA) which acts as an important carrier for transporting many ligands such as, hormones, fatty acids, amino acids and foreign molecules [66,67].Drug interactions at protein binding level will, in most cases, significantly affect the apparent distribution volume of drugs and also affect the elimination rate of drugs, protein-drug binding plays an important role in pharmacology and pharmacodynamics. In equilibrium dialysis we used Teflon microcells with two chambers separated by a semi permeable membrane. Insulin and NPI were introduced into the buffer compartment to check the absence of binding to albumin and to determine parameters of drug binding to serum and HSA. Drug was added to serum in a minimum volume of buffer to enable the comparison of percentage binding with those obtained by the other techniques which was initiated with drug in both chambers to avoid being trapped in the dialysis membrane. Experiments were repeated at different across-membrane drug concentration ratios (inner-outer) until drug passage through the dialysis membrane was minimal; indicating that the system was at equilibrium unbound fraction of drug (*f*u) was calculated as (100 – *f*b) % [23].

The physical characterization of the test NPI

Color, odor, taste, consistency, solubility and stability were assessed to describe the agent. The product is semi liquid at 10 °C under normal atmosphere.

Calorimetric estimation of NPI configuration

The most common methodology to establish heat release rate (HRR) is oxygen consumption calorimetry (OCC). It was originally based on the observation that HRR is proportional to oxygen consumption during the combustion of most organic liquid and gaseous fuels, thermal sensor element together with IR thermal camera for detection of temperature fields, emerged during tests on combustion process. In order to achieve further structural estimation of the product combustion test was performed by burning the mole of insulin product. Where ΔG to NPI was (3500) kcal / mol) which is significantly higher when compared to the original regular insulin (3000 kcal / mol) and blank group (1000 kcal / mol) [24].

Statistical analysis and software packages used

1- Linear, polynomial and exponential regression test (MATLAB statistics 2015)

2- Unpaired t-test (SPSS 20)

Meeting the ethical requisites

The ethical treatment of used animals has been considered based on the provisions of the global document followed at Faculty of Medicine / University of Kufa regarding reducing the number of animals to the minimum statistical limit and the use of animal husbandry controls and replacing the possible with the laboratory model and use of anesthesia when forced surgical intervention and euthanasia.

RESULTS

GC mass spectrophotometric findings

After running spectrophotometry by GC mass, there was a clear change of the charge coefficient to the weight in NPI when compared to regular insulin, figure (1).

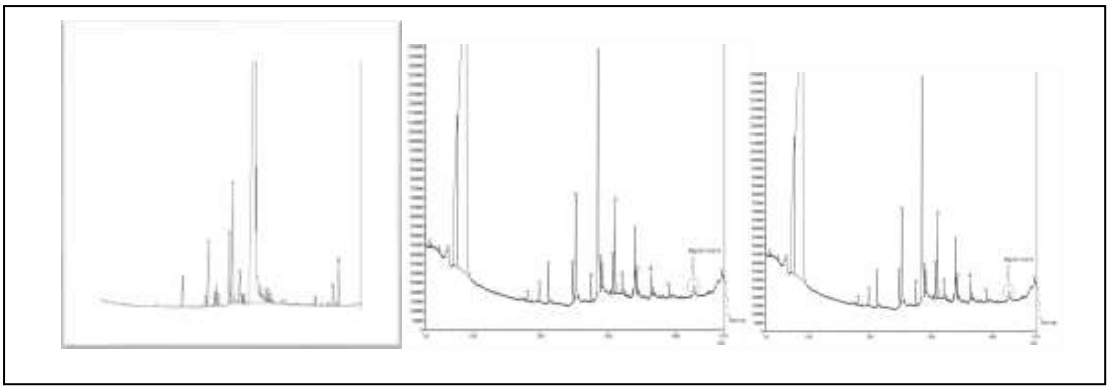


Figure 1: Chromatogram GC-MASS for blank (a), regular insulin (b) and NPI (c)

Finding of combined fluorescent electrophoresis

The insulin NPI designed in this study possesses high absorbency and moderate bioavailability as the entry of oleic into the insulin formula raised the degree of its solubility in fats, which increased its absorption through the intestinal membranes and improved its abundance in the blood, figure (2). Comparative fluorescence coloring FITC for insulin is to assess the absorption, spread, and passage of insulin from the intestinal canal of mice to the hepatic portal vein to the systemic circulation through the liver to assess percentage of bioavailability and rate of insulin resistance to digestion in the intestine and liver.

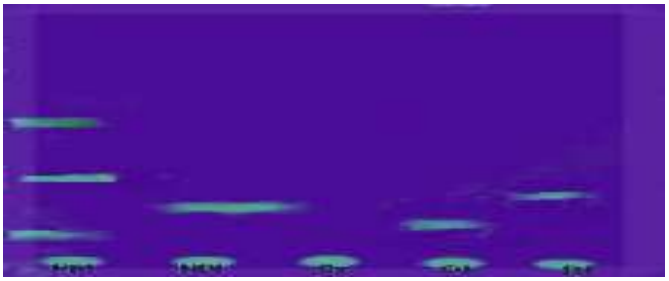


Figure 2: Combined fluorescent gel electrophoresis of regular insulin (RI), oil-mixed insulin (OI), insulin NPI in addition to the assessment of FITC trapping effect of the supernatant (Sx) and precipitant (Ppt)

Physical appearance of NPI insulin

The composition of nanoparticles NPI has physical properties that make it more acceptable than others for daily intake. It is distinguished by its golden transparent color and its oily taste with slight acidity and an acceptable odor, figure (3a). This is due to the presence of transcrocetinic acid and oleic acid. The addition of Transcrocetinate in the NPI composition enabled the formation of Nanoparticle polymerization and the occurrence of microscopic networks that make the general structure more stable and resistant to digestion and more sticky to the intestinal membranes and eliminated the need for insulin carriers such as liposomes with a harmful cumulative fatty effect over time. This suggests that NPI is safer than other oral formulations, more stable and abundant in blood.

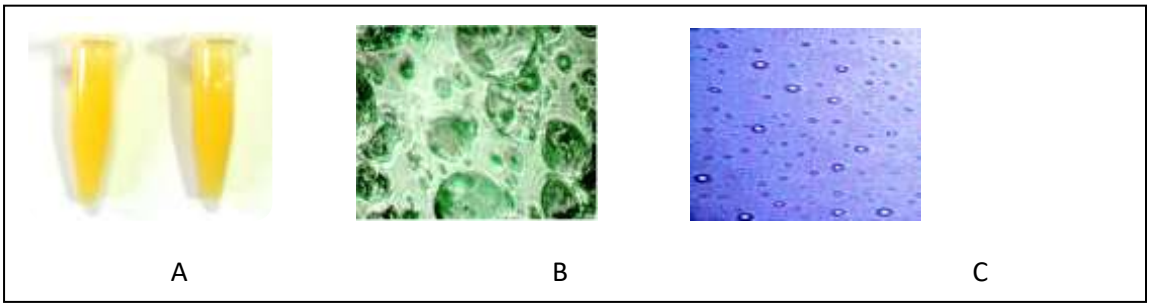


Figure (3-a): Liquid consistency and bright yellow appearance of the produced oral insulin,

Figure (3-b): Laser light microscopic image of the variable configurations of NPI ranging 250 nm to 1000 nm

Figure (3-c): Fluorescent picture of FITC-labeled regular insulin shows the fluorescent trapping within the protein mesh

Figure (3-d): Fluorescent microscopic mage of NPI shows the nanoparticles formulated from the modified insulin-oleate-Transcrocetinate and stearate in a diameter ranging 300-1200 nm.

FTIR findings

Oral NPI insulin has a strong resistance to stomach, intestinal and liver enzymes. It contains oleic acid in the sites of electronic affinity and alcohols side chains of amino acids in the structure of standard human regular insulin, as in the most recent displacement of absorption spectrum of alcohols and amines in FTIR to form esters and amides, respectively. This gives a molecular block in front of pepsin, trypsin, chymotrypsin and hepatic exopeptidases and end peptidases.

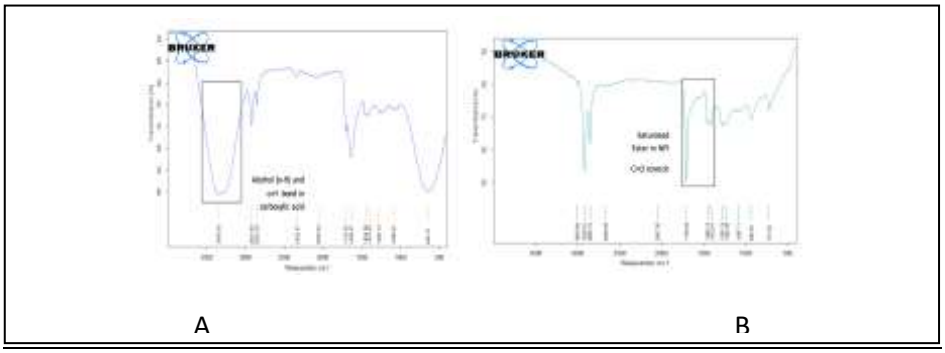


Figure 4: FTIR absorption spectrum for standard insulin [73] (a) and absorption spectrum of NPI [74](b).

Characterization of NPI in mice

The physicochemical properties (Table 1), pharmacokinetics (Table 2) and pharmacodynamics (Table 3) of NPI were conducted in mice using regular insulin as a control.

Table 1: Estimated physicochemical properties of NPI

Physicochemical and pharmaceutical properties	Regular insulin	NPI	Blank	Notes
1- Chemical composition	Recombinant human lispro insulin, DW, ZnO, NaCl, d-Cresol, phenol	Nano-dendrimer of lispro-transcrocetinate Oleate-Stearate	Transcrocetinate Oleate-Stearate	GC mass detected
2- Consistency	Thick liquid	Oily	Oily	-
3- Melting point	233 C ± 0.1	39 C ± 0.5	-5 C ± 0.1	By thermographic estimation
4- Heat of combustion	3000 kcal/mol	3500 kcal/mol	1000 kcal/mol	By calorimeter
5- Hydrophobicity	60%	95%	100%	By octanol/water Interface
6- Color	Transparent	Bright, translucent, yellow	Transparent, yellow	-
7- Odor	Phenol odor	Transcrocetinate odor	Transcrocetinate odor	-
8- Taste	-	Resin	Oil	-
9- Dosage form	Liquid vial	Oral oily solution	Oral oily solution	-
10- Concentration of insulin	3.5 mg/ml	2 mg/ml	0	Intentionally prepared
11- Acid-base nature	PH (5.5)	-0.3 relative to insulin	0.0	-
12- Acid-base stability	Stable	Stable	Stable	-
13- Density	1.09 g/cm^3	1.01	-	-

Table 2: Estimated pharmacokinetics of NPI

Pharmacokinetics	Regular insulin	NPI	Blank	Notes
1- onset of action a- I.P onset	a- 45 min ± 10 min	a- 30 min ± 15 min	a-no effect	By *in vivo*

b- oral onset	b- 45 min ± 15 min	b- 35 min ± 15 min	b-no effect	estimation of FBS*
2- Duration of action	4 hr± 1 hr	5 hr± 1.5 hr	No effect	In vivo FBS
3- Plasma $t_{1/2}$	5 min	-	-	
4- Hepatic metabolism First pass effect	60% (A = 40% for portal A = 24% for systemic absorbance)	40% (A = 50% for portal and A = 20% for systemic absorbance)	-	*In vivo* fluorescent assessment
5- Distribution	10%	15%	20%	Gel documentation
6- Albumin binding assay	10%	30%	-	-

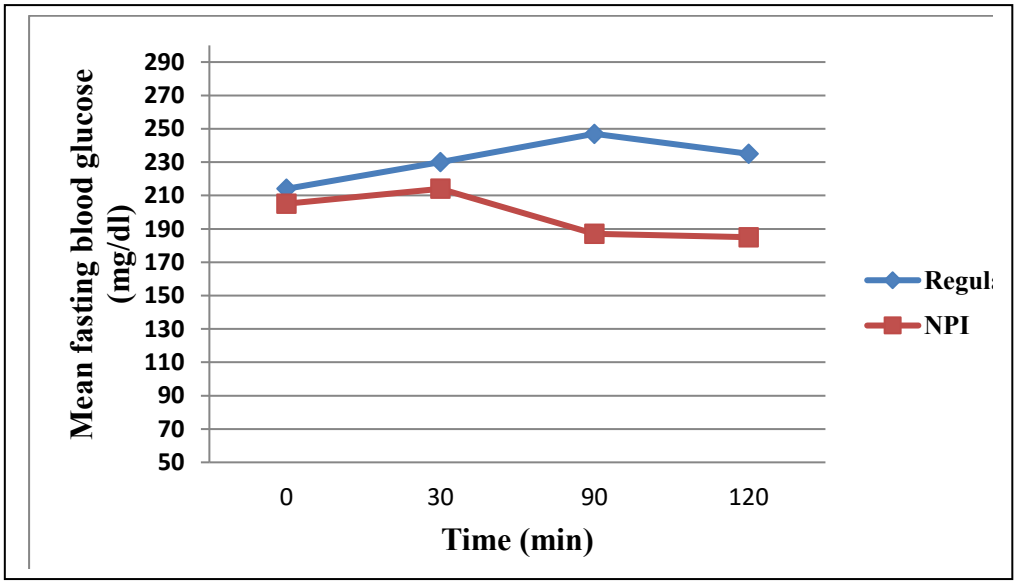
* FBS: fasting blood sugar

Table 3: Estimated pharmacodynamics of NPI

Pharmacodynamics	Regular insulin	NPI	Blank	Notes
1- Potency	Relative serum insulin 100%	70%	0%	-
2- relative efficacy a- I.P efficacy b- oral efficacy	 a-100% reference b- 10%	 a- 73% ± 5% b- 34% ± 4%	 No effect No effect	 P = 0.04 P = 0.03

Effect of the modified oral insulin on chicken blood glucose level

Regular insulin-treated group was given equivalent amount (10U/kg) of human regular insulin whereas NPI-treated group was given oral NPI (10U/Kg) with 30 min interval monitoring of tarsal blood sugar up to 120 min. It is obvious that NPI possesses an antihyperglycaemic protective slope -8.7 when compared with +8 for that of regular insulin (Figure 5).



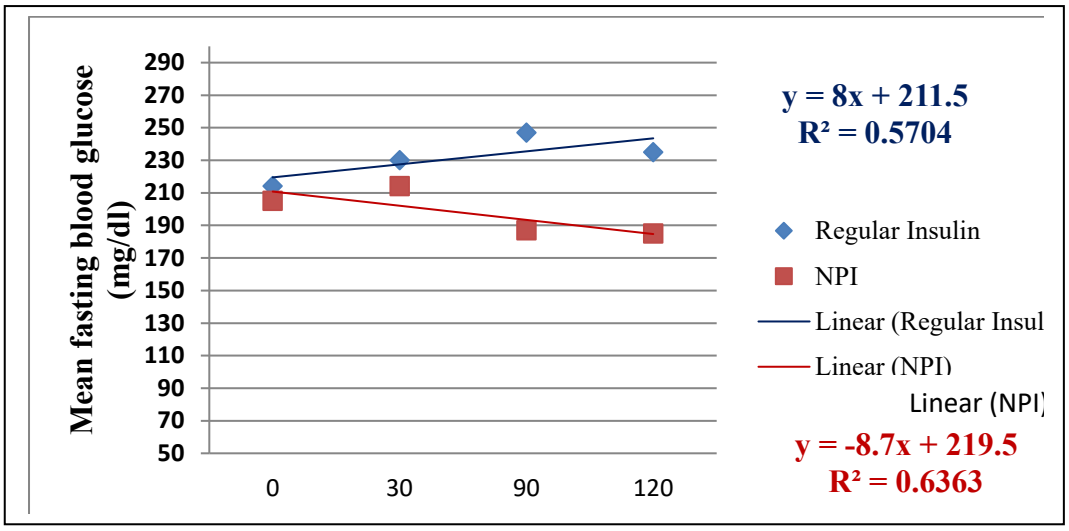


Figure 5: Accumulative response of chickens to the orally administered insulin. b, statistical model assessment of the effect of oral insulins on blood sugar

DISCUSSION

Since insulin dependent diabetes mellitus requires a lifelong parenteral replacement of insulin, this mode of drug administration is a potential error-producing condition and has many disadvantages especially in extreme ages. Of these drawbacks are the lack of dosage convenience and patient incompliance making both poor control of the illness and emotional feeling of frustration [75-76]. Both of those factors are major contributors in DM progress and complications particularly in children and some co morbidities. Many experiments and trials were designed to replace the invasive route of insulin delivery with more convenient ones like nasal, transdermal and oral routes [77].However, the problem of insulin efficacy in those routes remained the major challenge in front of researches. Many factors are real pharmacological obstacles in regard to oral route of insulin administration. Gastric pH, digestive enzymes, poor absorption of insulin through GIT mucosa, first pass effect of insulin (60% metabolized in the liver), adjunctive moiety toxicity and the lack of the native insulin efficacy are of the main kinetic and dynamic challenges of oral insulin supplementation[78- 81]. This current research had taken in consideration the preclinical design of oral insulin making full use of the advances in the field of nonmaterial to minimize the weak points concerning the oral insulin kinetics and to maximize the efficacy and the convenience of the oral dosage form.

NPI efficacy and potency

The substitution of insulin with Transcrocetinate had a rational anti-diabetic effect since Transcrocetinate structure property relationship correlates with the improvement of the compound absorption, protection against exopeptidases and end peptidases [82]. Moreover, Transcrocetinate is an approved natural dicarboxylic acid to have potent antioxidant and free radical trapping activity [83]. The Transcrocetinate moiety has many palatable physical properties like the bright yellow color and accepted flavor. Owing to its dicarboxylic structure, Transcrocetinate acts as a branching layer that improves the quality of the nano-dendrimer together with the oleate and stearate [84-85]. All these properties had improved the effectiveness, appearance and palatability of NPI oral dosage form.

The pharmacokinetic properties of the modified oral insulin

A comparative onset of action of the NPI was significantly earlier than that of the oral and intraperitoneal regular insulin (45 min ± 10 min, 45 min ± 15 min respectively for the regular) in comparison with (30 min ± 15 min, 35 min ± 15 min) that of NPI [86,87]. This relative reduction in the onset of NPI action might be attributed to the relative increase in hydrophobicity of acylated insulin [88] and its nanoparticulate size [89]. These factors are important biological enhancers for alpha phase of distribution so that NPI is expected to have more possible mechanisms of distributions [90]. This proposed explanation is further confirmed by the comparative fluoro-labeling assay estimated for FITC-labeled insulins upon passing into the portal circulation (Figure3). In terms of duration of action of NPI, it revealed a general agreement with the expected values and those researches which concerned with estimation of the duration of action of the acylated insulin (4 hr± 0.5 hr for the regular insulin and 5 hr± 0.45 hr for the NPI)[91,92]. This relative prolongation of NPI action can be interpreted as several pharmacokinetic factors had been modified. Of these factors is the presence of multiple acyl groups in the NPI that increased its plasma albumin binding rendering NPI more slowly released from this plasma reservoir [92-93]. The second important factor that may justify that increment in NPI duration of action is the protection of those acyl moieties against end peptidases and exopeptidases, the hepatic and tissue enzymes responsible for metabolizing insulin [94]. Although the general efficacy of the oral NPI was just 34% in comparison with the similar dose of the regular IP insulin, its duration of action could be a suitable replacement therapy in IDDM to cover the basal insulin requirement [95].

The Pharmacodynamics Properties of NPI

The linear regression method was used to compare the line slope and R factor of both NPI and the regular insulin in chicken and mice models of *in vivo* oral hypoglycemic assessment. The general agreement in both models was the increased relative oral efficacy of NPI in comparison with the orally supplemented regular insulin (34% for NPI in comparison with just 10% for regular insulin) [96].However, the intraperitoneal efficacy of the NPI was just 70% of IP regular insulin. Since the route of interest in this research was the oral one, this study may recommend further confirmation of the promising obtained results, because the structural modification of insulin into Nanoparticle NPI seems to improve significantly the absorption through GIT mucosa (P = 0.04) in addition to the protective effects against the digestive enzymes.

CONCLUSIONS

From the overall results, formulated NPI had favorable pharmaceutical properties including consistency, stability and constituents enable multiple formulations for a matter of convenience and compliance. Furthermore, NPI revealed significant and acceptable pharmacokinetic properties including its suitable and rapid onset of action within 30 min in addition to its longer duration of action throughout 5 hr which make it more suitable for meeting basal insulin requirement in IDDM.The overall oral efficacy of NPI was just 34% of that for parenteral regular insulin in both models. However, it was significantly higher than that of the comparable oral regular insulin.

RECOMMENDATIONS

1-Researchers can further modify branching layers on the core Transcrocetinate to assess further improvement in pharmacokinetic and pharmacodynamics properties. In addition to modifying the additive surfactants for the same purposes

2- Adoption of authenticated clinical trial for assessment of NPI throughout phase I of FDA approval is required

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