Pharmacological Study of Weight Losing of Liraglutide Loaded within Layered Double Hydroxide Carrier

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Abstract

The anti-diabetic drug liraglutide was loaded within LDH nanoparticles using the Fe^{+3}, Al^{+3}, Fe^{+2} and Ni^{+2} ions, the preparation of LDH was carried out via the titration of 2M of NaOH with trivalent and divalent ions in presence 0.5M HCl, the prepared compounds of liraglutide with LDH were tasted with selective groups of rats.

The compounds were characterized with Fourier transform infra-red (FTIR), X-ray diffraction (XRD), Atomic force microscopy (AFM), Zeta potential and Scanning electron microscopy (SEM).

The animals that injected with weekly dose of the prepared compounds shows the weight reduction comparative with that of the animals injected daily dose of pure liraglutide.

Keyword; *Liraglutide, double layered hydroxide, nanoparticles, antidiabetic drug.*

Introduction

Liraglutide is a fatty acid derivative of glucagon like peptide-1 [GLP-1]. It is formed by attaching a 16-carbon fatty acid molecule at position Lys26 and making an Arg34Lys substitution on GLP-1, and shares approximately 97% sequence homology with GLP-1 so retains the physiological activities of GLP-1[1, 2]. These structural modification of liraglutide increase chain aggregation, promote reversible non-covalent binding to other molecules, such as albumin, and resist dipeptidyl peptidase degradation. Liraglutide marketed as Victoza®, is a 1.8 mg daily subcutaneous injection that was initially approved by the FDA in 2010 as an adjunct therapy to diet and exercise for management of type 2 diabetes. Appetite suppression and delayed gastric emptying are thought to be responsible for the weight lowering effects of GLP-1. As a result, liraglutide was also developed as a weight loss agent [3].

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Layered double hydroxides (LDHs), also known as hydrotalcite-like compounds, represented by the general formula$[M^{II}_{1-x}M^{III}_{x}(OH)_2]^{x+}[(A^{n-})_{x/n} \cdot yH_2O]^{x-}$, where M^{II} and M^{III}, di-and tri-valent metal cations, respectively, are capable of occupying the octahedral interspaces of brucite-like sheet. A is anions between the layers can compensate the positive charges of the layer structures and these interlayer regions may contain water molecules[4]. Recently, particular attention has been focused on the LDH-based controlled release systems. Although many biomolecules/LDH hybrid complexes have been reported, only a few examples have been studied as drug delivery carriers. In the present study, liraglutide drug was selected as a model drug and intercalated into Fe^{+2}/ Fe^{+3} LDH, Fe^{+2}/Al^{+3}-LDH and Ni^{+2}/Fe^{+3}- LDH successfully by coprecipitation technique. This work focus on the structure and slow/ controlled release property of a synthesized drug–LDHs composite intended for providing basic data for organic–inorganic LDH hybrids[5].

Materials and Methods

1. Preparation of precursor and hybrids

Synthesis of Fe^{+2}/Fe^{+3} layered double hydroxides nanoparticles

The synthesis of Fe^{+2}/Fe^{+3} LDHs NPs was done by mixing 1:2 molar ratio of Fe^{+2}/Fe^{+3} (25 mL of 0.02 M of $FeSO_4.7H_2O$ solution and 25 mL of 0.04 M of $FeCl_3$ solution addition 2.1 mL 0.5 N HCl. Then a drop wise titration is done with 2 M NaOH with vigorous magnetic stirring at 80 ˚C until pH elevated from 2 to 9 with change in color of the solution from clear yellow to dark brown suspension which is then left at room temperature for 24 hours. The suspension is filtered and continuously washed with deionized water until the filtrate became neutral. The obtained solid residue was dried at 50˚C in oven for 4 hours [6].

Synthesis of Fe^{+2}/Al^{+3} Layered Double Hydroxides nanoparticles

In the present work Fe^{+2}/Al^{+3} LDHs NPs was synthesized by mixing 25 mL of 0.06 M of $Al(NO_3)_3$ solution and 25 mL of 0.02 M of $FeCl_3$ (3:1 ratio) respectively. To which 2.1 mL of 0.5 N HCl was added, then titrated drop by drop with 2 M NaOH with vigorous magnetic stirring at 80˚C until the pH elevated from 2 to 9 with change in the color of the solution from clear yellow to light brown suspension which is then left at room temperature for 24 hours. The resultant suspension filtered and washed with deionized water several times, until the filtrate became neutral [7].

Synthesis of Ni^{+2}/Fe^{+3} Layered Double Hydroxides nanoparticles

Ni^{+2}/Fe^{+3} LDHs NPs was synthesized by mixing 25 mL of 0.75 M of $Ni(NO_3)_2$ solution and 25 mL of 0.25 M of $FeCl_3$ (3:1 ratio) respectively. To which 2.1 mL of 0.5 N HCl was added, then titrated drop by drop with 2 M NaOH with vigorous magnetic stirring at 80˚C until the pH elevated from 2 to 9 with change in the color of the solution from clear yellow to brown suspension which is then left at room temperature for 24 hours. The resultant suspension filtered and washed with deionized water several times, until the filtrate became neutral [8].

Synthesis of Fe^{+2}/Fe^{+3} Layered Double Hydroxides loaded liraglutide

The method used to synthesis the liraglutide loading with LDH can be describe as: In beaker 500 ml in size put 10 ml (0.00121g, 0.0008 M) of $FeSO_4.7H_2O$ solution mixed with 10 mL (0.00064g, 0.0004 M) $FeCl_3$ solution. The mixture were treated with 2.1ml 0.5 HCl and 1ml of liraglutide (0.006g, 0.0016 M) at the same time, the produced mixture was titrated with 2M, NaOH solution at room temperature, the PH was rapidly elevated to C_a 9 and the colour of mixture observed change from yellow to dark brown suspension [9].

Synthesis of Fe^{+2}/Al^{+3} Layered Double Hydroxides loaded liraglutide

The method used to synthesis the liraglutide loading with LDH can be describe as: In beaker 500 ml in size put 10 ml(0.0017g, 0.0008M) of Al $(NO_3)_3$ solution add to 10 ml of (0.00064g, 0.0004M) $FeSO_4$ solution. The mixture were treated with 2.1ml 0.5 HCl and 1ml of liraglutide (0.006g, 0.0016 M) at the same time, the produced mixture was titrated with 2M, NaOH solution at room temperature, the PH was rapidly elevated to C_a 9 and the colour of mixture observed change from pale yellow to light brown suspension [10].

Synthesis of Ni^{+2}/Fe^{+3} Layered Double Hydroxides loaded liraglutide

The method used to synthesis the liraglutide loading with LDH can be describe as: In beaker 500 ml in size put 10 ml (0.00146g, 0.0008M) of $Ni(NO_3)_2$ solution mixed with 10 mL (0.00064g, 0.0004M) of $FeCl_3$ solution. The mixture were treated with 2.1ml 0.5 HCl and 1ml of liraglutide (0.006g, 0.0016 M) at the same time, the produced mixture was titrated with 2M, NaOH solution at room temperature, the PH was rapidly elevated to C_a 9 and the colour of mixture observed change from yellow to brown suspension [9].

Animals and study design:

Thirty adult's male wistar rats (weighing 220-248 gm) were utilized in this study. Animals were attained and placed in animal house of College of Pharmacy/ Mustinsiryiah University, they received water and ordinary pellets for one week for acclimatization, after that they feeding lard [11] for 12 weeks to develop obesity were divided into 5 groups:

- (Group A) was receive 0.5ml/kg/week normal saline.

- (Group B) was receive 600µg/kg/day of pure liraglutide.
- (Group C) was receive 650µg/kg/week IP of Fe^{+2}/Fe^{+3} LDH loaded liraglutide.
- (Group D) was receive 655µg/kg/week IP of Ni^{+2}/Fe^{+3} LDH loaded liraglutide.
- (Group E) was receive 658µg/kg/week IP of Fe^{+2}/Al^{+3} LDH loaded liraglutide.

Animals were kept in plastic cages each with dimension (20x25x35 cm) that harbor three rats, animals within same cage were discriminated by back fur marking by using waterproof colored marker. The animals were placed under controlled condition of room temperature (25 $\pm 1^0$C) and light stream of 12 hours light/ 12 hours dark cycles. Diet and water were attainable easily to animals. Animals study design started in February /2019 and ended in Jun /2019. Ethics committee of the College of Pharmacy/ Mustansiriyah University was given their approval to begin the study.

Results and Discussion

Characterization of prepared compounds

FT-IR spectra for LDHs and LDHs loaded liraglutide

The FT-IR spectra of the synthesized compounds displays the characteristic absorption bands by which the functional groups were identified [12]. The assigned bands of LDHs spectra and LDHs loaded drug spectra refer to the type of binding between the molecules as hydrogen bonding only as a physical tied and easy liberate in target site[13].

X-ray diffraction (X-RD)

A general analysis of these patterns shows that the LDHs loaded liraglutide shows the peaks at 33 two theta assigned to the dihedral geometry as amorphous structure with small difference between LDHs types. Such difference is attributed to the higher similarity of ionic radii for the ion pair.

Atomic force microscopy (AFM) Results:

Results of the AFM images of the LDHs typical surface (in three and two dimensions), the spectrum of AFM pictures of prepared compounds before and after loading the liraglutide since the difference between the arrangements of layered after loading is very clear refer to the affecting of binding of drug with the LDH, from the study of surface and layered of nanocompounds were arrange as vertical cylinders this shape make the molecules easy to liberate the liraglutide.

Zeta potential measurement

Zeta potential measurement for the prepared LDHs loaded liraglutide. The criteria of stability of NPs are measured when the values of zeta potential ranged from higher than +20 mV to lower than -20 mV. The values of the zeta potential of the prepared compounds are listed in table-1 provided satisfactory evidence about their little tendency towards aggregation when its zeta potential in the negative scale and below -20 mV. These results suggested that the prepared LDHs loaded drug particles were stable with no tendency to aggregates and this in agreement with the results reported for colloidal nanoparticles behavior [14].

Table 1: Zeta potential values for the prepared LDHs NPs loaded liraglutide

LDHs NPs Type	Zeta Potential (mV) ± SD	Mobility (µ/s)/(V/cm) ±SD
Fe+2/Fe+3 LDHs loaded liraglutide	-32.82	-2.56
Al+3/Fe+2 LDHs loaded liraglutide	-26.45	-2.07
Ni+2/Fe+3 LDHs loaded liraglutide	-28.00	-2.19

Scanning electron microscopy (SEM)

The pictures shown in figure-1 represented the SEM images of LDHs loaded liraglutide. The images confirmed the layered shape of LDHS with multiple agglomeration areas, due to the hydrogen bonding attraction between multiple hydroxyl groups those had not been hydrolyzed by acidic hydrolysis [15].

In vivo

The change in the initial and final mean of body

weight of group A (0.5ml/kg/week) normal saline intraperitoneal after four weeks was small that indicate there is no significant difference ($p = 0.07$). While body weight of group B was significantly decreased ($p < 0.05$) after daily administration of 600µg/kg liraglutide plus 0.5ml/kg normal saline intraperitoneal for four weeks. This result in weight reduction compatible with result that reported by Astrup *et al*. [16]. Liraglutide lead to slows gastric emptying time and decrease food intake, so cause weight reduction [17].

The body weight of prepared compounds groups C, D and E was significantly decreased ($p< 0.05$) after weekly administration of prepared LDHs loaded liraglutide plus 0.5ml/kg normal saline intraperitoneal for four weeks.

Reduction in the weight of groups C and E about 18% of body weight after four weeks this results very close to results of group B (daily dose of pure liraglutide) that mean Fe^{+2}/Fe^{+3} LDH and Fe^{+2}/Al^{+3} LDH are more suitable for loading drugs because these compounds have higher loading capacity than group D as shown in (figure-2). The statistical analysis of rat ´s body weight represented as mean ± standard error of mean in groups (n=6 for each group).

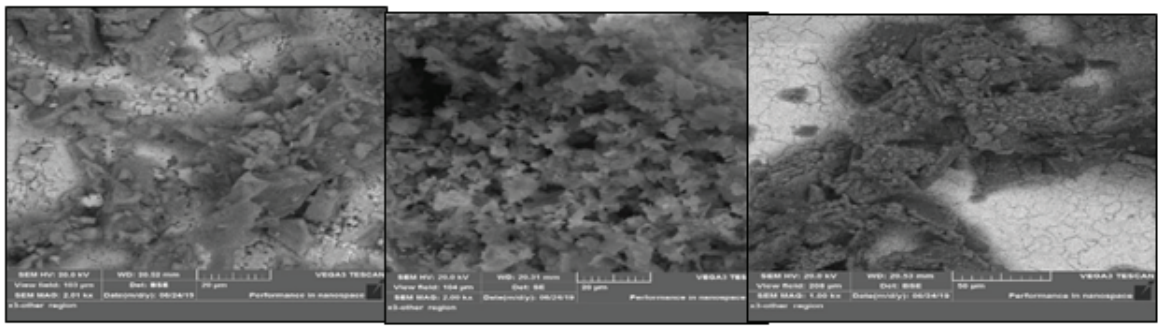

Figure 1: SEM images of the prepared LDHs loaded liraglutide in different measurements.

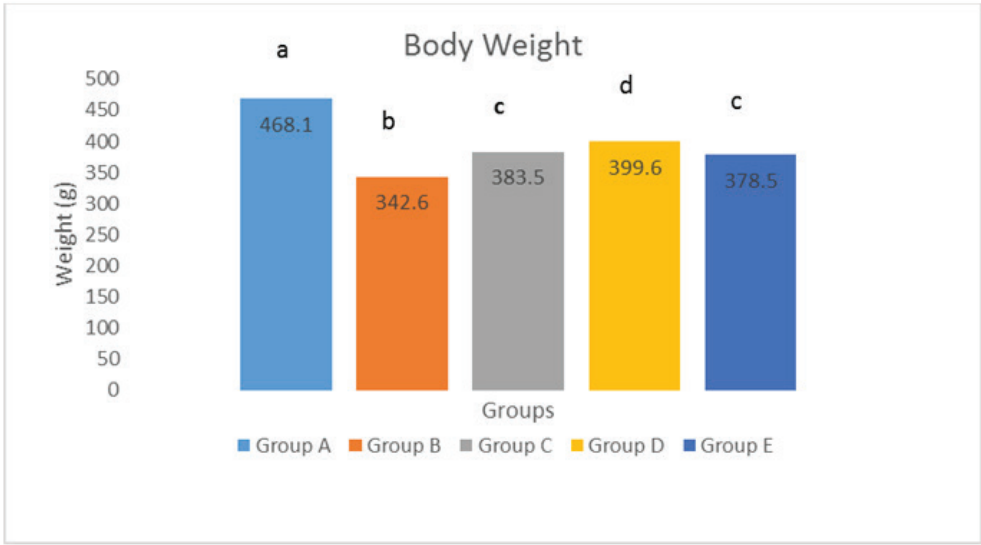

Figure 2: Bar chart illustrate the changes in body weight after four weeks of receiving these prepared compounds:

Different small letters indicate significant differences among the treatments group $p< 0.05$.

Conclusion

1. Characterization and identification of the synthesized compounds were confirmed by determination of FT-IR spectroscopy.

2. The size of the nanocarrier was successfully confirmed by AFM, SEM and XRD.

3. The zeta potential analysis provided a satisfactory evidence about the prepared LDHs little tendency towards aggregation.

4. The Preliminary study of the weight reduction activity by intercalation of liraglutide drug within LDHs NPs via co-precipitation method provide a promising nanocarriers to prolong duration of action of liraglutide.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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