Nanoformulation of *Leonotis leonurus* to improve its bioavailability as a potential antidiabetic drug

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Abstract Nanostructured lipid carriers (NLCs) of *Leonotis leonurus* were successfully produced using high-pressure homogenisation (HPH) on a LAB 40 homogeniser. The particle size was determined for the formulation as well as short-term stability study. The formulation was exposed to Chang liver cells for a glucose uptake study and to INS-1 cells for a chronic insulin release study under normoglycaemic and hyperglycaemic conditions. The particle size of the extract NLC was 220 nm with a PdI of 0.08 after homogenisation at 800 bar. The formulation was stable at the tested temperatures. The extract NLC formulation at 1 µg/ml improved glucose uptake, relative to the control liver cells. Insulin release in INS-1 cells was also elevated under hyperglycaemic conditions when exposed to the NLCs, in comparison with the control untreated cells and the non-formulated extract. The plant extract encapsulated in NLC improved the uptake of glucose and enhanced the insulin sensitivity in vitro, compared to the extract.

Keywords *Leonotis leonurus* · Nanostructured lipid carriers · Type 2 diabetes · Insulin

Abbreviations

NLC Nanostructured lipid carriers
PCS Photon correlation spectroscopy

LD Laser diffractometry
PdI Polydispersity index
HPH High-pressure homogenisation

Introduction

Type 2 diabetes has reached epidemic levels and is increasingly becoming a global health problem (Stumvoll et al. 2005; Zozulinska and Wierusz-Wysocka 2006). In 2011, it was reported that an estimated 285 million people worldwide had diabetes, with this figure set to rise to 595 million in the year 2035 or roughly 10% of the world population (IDF 2013). Insulin resistance, a lack of insulin sensitivity, progressive pancreatic beta cell failure (Skelin et al. 2010) as well as hyperglycaemia (Das et al. 2012b; Preissig and Rigby 2009) have been known to be some of the underlying causes of the disease. The lack of insulin sensitivity often results in the cells not being able to respond to glucose, hence a decrease in glucose uptake, resulting in hyperglycaemia and glucotoxicity (Leahy 2005). Long-term consequences of Type 2 diabetes include, among others, macrovascular complications (e.g. atherosclerosis) and microvascular complications (e.g. retinopathy, neuropathy and nephropathy) (Wilmot and Idris 2014).

Nanosuspensions represent an interesting and potentially useful method for improving the bioavailability of hydrophobic drugs. Nanosuspensions are defined as unique liquid submicron colloidal dispersions of nanosized pure drug particles that are stabilised by a suitable polymer and/or surfactant (Wang et al. 2013). Lipid nanoparticles are made from highly purified lipid blends. The particle size is

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mainly between 150 and 300 nm, smaller sizes, e.g. < 100 nm or larger sizes up to 1000 nm can be obtained as well for special needs (Müller et al. 2011a). Nanostructured lipid carriers (NLCs) consist of a lipid matrix with a nanostructure to improve drug loading and firmly incorporates the drug during storage. The NLCs can be produced by high-pressure homogenisation (HPH), and the process can be modified to yield lipid particle dispersions with solid contents from 30 to 80% (Radtke et al. 2005). NLCs are made from a blend of solid and liquid lipids, with the blend being solid at room temperature. NLCs are commonly used in the pharmaceutical industry as drug formulations to increase the oral bioavailability of poorly soluble drugs (Müller and Keck 2004; Müller et al. 2011b). Smaller particle sizes, in the nanometre range, give a surety of them becoming bioavailable and thus can be administered intravenously without any side effects (Rabinow et al. 2007).

Leonotis leonurus is a shrub which is commonly known as wild dagga and lion's ear by its vernacular names in South Africa and belongs to the family *Lamiaceae* (Van Wyk et al. 2000). The major chemical constituents of the plant are the diterpenoid labdane lactones; premarrubiin; and marrubiin (Laonigro et al. 1979). These chemical constituents have been known to contribute to the anticoagulant (Kee et al. 2008) and antidiabetic properties (Mnonopi et al. 2012a). Studies showed the extract and its constituent, marrubiin, increased insulin sensitivity in INS-1 cells (Mnonopi et al. 2011a). Glucose-stimulated insulin secretion (GSIS) studies on INS-1 cells conducted in our laboratory indicated that the organic extract and marrubiin have the potential to induced insulin secretion under hyperglycaemic conditions (Mnonopi et al. 2012a). Mnonopi et al. (Mnonopi et al. 2011a, b) found that marrubiin increased the gene expression of insulin and GLUT-2 in INS-1 cells. The plant extract has also been shown to possess anticoagulative activity, antiplatelet activity, anti-inflammation effects (Mnonopi et al. 2011b). In the field of phytomedicine, some of the most common problems are that the active components in the extract are present in low concentrations and it is easily degraded if consumed orally as it passes through the digestive tract before being absorbed in the colon as many of the compounds are poorly soluble.

In order to improve the stability and the absorption of the active compounds of *L. leonurus*, this study aimed at producing and characterising a lipid-based nanoformulation of *L. leonurus* leaf extract, i.e. nanostructured lipid carriers (NLCs). Its production, characterisation and antidiabetic properties are investigated in an in vitro cell culture system to determine its biochemical properties.

Materials and methods

Materials

Leaves of *L. leonurus* were obtained and identified by the curator of the herbarium of the Department of Botany, Nelson Mandela Metropolitan University, with reference number 19302. Plantacare® 2000 was obtained from Cognis GmbH (Germany). Sodium chloride (0.9%) solution was from B. Braun Melsungen AG, Germany. TagoCare® 450 and Miglyol® 812 (Caprylic/Capric Triglyceride, Sasol, Germany), Cutina® CP (Cognis, Düsseldorf, Germany), RPMI-1640 (Gibco, South Africa), glutaraldehyde (Merck, Germany), freshly prepared deionised water (Milli-Q Millipore/Merck, South Africa), sodium pyruvate, HEPES and β -mercaptoethanol (Sigma, USA), Chang liver and INS-1E cells (ATCC, VA, USA), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Duchefa Biochemie B.V) were used. D-glucose was from Merck; glucose oxidase reagent consisting of phenol, 4-aminoantipyridine, EDTA, glucose oxidase and peroxidase was from Sigma. Sulfonylurea was a gift from the Department of Pharmacy, NMMU, South Africa).

L. leonurus leaf extraction procedure

L. leonurus was collected at Nelson Mandela Metropolitan University (NMMU) Port Elizabeth (South campus) and identified by Mr C W Thomas (curator of the herbarium, Department of Botany, NMMU). The extraction procedure was a modification of the method used by Light et al. (2002). Briefly, the leaves were rinsed with distilled water and dried at 50 °C in an oven. The dried leaves were crushed and immersed in 10 ml of acetone per gram of plant material. The plant suspension was sonicated for an hour at room temperature and filtered through Whatman No 1 filter paper. The solvent was removed from the filtrate using a rotary evaporator at 60 °C. Thereafter, the residue was freeze-dried and stored in the dark at 4 °C in a desiccator, until further use (Light et al. 2002).

Preparation of the *L. leonurus* NLC

The organic leaf extract of *L. leonurus* was engineered into NLC by using high-pressure homogenisation (HPH) on Micron LAB 40 (batch size 40 ml, APV Deutschland GmbH, Germany). TagoCare® 450 and Cutina® CP (1:8, w/w) were used as the solid lipids. Liquid lipid, Miglyol® 812, was used at a final concentration of 2% w/w and the emulsifier, Plantacare® 2000 UP (decyl glycoside), at 1% w/w concentration. The solid lipids were melted to a

temperature of 80 °C, and liquid lipid was added. This was followed by the addition of the *L. leonurus* extract (1% w/w). The hot lipid solution was added to the aqueous surfactant solution under stirring using Ultra-Turrax T25 (Janke and Kunkel GmbH, Germany) for 30 s at 800 rpm. A total of 40 ml of the formulation was prepared. The solution was then homogenised for five cycles at a pressure of 800 bar and was immediately filled in vials, sealed and allowed to cool down at room temperature.

Photon correlation spectroscopy (PCS) characterisation of the extract NLC

PCS is a widely used technique for measuring the size of particles in the submicron range (Thode et al. 2000). The encapsulated *L. leonurus* NLC particle sizes were determined using a Zetasizer Nano ZS (Malvern Instruments, UK). The results are given as the average weighted diameter of the particles (z-average) and the polydispersity index (PDI), a measure of the width of the size distribution. Readings were completed at 25 °C, and the mean value of ten measurements was obtained.

Laser diffractometry (LD)

LD is a technique for measuring the particle size of nanoparticles and microparticles. The size determinant for the *L. leonurus* NLCs was also performed using a Mastersizer 2000 (Malvern Instruments, UK) in deionised water. The LD volume-weighted diameters $d(v)10\%$, $d(v)50\%$ and $d(v)90\%$ were used to characterise the particle size of the NLCs.

Zeta potential (ZP) measurements

The ZP gives a prediction of the long-term stability that a dispersed system will have. The surface charge properties of the NLCs produced were analysed by using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). The zeta potential was analysed in conductivity adjusted Milli-Q water (50 $\mu\text{S}/\text{cm}$), using 0.9% NaCl solution. Zeta potential was analysed in the original surfactant. All measurements were performed at 25 °C.

Light microscopy

L. leonurus NLC was visualised under light microscope (Orthoplan, Leitz, Germany) on samples immediately after production and also during a storage study (see later section) to visualise possible particle size aggregation. A magnification of 1000-fold was sufficient in the visualisation of particle sizes and aggregates.

NLC observation by scanning electron microscopy

Scanning electron microscope was used for image analysis of the NLCs. A few drops of the dispersion was placed on a stub and fixed in 2.5% glutaraldehyde in 0.1 M PBS (pH 7.4). The sample was placed on a sample holder and vacuum-dried, particles were coated with gold and examined using a scanning electron microscope (Carl Zeiss Evo MA 15), and images were loaded using Vega TC software (Tescan digital microscopy imaging).

Short-term physical stability study

The plant extract encapsulated in NLC was subjected to a short-term stability test to determine physical stability and optimal temperature storage conditions. The NLC product of the *L. leonurus* extract was stored at three different temperatures (4 °C, room temperature, i.e. 25 and 40 °C) for 2 months in sealed vials. During the course of storage, samples were analysed for growth in particle size and changes in surface charge properties (zeta potential analysis). Observations were noted on the day of production, namely the 3rd, 7th, 14th, 28th and 60th day.

Cell culture and maintenance of cell lines

Chang liver cells and INS-1 cell lines represent liver cells and insulin producing β -cells, respectively. These cell lines were used as diabetic models in the following experimentation. Chang liver cells and INS-1 cells were maintained in a controlled humidified atmosphere at 37 °C in 5% CO_2 . Cells were routinely maintained in a 10-cm culture dish until confluent and then seeded in a 48-well plate for experimentation. INS-1 cell lines were maintained in RPMI-1640 supplemented with GlutamaxTM-I (Gibco), 1 mM sodium pyruvate, 10 mM HEPES, 50 mM β -mercaptoethanol and 10% FBS. Prior to experimentation, cells were seeded in a 96-well plate.

Cell cytotoxicity study

Cell cytotoxicity of the *L. leonurus* NLC formulation was investigated using INS-1 and Chang liver cell lines using the MTT assay (Mosmann 1983). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a yellow dye, is reduced to purple formazan in the mitochondria of living cells. INS-1 cells were seeded in a 24-well plate at a density of 30×10^3 in a 24-well plate. The cells were exposed to *L. leonurus* NLC formulation at concentrations of 0.2, 0.5, 1.0, 5.0 and 10 $\mu\text{g}/\text{ml}$, a non-formulated *L. leonurus* extract and an NLC blank. A control sample used only contained culture media. After 48 h exposure, MTT (0.5 mg/ml) was added and incubated at

37 °C for 3 h. DMSO was added to dissolve the crystals formed, and the absorbance was read at 540 nm on a microtitre plate reader (BioTek PowerWave XS microtitre plate reader).

Insulin-stimulated glucose uptake assay in Chang liver cells

Chang liver cells were seeded in a 24-well plate at a density of 2×10^3 cells/well and were allowed to attach overnight at 37 °C. The wells were divided and treated with the *L. leonurus* NLC formulation at varying concentrations: 0.2, 0.5, 1.0, 5.0 and 10 µg/ml. After 48 h of exposure, cells were starved with glucose-free media RPMI 1640 (Hyclone) for an hour. Insulin-stimulated glucose uptake was then determined by incubating the cells in RPMI 1640 containing 8 mM D-glucose and 60 µU/ml insulin for 1 h. Glucose oxidase reagent (phenol, 4-aminoantipyridine, EDTA, glucose oxidase and peroxidase, Sigma) (Young 2001) was used to detect the presence of glucose present by determining the absorbance spectrophotometrically at 492 nm (BioTek PowerWave XS microtitre plate reader).

Insulin secretion in INS-1 cells

Insulin release was investigated over a 48-h period and was conducted using INS-1 cells. Cells (30×10^3 /well) were seeded in 24-well plates. Cells were cultured in both normoglycaemic (11.1 mM glucose) and hyperglycaemic conditions (33.3 mM glucose). The NLC drug dose was added to the cells at a range of concentrations between 0.25 and 10 µg/ml, and treatments were performed for 48 h. The procedure for measuring the insulin content was conducted with a RIA kit (Millipore, US), according to the manufacturer's instructions (www.Millipore.com). Sulfonylurea (gliclazide) (10 µg/ml) is a drug that is used for the treatment of cells to respond to insulin and was used as a positive control for insulin release. A standard curve was constructed using commercial insulin (provided by the kit) in the concentration range 0.25–10 µg/ml.

Statistical analysis

The results are presented as mean $\pm$ standard error of the means (SEM) for the number of determinations (n) indicated. Data was analysed by the student's t test: two samples were assuming equal variance and considered statistically significant for $P < 0.05$. The results were compared to control samples.

Results and discussion

Production of NLC

NLCs consist of a lipid matrix with a special nanostructure that improves drug loading and firmly integrates the drug during long periods of storage. In this study, the properties of the extract NLC and the antidiabetic effects were investigated. Smaller particle size results in a larger surface area as well as greater solubility. This results in an increase in dissolution velocity and bioavailability (Junghanns and Müller 2008). HPH technique was found to be suitable for the production of nanometre particles of the plant extract. The selected pressure and the number of HPH cycles determined the particle size of the lipid nanoparticle. Previous studies have shown that with lipids, higher homogenisation pressures and more homogenisation cycles are not necessary for NLC production (Müller et al. 2011b). This often leads to an additional input that causes high kinetic energy of the droplet resulting in coalescence (Mehnert and Mäder 2001). Thus, the homogenisation cycles were optimised to 800 bar and five homogenisation cycles.

PCS and LD diameters of particles

Figure 1a shows formulation particle size, as measured by photon correlation spectroscopy (PCS) and laser diffraction (LD), respectively. After production, the mean particle size was reported to be 220 nm with a PdI of 0.08. The PdI is an indication of the width of the particle size distribution, and it ranges from 0 to 1. A PdI of < 0.2 is considered to have a narrow size distribution (Das et al. 2012a). The particles generated, as determined by LD $d(v)50\%$, were found to be below 0.2 µm (Fig. 1b), which corresponds to the PCS measurements. The particle size of the $d(v)95\%$ was about 0.4 µm, which indicated that the majority of the particle sizes were in the nanometre range (Fig. 1b). This was confirmed by light microscopy of the final image (Fig. 2a), which illustrates a homogeneous sample after the final preparation of HPH (five cycles). No aggregation or particle clumps was noted at $\times 100$ magnification. Light microscopy is useful for the determination of larger particles sizes over 10 µm which is clearly visible under a light microscope. SEM (Fig. 2b) confirmed these results. Thus, it was concluded that the preparation of the *L. leonurus* NLC was effectively incorporated into the lipid matrix.

Fig. 1 PCS (a) and LD diameters (b) of *L. leonurus* NLC produced by HPH after five homogenisation cycles, at 800 bar at 80 °C. Mean particle size and polydispersity indices (PDI) are shown

Fig. 2 A photomicrograph (a) and a scanning electron micrograph (b) of *L. leonurus* encapsulated NLC. SEM magnification: $\times 4.61$; scale bar = 10 μm

ZP analysis

The ZP measurements of a particle are a good determinant of the stability of a particle and refer to the surface charge of a particle. It indicates the degree of repulsion between close and similar charged particles in a dispersion medium, and this repulsive force prevents the aggregation of particles (Freitas and Müller 1998). ZP measurements of particles are normally performed in water (to determine the stern potential) and in the surfactant medium (to determine the thickness of the diffuse layer). The stern potential relates to the Nernst potential, which gives an indirect determination of the charges of a nanoparticle. Thus, the higher the charge, the more stable a particle will be. During storage of formulations, it is generally observed that at higher temperatures, the sizes of particles grow due to aggregation (Wang et al. 2013). The ZP values are thus also lower. The instability at higher storage temperatures is due to stressful factors which have a destabilising effect,

e.g. reduction in viscosity in stabiliser layers (Moschwitzter 2013; Müller et al. 2011c). The ZP of the *L. leonurus* NLC after production was reported to be -32.7 mV in water, and in the original dispersion medium, -52.3 mV, indicating that they had a good physical stability when prepared and this was maintained during the storage period tested (Fig. 3).

Short-term stability study

Short-term stability test was performed on the *L. leonurus* NLC formulation to determine its physical stability at three different temperatures. This stability test provided a prediction of the shelf life of the nanoformulation as well as the suitable storage temperature. A slight increase in particle size was observed for all the storage temperatures as is noted in Fig. 3. Within 60 days of storage, the particle size of the samples did not show any significant increase. The polydispersity indices of the particles rather remained

Fig. 3 Short-term physical stability profile of the particle size diameter of *L. leonurus* extract NLC at temperatures, 4 °C, room temperature (± 25 °C) and 40 °C at the day of production and at the 7th, 14th, 28th and 60th day. Mean particle sizes are not significantly different at temperatures tested

constant and below 0.3, a good indicator of a homogeneous particle. If samples are not stable during the storage period, it could be attributed to the fact that there was an alteration in chemical composition, e.g. the decomposition of a stabiliser (Mitri et al. 2011). Thus, the samples stored at the different storage conditions were all stable (Fig. 3), indicating that temperature did not have a significant effect on the stability of the NLC. The ZP on the final day of the storage study was reported to be -56.7 , -54.4 and -53.2 at temperatures, 4, 25 and 40 °C, respectively. The surfactants, along with the lipids used for the NLC and nanoemulsion preparation, contributed largely to the stability of the formulations, as reported in the literature for many NLC and emulsion products (Zhang et al. 2014; Wu et al. 2014; Miller et al. 2014; Moschwitz 2013; Gao et al. 2013; Müller et al. 2011a).

Cell cytotoxicity study

To determine the toxicity of the *L. leonurus* NLC, a cytotoxicity assay was performed on both INS-1 β -cells and Chang liver cells. The concentration range of the extract tested was between 0.25 and 10 $\mu\text{g/ml}$. This range was chosen based on the previous study with the *L. leonurus* extract (Mnonopi et al. 2012a). Figure 4a illustrates that there was no significant effect of the nanoformulations on the Chang cells exposed to the formulation when compared to the control cells. A decrease in the number of viable cells was noted in a concentration-dependent manner from 0.25 to 10 $\mu\text{g/ml}$. However, these changes were not significantly observed, in relation to the control. The lowest cytotoxic level obtained was 80% viability of the cells treated with 10 $\mu\text{g/ml}$ plant extract alone. Similarly, the INS-1 cell lines (Fig. 4b) had no significant cytotoxicity when the formulation was

compared to the pure extract and/or the control cells. Studies on INS-1 cell lines (Mnonopi et al. 2012b) used similar concentrations of the plant extract, and no cytotoxic effects were observed, supporting the current findings of the plant extract and the extract nanoformulations having no cytotoxic effect in the cell lines investigated. To determine if the formulation alone had any contribution to cytotoxicity, a blank formulation (NLC B1) was tested and no significant cytotoxicity was observed (Fig. 4a, b). Nanoformulations (nanocrystals, NLC and nanoemulsions) have been prepared for various other medicinal drugs. One such drug is tripterine, which was prepared for delivery as an NLC drug (Chen et al. 2012). The NLC tripterine showed no cytotoxicity when investigated in Caco-2 cell lines. Less cytotoxicity was observed for the NLC tripterine in comparison with the non-formulated tripterine, reducing its side effect in the intestinal cell line model. This evidence supports the current finding of less cytotoxicity observed with the three formulations investigated. The decrease in cytotoxicity which is often associated with most nanoformulated drugs can be explained by their controlled release once administered to their target tissues (Das et al. 2012b).

Insulin-stimulated glucose uptake in Chang liver cells

As the concentrations tested showed no cytotoxicity, their ability to affect glucose uptake on Chang liver cells was investigated. Firstly, insulin-induced glucose uptake was performed and indicated an elevated uptake of glucose into the cells which were significantly relevant to the control. A concentration range of the *L. leonurus* NLC from 0.25 to 10 μg was found to compare favourably to the *L. leonurus* extract (Fig. 5). Metformin, the positive control, is a drug that is used for the treatment of hyperglycaemia in patients with T2DM (Foretz et al. 2010; Mahrouf-Yorgov et al. 2009). Treatment of the cells with this drug showed a significant increase ($P < 0.01$) when compared to the untreated control cells. All the treatments showed a significant increase ($P < 0.05$) in glucose uptake in relation to the untreated cells. We observed that at the highest concentration of the extract NLC formulation (10 $\mu\text{g/ml}$) increased glucose uptake by 18% relative to the control. A similar effect was observed for the non-formulated extract. Metformin increased glucose uptake significantly in comparison with the formulations and in comparison with the control ($P < 0.01$) by 51%. The plant extract NLC reported a slight increase in insulin-stimulated glucose uptake, in relation to the extract non-formulated; however, this change was not significantly observed. The Chang liver cells were normalised to 50,000 cells/well using the MTT assay using cell numbers to obtain a standard curve (data

Fig. 4 MTT assays of *L. leonurus* extract NLC (0.25–10 µg/ml). Extract concentration used was 10 µg/ml. NLC blank formulation at 10 µg/ml in INS-1 cells (a) and Chang liver cells (b), Met metformin, Ext *L. leonurus* extract

Fig. 5 Insulin-stimulated glucose uptake measured in the presence of 60 µU/ml insulin and *L. leonurus* NLC (0.25–10 µg/ml). Met (1 µM) was used as a positive control and the Ext that represents extract (10 µg/ml) was used as a reference treatment in relation to the formulations. The untreated samples only had the presence of media. Error bars represent $\pm$ SEM of $n = 3$, * $P < 0.05$ versus untreated (control). # $P < 0.01$ versus untreated (control)

not shown). The results of this study provide novel findings of glucose uptake in cell lines by the *L. leonurus* extract NLC. The extract NLC was able to stimulate glucose uptake in Chang liver cells.

Insulin secretion in INS-1 cells

The effect on insulin secretion was performed on INS-1 cells, a model for β -cells. Insulin secretion occurs when glucose is metabolised in the β -cells and the ATP/ADP ratio increases. This leads to closure of ATP-sensitive K^+ -channels, depolarisation of the cell membrane and opening of voltage-gated Ca^{2+} -channel resulting in a rise in intracellular Ca^{2+} , which promotes insulin secretion (Ashcroft et al. 1994). Based on the experimental procedures of previous findings (Mnonopi et al. 2012a), we performed a similar experiment whereby the chronic insulin stimulation was determined after 48 h of exposure with the extract

NLC under normoglycaemic (11.1 mM glucose) and hyperglycaemic (33.3 mM glucose) conditions (Fig. 6). The highest concentration of the formulation 10 µg/ml showed a significant increase ($P < 0.05$) in insulin stimulation in the INS-1 cells when compared to the control hyperglycaemic condition. The positive control, as well as the non-formulated extract, showed a significant increase ($P < 0.05$) in insulin stimulation in relation to the control hyperglycaemic condition. Under normoglycaemic conditions, the extract NLC (10 µg/ml) and the positive control (sulfonylurea) showed an increase in insulin secretion, relative to the control untreated. There was an increase in insulin secretion in a dose-dependent manner (0.25–10 µg/ml), under normo- and hyperglycaemic conditions. However, the increase was not significantly observed, in comparison with the control normoglycaemic group. The insulin increase under hyperglycaemic conditions was significantly observed for the NLC extract at 0.25 µg/ml in

Fig. 6 Chronic insulin release in INS-1 cells completed under normo- and hyperglycaemic conditions for a 48-h exposure of the NLC at varying concentrations (0.25–10 µg/ml). Data are expressed as the mean $\pm$ SEM ($n = 3$), # $P < 0.05$ versus control normoglycaemia (11.1 mM) treatment, * $P < 0.05$ versus untreated (control) hyperglycaemia (33.3 mM). Sul sulfonylurea, positive control. Ext *L. leonurus* extract 10 µg/ml

comparison with the normoglycaemic insulin increase at the same concentration. An extract from *Leonotis sibiricus* has also been shown to enhance insulin secretion in INS-1E cells and also to promote proliferation of the cells (Schmidt et al. 2013). This supports the current findings of the effect of other *Leonotis* species on insulin release. The increase in insulin release by the extract NLC could be due to the small particle size and bioavailability of the formulation (Jin et al. 2013), which results in an effective cellular uptake to achieve its effect. Subsequently, the solubility, the dissolution rate and the bioadhesion to the cells or tissues are improved (Gao et al. 2013).

Our observation shows that the formulated plant extract was able to promote the secretion of insulin in INS-1 cells at 33.3 mM in a concentration-dependent manner. The formulation may thus be a potential means of treatment or administration for patients suffering from Type 2 diabetes, a formulation providing increased solubility and bioavailability. These findings, therefore, provide a starting point for the development of improving delivery of not only *L. leonurus*, but other medicinal plant extracts.

Conclusion

We were able to prepare a nanoformulation from the organic leaf extract of *L. leonurus* that was stable with a particle size in the nanometre range. The formulation was not cytotoxic to the Chang liver cells and INS-1 cells at the concentration range tested. The formulation did not alter the previous biological activity obtained from in vitro studies as it was able to increase the uptake of glucose in Chang liver cells as well as being able to stimulate insulin release in INS-1 cells under hyperglycaemic conditions, as determined by the insulin release.

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Compliance with ethical standards

Conflict of interest The authors report no conflict of interest for this work.

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