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Preparation and Evaluation of Oral Capsule-Based Niosomes for the Anti- Cancer Drug Axitinib

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Abstract

Niosomes are bilayer vesicles formed by self-assembling non-ionic surfactants. They act as a potential oral drug delivery system for poorly soluble drugs to enhance their dissolution and oral bioavailability. Axitinib, a tyrosine kinase inhibitor, works by blocking the vascular endothelial growth factor receptors (VEGFR), which suppress tumor growth. It is classified as a class II drug, which has poor solubility and high permeability. In this study, axitinib (AXT) capsule-based niosomes were formulated by the thin film hydration method as liquid niosomal dispersion using span 60: cholesterol (1:1 weight ratio), 5mg dicetyl phosphate (DCP) and 5 mg drug, with an entrapment efficiency of 96.5% and a particle size of 64.5nm. They were then converted to dry powder via silicified microcrystalline cellulose (SMCC) and evaluated for flow properties, in-vitro drug release, permeation study, cytotoxicity study and bioavailability investigation. The results showed good flow properties of the powder content of prepared capsule-based niosomes. The drug release from capsule-based niosomes was rapid in the initial phase, where 40.61% of the drug was released from capsule-based niosomes in an acidic medium (stomach) followed by continuous release (in the intestine), and compared with the drug release of marketed tablets. The permeation results showed enhancement of drug permeation when prepared as noisomes. The study of the in-vitro cytotoxicity effect showed that the cytotoxic effect of prepared capsule-based niosomes is significant ($p<0.05$) and higher than that of pure drugs, increasing with time. The bioavailability investigation of capsule-based niosomes compared with marketed tablets revealed that capsule-based niosomes enhanced the oral bioavailability of axitinib. The pharmacokinetic results showed that the capsule-based niosomes revealed a higher peak drug concentration (C_{max} = 5.23±0.07 µg/ml) with a maximum peak time (T_{max} =4 hrs). In contrast to the C_{max}= 4.8±0.025 µg/ml and T_{max} = 6 hrs for conventionally marketed tablets, the AUC 0-24 for capsule-based niosomes was 70.092 µg/ml.hr, which is highly significant ($p<0.05$) in comparison with the AUC 0-24 for marketed tablets, which was 53.714 µg/ml.hr. This substantial difference suggests improved drug solubility dissolution, eventually improving oral bioavailability when loaded in niosomes. The results indicated that niosomes are successful nano-carriers for enhancing drug dissolution and bioavailability.

Keywords: Axitinib, Anti-cancer, Bioavailability, Niosomes, Oral capsule.

Introduction

The success of most cancer chemotherapy depends on many factors, such as the active agent, patient status, chemotherapy schedule, dosage form and route of drug administration [(1)].The administration of the drug by the oral route is widely prevalent due to several advantageous factors, including the convenience associated with oral drug administration, patient preference, cost-effectiveness and the ease of large-scale manufacturing of oral dosage forms, as the adherence of patients to oral formulations appears to be somewhat higher compared to parenteral strategies or inhalation dosage forms [(2)] .With these advantages, the improvement of oral formulations confronts various problems, such as the physicochemical characteristics of drugs [(3)], limited water solubility and membrane permeability, first-pass metabolism and drug efflux resulting in erratic absorption and low oral bioavailability [(4)].

Niosomes are one of the drug delivery systems, defined as non-ionic surfactant vesicles that contain an aqueous phase and can encapsulate a wide variety of molecules within the aqueous spaces and their lipid membranes [(5)].This vesicle of drug delivery provides many advantages compared to alternative conventional methods, such as dose reduction, enhanced drug stability and compatibility with non-ionic surfactants, accessible modification, and delayed clearance [(6)].Applying colloidal nano-carriers as an oral drug delivery system improved the surface area, enhanced the drug dissolution rate, and

substantially improved the oral absorption process [7].Axitinib belongs to the specific second-generation inhibitor of vascular endothelial growth factor receptors (VEGFR1, 2, and 3), inhibiting the growth of new blood vessels in several types of tumours such as metastatic renal cell carcinoma (mRCC), thyroid cancer, and non-small cell lung cancer [8] , with absolute bioavailability (58%) [9]. This study prepared axitinib as oral hard gelatin capsule-based nano-sized niosomes (no previous study developed this anti-cancer drug as oral capsule-based niosomes) to enhance drug solubility and dissolution. Then, the contribution of the niosomes in improving oral bioavailability will be studied through an in-vivo study using animal models for such prepared capsules compared to the available marketed conventional tablets with similar doses.

Materials and method

Materials

Axitinib, span 60, cholesterol, dicetyl phosphate (DCP), silicified microcrystalline cellulose (SMCC), and anhydrous calcium chloride were purchased from Tunchem Pharm Company, China. Monobasic sodium phosphate, dibasic sodium phosphate, and tribasic sodium phosphate were purchased from India (Alpha Chemika). Organic solvents (methanol, chloroform) were obtained from Merck (Darmstadt, Germany)—other chemicals with analytical grade.

Preparation of niosomal powder from the niosomal dispersion

A liquid niosomal dispersion (containing span60: cholesterol 1:1 weight ratio, 5 mg of dicetyl phosphate (DCP), and 5 mg of axitinib) was prepared by the thin film hydration method by dissolving the cholesterol, surfactant, and DCP in an organic solvent (a mixture of chloroform and methanol in a proportion of 1:1 v/v) in a rounded bottom flask. The drug was dissolved in 10 mL of methanol and added to the first mixture. The organic solvent was evaporated using a rotary evaporator rotated at 130 rpm at 60°C till a smooth, dry lipid film was formed. The film was then hydrated with 10 ml of phosphate buffer pH 7.4 at 60°C, rotated for 1 hour, and sonicated using ultrasound probe sonication for 4 minutes.

The obtained milky dispersions were kept in a refrigerator at 4°C [10]. The entrapment efficiency of niosomal dispersion was determined using the centrifugation method, where the formula was placed in an eppendorf tube and then rotated for 60 minutes at 10000 rpm at 4°C using a cooling centrifuge to get a pure supernatant. HPLC analysis determined the free drug in the supernatant [11]. The dispersion was converted into a powdered form by adding the liquid niosomal dispersion to 300 mg of silicified microcrystalline cellulose (SMCC) powder [12]. The resulting mixture was then left for 15 minutes at room temperature, followed by agitation using a vortex mixer for five minutes. Subsequently, the mixture was left overnight in a desiccator that contained anhydrous calcium chloride until complete dryness [12]. The powder obtained was filled in hard gelatine capsules size 0 [13].

Flow properties determination for the dried powder

A. The measurement of the angle of repose

The angle of repose for the powder formulation was determined using the fixed funnel method. In this method, a funnel was placed at a fixed distance of approximately two centimetres above a circular piece of paper set horizontally. The powder was poured down the funnel, then the diameter of the powder cone was determined, and subsequently, the angle of repose (θ°) was obtained employing the following equation [14].

$$\tan\theta = \frac{h}{r} \quad \text{..... Equation. (1)}$$

Where: h = powder cone height; and r = powder cone radius.

b- Compressibility index or Carr's index

This approach involves carefully pouring the powder into a graduated cylinder, measuring its mass and volume, and then computing the bulk density. After that, the powder settles after 100 taps on the cylinder. The process of tapping causes a change in density, or compressibility [15]. The following equation may be used to compute it and associate it with flow.

$$\text{Carr's Compressibility index} = \frac{\text{Tapped Density (T)} - \text{Bulk density(B)}}{\text{Tapped density}} \quad \text{.... Equation....(2).}$$

Where: B = freely settled bulk density of the granules and

T = tapped bulk density of the granules. Powders with a Carr's index greater than 25 are considered to have poor flowability [15].

c- Hausner ratio

It is a ratio of a powder's tapped bulk density to its poured (treated) bulk density. This ratio can be applied to provide an index of the flow character of a powder. In order to calculate the Hausner ratio (H), the tapped bulk density (ρt) and aerated bulk density (ρa) of a powdered substance are obtained according to the following equation [16].

$$H = \frac{\rho t}{\rho a} \quad \text{.....Equation. (3)}$$

d- Study the morphology of the produced niosomes

Field emission scanning electron microscopy (FE-SEM) was employed to study the shape and morphology of niosomes. It gave an idea about the surface structure of the niosomes and measured the size of the vesicles [17].

Study the in-vitro drug released from the prepared capsules

To study dealt with the drug release profile of the prepared capsule-based niosomes, USP dissolution apparatus II (paddle type, Copley dissolution 8000, UK) was used in comparison to the marketed axitinib (Inlybest®), where the capsule and marketed tablet were immersed (separately) in a jar containing 500 ml of 0.1 N HCl (pH 1.2) containing 2% sodium lauryl sulphate (SLS) and the release was followed for 2 hours, then adjusted the pH to 6.8 via tribasic sodium phosphate and the release followed for 4 hr. The temperature was maintained at 37 °C while the paddle was set to rotate at a speed of 100 rotations per minute. Samples of 5 mL were taken out and replaced with a newly prepared medium at predetermined time intervals. Subsequently, [18]. The sample was subjected to analysis for the content of the drugs using HPLC. The sample was subjected to analysis for drug content using an HPLC-UV detector (the mobile phase is composed of methanol: water at 85:15% v/v) in column C18 (250×4.5 mm, 5 µm) with a flow rate of 1.0 ml/min. The temperature was maintained at 30°C, the injection volume was 20 µl, and the detection of the sample was done at 330 nm [11].

FTIR Spectroscopy

A FTIR study was done to determine the compatibility and interactions between the drug substance and excipients of the powder-based niosome capsules, where the powder-based niosomes, pure drugs, span60, cholesterol, DCP, and SMCC were mixed with potassium bromide (each one separately) and compacted to form a disc. The disc was investigated by FTIR spectroscopy (IR Affinity-1, Shimadzu, Japan), where the spectral range was 4000 cm^{-1} to 400 cm^{-1} [19].

Differential Scanning Calorimetry (DSC) analysis

The differential scanning calorimetry (DCS) technique is employed for thermal investigation to assess axitinib's physical characteristics in its solid-state formulation and other content (cholesterol, span 60, DCP, and SMCC) to evaluate its compatibility. The procedure for the experiment involves the placement of the solid sample within hermetically sealed aluminium pans, followed by the gradual increase of temperature within the ambient temperature range to 400°C. This temperature elevation is carried out at a consistent scanning rate of 10 °C per minute under constant nitrogen purging of 20 ml/min at a rate of 20˚C per minute in the differential scanning calorimetry (DSC) device (131 Evo, Setarm, France) [20].

Drug content determination

The drug content of the prepared capsules was determined by HPLC (Sykamn, Germany), with the mobile phase composed of (Acetonitrile:0.01 M KH_2PO_4 of 60:40 V/V), using column C18 (250×4.5 mm, 5 µm) with a flow rate of 1.0 ml/min. The temperature was maintained at 30°C [21], the powder was sonicated with 10 ml of methanol for 30 minutes, then filtered, and the drug concentration was measured [22]. The percent of drug content was calculated from the following equation [23].

$$\% \text{ Drug content} = \frac{\text{Actual amount}}{\text{theoretical amount}} \text{x}100 \ldots.$$

Equation…….. (4)

Ex-vivo permeation test

This investigation was carried out on marketed tablets that used Franz cell technology and powder-based niosome capsules. The small intestine of the young male sheep was removed, cleaned, and socked in PBS (pH 7.4) at 5±3○C for 12 hours. The small intestine was then sliced into 3.5 cm pieces and mounted on the Franz cell's glass ring [24] , after the remaining corners were trimmed. With the apical surface in contact with the donor chamber and the basal surface in contact with the receiving medium, the intestine segment was positioned between the donor and receptor compartments. Within the receptor compartment were 30 mL of pH 7.4 PBS.

In comparison, the donor compartment was filled with powder-based niosome capsules equivalent to 5mg of axitinib and marketed tablets (each separately) after adding 10 ml of PBS pH 6.8 containing 2% SLS. The cell contents were stirred using a magnetic bar at 100 rpm, and the temperature was maintained at 37 ±1°C [25]. Aliquots of 5 ml were withdrawn (from the receptor compartment) at regular intervals and replaced with an equivalent amount of fresh medium. The amount of drug permeated was quantified using HPLC. The cumulative drug amount was plotted against time to estimate the percentage of the dose permeated [25].

Cytotoxic activity of axitinib capsules-based niosomes

A549, a cell line derived from lung carcinoma, was procured from the American Type Culture Collection (ATCC) located in Middlesex, United Kingdom. It was preserved in the cell bank of Mustansiriyah University's Biomedical Research Centre in Iraq.

Cell Viability and Inhibitory Concentration (IC50) by MTT Assay

To determine the vitality of A549 cancer cells, prepared niosomes for axitinib and the drug in its pure form were utilised in conjunction with the MTT assay. The cell suspensions (A549) were dispensed at concentrations of 5 x 103 cells per well into 96-well flat-bottom tissue culture plates (Falcon, USA). Under standard conditions, the plates were incubated for 24 hours. For 48 hours, the plates were incubated with 3 x 103 cells per well. All incubation times were maintained at 72 hours. A549 cells at 90-100% confluency were trypsinized by 0.25% trypsin in phosphate-buffered saline (PBS, pH 7.4), seeded into a 96-well plate at a density of 5000 cells per well, and incubated at 37ºC in a 5% CO_2 atmosphere for 24 hours. Serial dilutions of

working concentrations were prepared, ranging from 500 to 1 µg/ml. When cells reached 50-60% growth, they were treated with either pure axitinib (dissolved in dimethyl sulfoxide (DMSO) and subsequently diluted with the culture medium) or capsule-based niosomes. The 96 well plates were incubated in the cell culture incubator for 24, 48, and 72 hours. Subsequently, a solution of MTT (5 mg/mL) was introduced into each well. The supernatants were extracted from the wells, after which the formazan crystals were dissolved with dimethyl sulfoxide (DMSO). The optical density (OD) was then measured using a microplate reader at 560 nm and 600 nm wavelengths for the blank sample [26].

Dimethyl sulfoxide (DMSO) was employed as a negative control in the experimental setup, including cells treated with pure axitinib. Conversely, untreated cells were utilized as the negative control in the experimental setup involving cells treated with axitinib niosomes. The dose-response curve, time response curve, and IC50 value were obtained using GraphPad Prism software, version 9.0. The cell viability percentage was calculated using the following formula:

$$\textbf{Cytotoxicity}\% = 100 - \text{Cell viability} \quad \text{Equation (5).}$$

$$\textbf{Cell viability}\ \% = \frac{\text{OD560}-600(\text{sample})}{\text{OD560}-600(\text{control})} \times 100 \quad \text{Equation (6).}$$ [27]

Bioavailability investigation

The study protocol received approval from the animal care committee at the Iraqi National Centre for Drug Control and Research. In this study, three male rabbits, with weights ranging from 1.5kg to 2kg, were captured in a rabbit hutch to collect blood samples.

The rabbits were fasted overnight for 12 hours with free access to water and a washout period of 2 weeks. During the investigation day, the rabbits were arranged in a horizontal position on a table. The oral dose for rabbits was determined according to (equation 7 below) and subsequently administered to the animals orally via gavage at 8:00 a.m. [28].

The experiments were conducted as a cross-sectional animal study. The experiment included the administration of axitinib conventional tables (containing 10 mg of drug) dispersed in 10 ml of phosphate buffer pH 7.4, administered orally to rabbits, followed by collecting (1 ml) of blood samples taken from the marginal ear vein at 0.5, 1, 2, 4, 6, 12, and 24 hr. using EDTA tubes to prevent the coagulation process. Samples were centrifuged for plasma separation. The separated plasma was immediately put in a refrigerator and kept at freezing temperatures until the samples had become ready for analysis [29,30]. Following a washout period of 2 weeks, the capsule-based niosomes (containing 10 mg of axitinib) dispersed in 10 ml of phosphate buffer pH 7.4 were administered orally to the same set of rabbits. The analysis of the samples was done by HPLC [31]. To prepare the plasma sample for HPLC analysis, 250 µL of plasma, 50 µL of internal standard (crizotinib), and 2 mL of acetonitrile was added. The above mixture was subjected to the cyclomixer for 15 seconds, vortexed for 2 minutes, and finally centrifuged for 3 minutes at 3200 rpm. After centrifugation, the organic layer was collected, and 10 µl was directly injected into HPLC [31].

The calculations of the oral dose for rabbits were conducted using the body surface area (BSA) normalization approach and the human equivalent dose (HED) of pharmaceuticals, which incorporates the species-specific factor. This factor, indicated as Km, is calculated by dividing the body weight of the rabbit in kilogrammes by its BSA in square metres. The equation used for this calculation is as follows:

$$Animal\ equivalent\ dose\ (\frac{mg}{kg}) = Human\ dose(\frac{mg}{kg}) \times Human\ Km/ Animal\ Km \ldots.\ \text{Equation} \ldots\ldots\ (7).$$

The maximum effective single daily dose of axitinib for adult humans was 0.167 mg/kg and 0.515 mg/kg for rabbits, the values of Km for adult humans and rabbits were 37 and 12, respectively [32].

Statistical analysis

Data are expressed as the mean ± standard deviation. Statistical analysis was conducted using SPSS software (Statistical Packages for Social Sciences, version 24). Unpaired *t*-test and one-way analyses of variance (ANOVA) were used to compare between two and multiple groups, respectively. $P < 0.05$ was considered significantly different.

Result and discussion

Flow properties determination for the dried powder

The results of the angle of repose, Carr's index, and Hausner ratio are shown in **Table 1**. The results showed good flow properties of the dried powder, attributed to the presence of SMCC in the formulation, which is characterized by excellent flow properties. It gives satisfactory tensile strength with less compaction force and absorbs much less moisture from the environment [33].

Table 1. Flow property parameters of the prepared dried powder-based niosomes

Repose Angle (θ)	Flow property	Carr's index	Flow property	Hausner ratio	Flow property
32.3± 1.1*	good	12±0.58*	good	1.12±0.01*	good

* n=3 (experiments were done as triplicate), mean± SD

Study the morphology of the produced niosomes.

The FE-SEM photograph of the produced niosomes presented in **Figure 1** showed that niosomes were spherical in shape without aggregation, with a smooth surface and a diameter within the nanoscale [34].

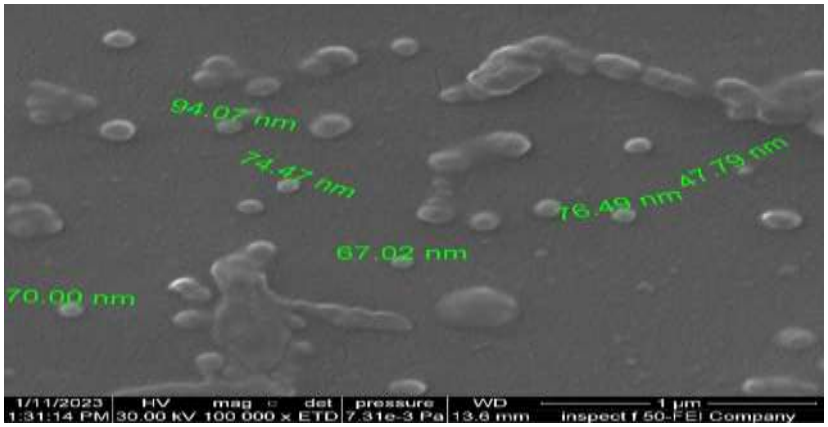

Figure 1. Morphology of niosomes by field emission scanning electron microscopy (FESEM)

Study the in-vitro drug release from the prepared capsules

The drug was released completely from conventional tablets in an acidic medium (0.1N HCl), where 100% of the drug was released within 20 minutes, while the release from capsule-based niosomes was biphasic, with a fast initial phase where 40.45% of the drug was released within 2 hours in the acidic medium, as shown in **Figure 2.** Due to that, the drug is entrapped in the lipophilic region of niosomes (nisomes act as protective carriers for the drug), so it was not released completely in stomach media. However, it is highly soluble in an acidic environment [35,36] followed by slow continuous release from niosomes in the intestinal medium (100% of the drug released within 4 hours). The initial phase of release may be attributed to the desorption of the drug from the outer surface of the niosomes. In contrast, the slow, continuous phase is probably dependent on the gradual diffusion of the drug across the membrane. [37], the niosome vesicles act as depots to release the drug slowly [38].

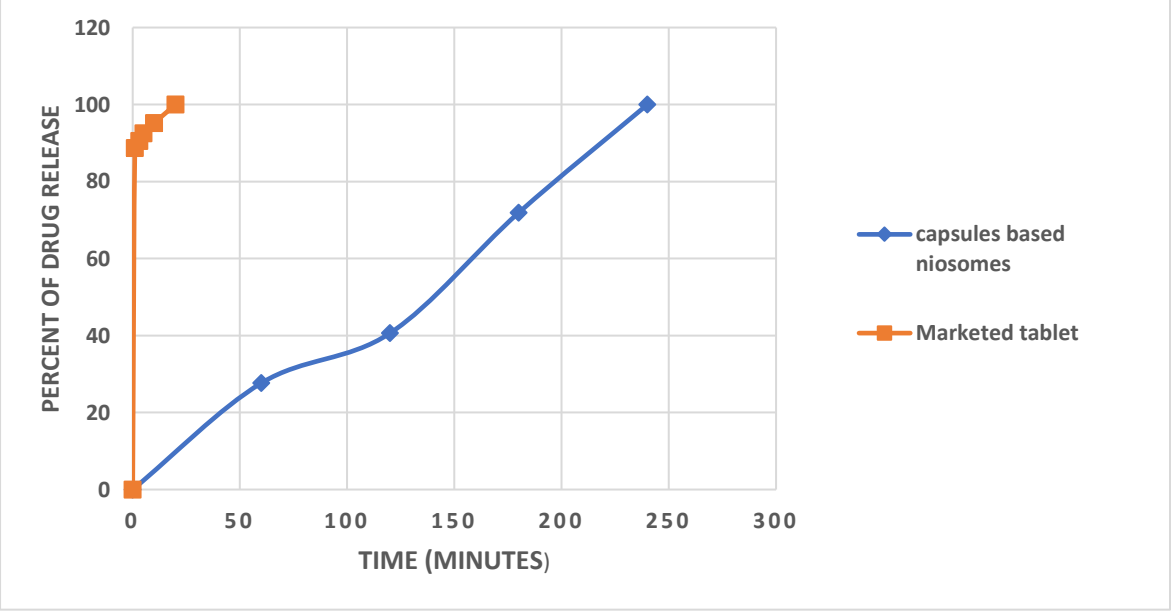

Figure 2.In vitro drug release profile of axitinib capsules based niosomes and marketed tablet (n=3 (experiments were done as triplicate), mean± SD

FTIR spectroscopy

Axitinib exhibits characteristic absorption peaks [39], as shown in **Table 2**.

Table 2. The characteristic absorption bands of pure axitinib by FTIR

Main characteristic bands for pure drug (axitinib)	Absorption bands groups in pure Axitinib	Absorption bands group of axitinib in dried powder	Range of absorption spectrum
C=O (Amide C=O-NH)	1635 cm^{-1}	1647	(1600-1800)
N-H	3259cm^{-1}(3200-3400)	3336.85	(3200-3400)
C=C (Aromatic)	1585cm^{-1}(1600-1800)	1554.63	(1600-1800)

Possible overlap between the N-H stretching band of the pure drug axitinib and a hydroxyl group (OH) of other niosome components led to H-bond formation and shifting in the amide absorption band from 1635 to 1647 as well as the amine band from 3259 to 3336.85 of the pure drug in dried powder-based noisomes (due to the presence of the OH group in span60 and cholesterol), which confirms the successful encapsulation of axitinib by the niosomes and forms the vesicles [40]. Also, the relative drug amount in the formulations was small compared with other components, which could cause a lack of the characteristic peaks of pure drugs. Also, more than one band shared the same region of the spectrum. However, the presence of the main peak position (amide C=O-NH) of the pure drug in dried powder (content of the capsule) implies the absence of the interaction between the excipients and the medication [41], as shown in **Figure 3.**

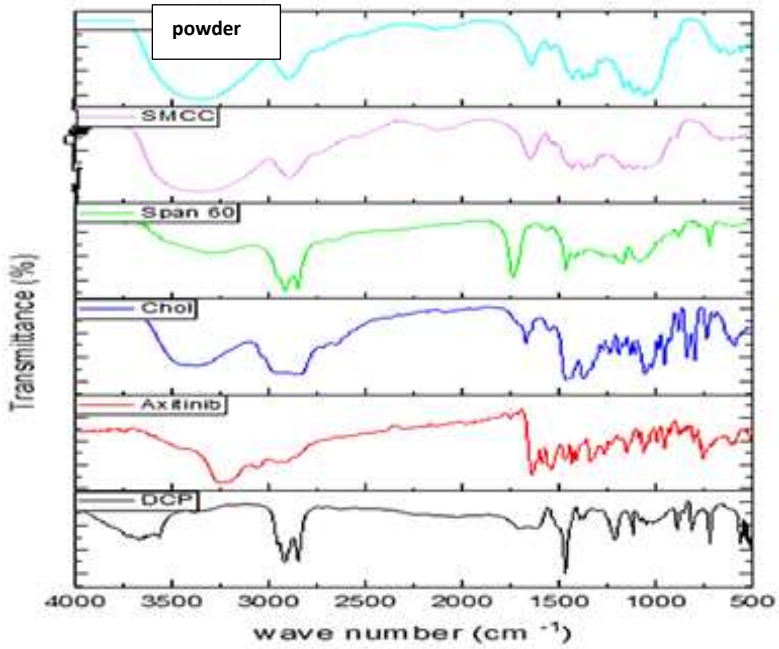

Figure 3. FTIR spectrum of the pure drug (axitinib), cholesterol, span60, DCP, SMCC, and prepared powder content of capsules-based niosomes.

Differential scanning calorimetry (DSC) analysis

The DSC analysis for powder content of capsule-based niosomes, pure axitinb, span60, cholesterol, DCP, and SMCC is shown in **Figure 4.** The drug in its pure form had a distinct peak at 225°C, which aligns with its known melting point. This observation serves as an indication of the drug's purity upon receipt. While the powder content of capsule-based niosomes exhibited broad endothermic peaks at 88°C and 248°C, the absence of the endothermic peak of the pure axitinib from DSC curves of the powder content of capsule-based niosomes suggested the incorporation of the drugs into the niosomes with an amorphous property that was homogeneously and molecularly dispersed in the nanoparticle matrix. Also, the dilution effect was considered an additional factor in the drug peak disappearing. Similar results were observed with acyclovir niosomes [42].

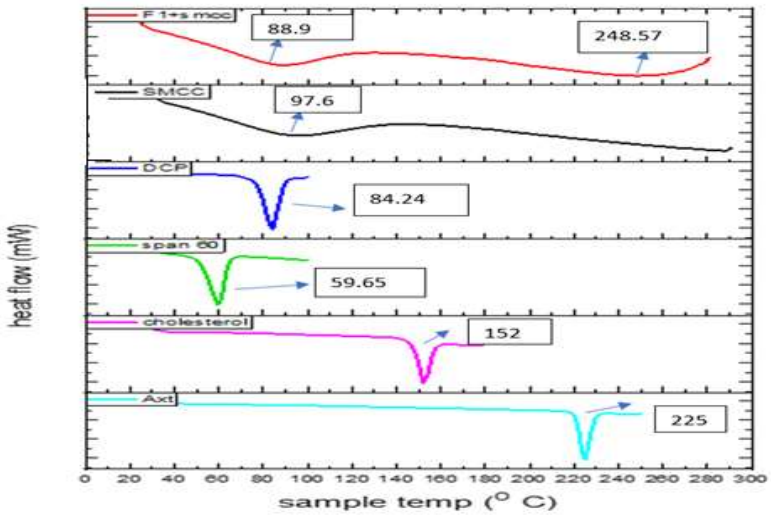

Figure 4. DSC thermograms of pure drug (axitinib), span60, DCP, cholesterol, SMCC, and prepared powder content of capsule-based niosomes.

Drug content determination

Generally, the determination of drug content is essential to ensuring the presence of an effective therapeutic dose. The result of the percent of drug content in axitinib niosome capsules was 97.23%, which agreed with USP requirements [43], indicating the drug content uniformity in the prepared capsules and the adequacy of the preparation method [44].

Ex-vivo permeation study

The test was conducted for the powder content of capsule-based niosomes and marketed tablets. The results in **Table 3** revealed that the drug's intestinal permeability was found to be significant ($p < 0.05$, $p = 0.0021$) higher in powder-based niosomes than in marketed tablets. The results showed that after four hours, 78% of the drug in powder-based niosome capsules had permeated the intestine, compared to 58% of it permeated in the marketed tablets. The permeation enhancement of the drug for the content powder of capsule-based niosomes indicated the role of vesicles (niosomes) in improving the drug's intestinal permeation because of the vesicles' large surface area for interactions and their nanoscale particle size. Thermodynamic interactions between these vesicular niosomes and the intestinal membrane may be the mechanism underlying the improved drug penetration and absorption. [45,46].

Table 3. Permeation study parameters of the powder content of capsule-based niosomes and marketed tablets

parameters	Marketed tablets	Powder content of the capsule-based niosomes
Permeated amount after 4 hr (mg/cm^2)	0.923±0.043*	1.242±0.12*
Permeation percentage	58%	78%

***** n=3 (experiments were done as triplicate), mean ± SD.

Cytotoxic Activity of axitinib capsules based niosomes comparison with pure drug

A549 cells were treated with a pure DMSO dissolved in DMSO, with the knowledge that DMSO exhibited negligible or no cytotoxic activity (as measured by inhibition rate percent IR%) towards both normal and tumour cells. Axitinib-loaded niosomes showed a lower IC50 (80.26 µg/ml) than pure axitinib (IC50= 120 µg/ml), as shown in **Figure 5.** Similar findings were obtained with noisome loaded with gemcitabine and cisplatin for the treatment of lung cancer cells [47].

The dose-response curve **(Figure 6)** shows that axitinib capsule-based niosomes' cytotoxicity is higher than pure axitinib. The cytotoxicity increased with increased concentration, so at the concentration of 500µg/ml of capsule-based noisome, the percentage of cell survival is 15.675%. In comparison, the cell survival percent of the pure drug was 25.987% at the same concentration, the same result observed with niosomes loaded with nintedanib against non-small-cell lung cancer [48].

The time-response curve **(Figure 7)** showed that the cytotoxicity of prepared capsule-based niosomes was higher than that of pure axitinib and increased with time. The percent of cell survival of capsule-based niosomes in concentration 250 µg/ml at 24 hr. was 25.87%, and at 72 hr., it was 18.78%. In comparison, the cytotoxicity of the pure drug at 24 hr was 30.35%, and at 72 hr, it was 25.56% at the same concentration. The same results were observed with buckysomes loaded with pemetrexed against non-small-cell lung cancer [49].

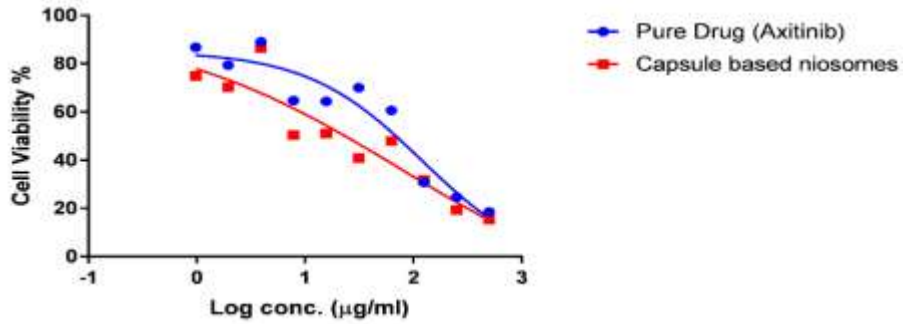

Figure 5. IC 50 determination for the prepared axitinib capsules based on niosomes and pure axitinib on A529 cells.

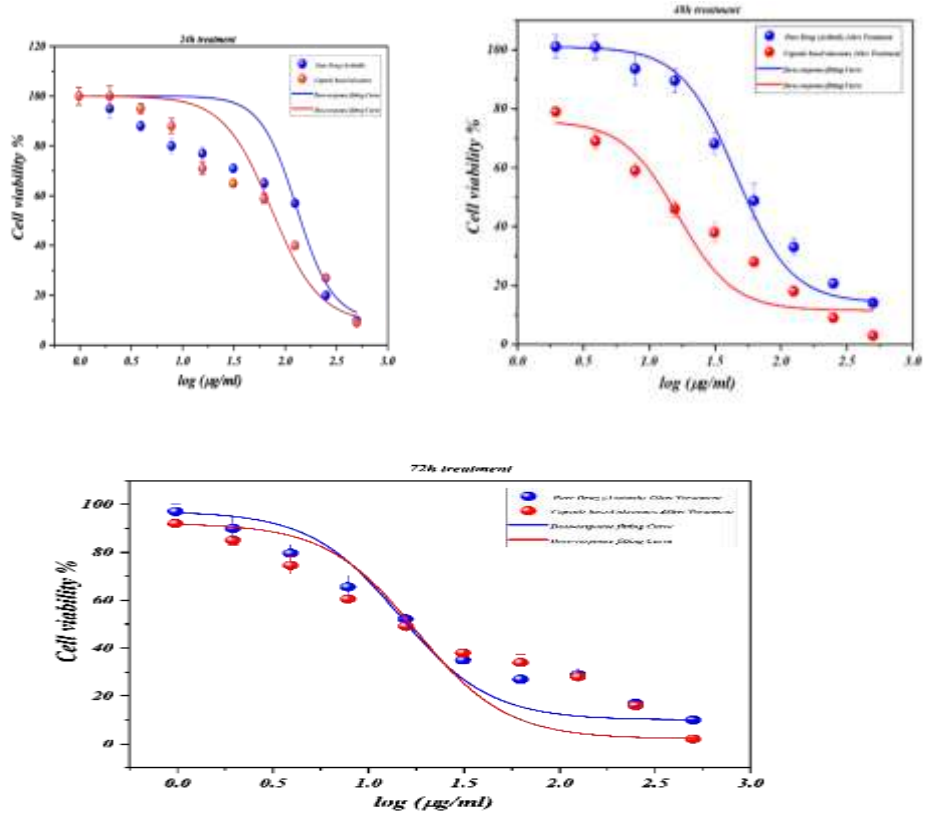

Figure 6.Dose-response curve at different concentrations in lung cancer line A549 of pure drug and capsule-based niosomes.

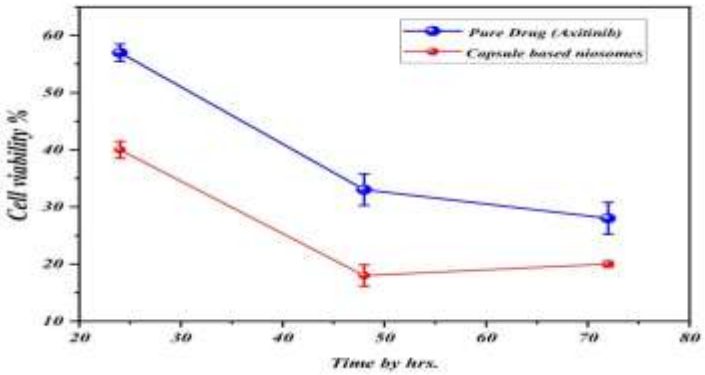

Figure 7. Time-response curve at different times in lung cancer line A549of pure drug and capsule-based niosomes.

Bioavailability investigation

The concentration of axitinib in rabbit blood samples was determined using the HPLC method to evaluate the pharmacokinetic behaviour of the axitinib capsule-based niosomes compared with the marketed conventional axitinib tablets. The axitinib was detected with a retention time of 5.94 min., the crizotinib (internal standard) retention time of 7.89 min., and the control (plasma without drug) retention time of 3.89 min. The mean plasma drug concentrations (µg/ml) of axitinib at the selected time intervals for 3 rabbits with body weight (1.5-2kg) and receiving a single dose of axitinib capsule-based niosomes and commercial Inlybest® tablets with a one-week washing period using a cross-over design. The mean plasma concentration-time profile is presented in **Figure 8.**

The pharmacokinetic parameters of axitinib capsule-based niosomes and marketed tablets after oral administration were determined using PK Solver. (version 2.0) software, where a linear trapezoidal rule calculated the area under the curve (AUC) of the plasma drug concentration-time from 0 to 24 hr [50]. The peak concentrations (Cmax) of capsule-based niosomes and marketed axitinib were found to be 5.28 µg/ml and 4.83 µg/ml, respectively. The areas under the plasma concentration-time curves (AUC0-24) for capsule-based niosomes were 70.92 µg/ml, significantly ($P < 0.05$, $p = 0.0032$) higher than the area under the concentration-time profile of marketed axitinib tablets, which was 53.714 µg/ml.hr., as shown in **Table 4.** It indicates a higher amount of drug absorbed from the capsule-based niosomes. Also, the time required to achieve maximum concentration (T max) for niosome-based capsules (4 hr) and 6 hr for marketed tablets indicated a faster absorption rate from capsule-based niosomes than the marketed tablets. The relative bioavailability (F-value) of capsule-based niosomes to the marketed tablets was found to be 130.5%, indicating the contribution of niosomes in improving the rate and extent of drug absorption and hence their oral bioavailability. Similar results were observed with clarithromycin, where niosomes enhanced its oral bioavailability compared to conventional oral dosage forms [51].

The efficacy of the noisome could be attributed to its nano-size vesicles and high surface area exposed to a dissolution medium that improved drug solubility, dissolution, and permeation [7].It was reported that the surfactant components present in the formulations have the potential to inhibit the activity of P-glycoprotein (P-gp), hence reducing its ability to facilitate efflux. Additionally, these surfactant components may also lead to a decrease in the activity of CYP enzymes, which play a crucial role in the process of first-pass metabolism due to the modulation of membrane fluidity [52]. Also, the niosomes might cross the anatomical barrier of the gastrointestinal tract via transcytosis of M cells in the intestinal lymphatic tissues [53].

Table 4. Pharmacokinetic parameters of a single dose of capsule-based niosomes and marketed tablets.

Parameters	Marketed tablets	Niosomes
AUC 0-∞ (µg/ml.hr)	53.714	70.092
C max (mcg /ml)	4.8±0.025	5.23±0.07
T max (hr)	6 hr	4 hr
Clearance (observed) (mg)/(µg/ml)/h	0.186168506	0.142797332
Relative bioavailability F % = (AUC niosomes based formula / AUC marketed tablets = 130.5%		

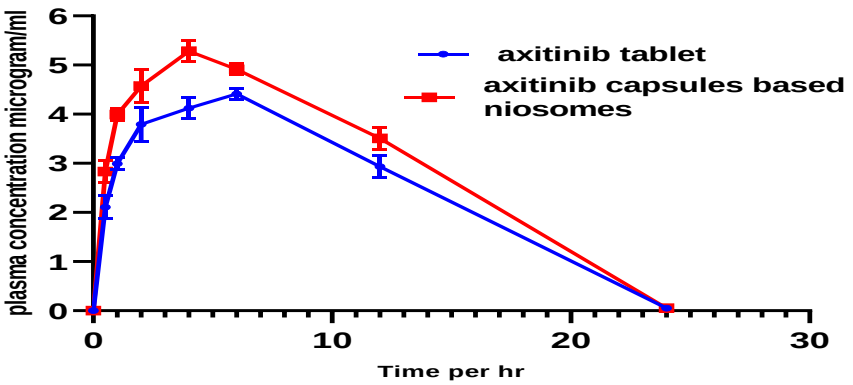

Figure 8. Plasma concentration-time profile for capsule-based niosomes and marketed conventional axitinib tablets after single dose administration to rabbit (n=3 experiments were done as triplicate, mean ± SD).

Conclusion

Axitinib was successfully loaded in nano-carrier vesicles and prepared as oral capsule-based niosomes, which act as protective carriers for the drug in an acidic medium compared to conventional tablets, which release the drug completely in the stomach. The in-vitro cytotoxicity investigation revealed an enhanced cytotoxic effect on A549 lung cells compared to the pure drug. The bioavailability investigation revealed that the capsule-niosomes had a higher bioavailability compared to marketed tablets, which was attributed to the colloidal structure of niosomes, which are nano-vesicles that possess a larger surface area, which led to improved drug dissolution and absorption, ultimately leading to enhanced oral bioavailability of the drug.

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Conflicts of Interest

The authors declare that there is no conflict of interest.

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Ethics Statements

The study protocol was approved by the scientific and ethical committees of the College of Pharmacy /Mustansiriyah University (Ref number: 87 and date: 4/9/2023).

Author Contribution

The authors confirm the contribution to the paper as follows: study conception and design: Ibtihal Abdulkadhim and Nidhal k. maraie; formulation, analysis and interpretation of results and draft manuscript preparation: Ibtihal abdulkadhim; supervision and review: Nidhal K. maraie. Both authors reviewed the results and approved the final version of the manuscript.

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تحضير وتقييم الكبسولات الفموية على اساس النايوسومات المحملة بدواء الاكستنيب المضاد للسرطان

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الخلاصة

نايوسومات عبارة عن حويصلات ثنائية الطبقة تتكون من التجميع الذاتي للمواد الخافضة للتوتر السطحي غير الأيونية، وهي تحظى باهتمام متزايد كنظام ذو امكانية لتوصيل الدواء عن طريق الفم خصوصا للأدوية شحيحة الذوبان لتعزيز ذوبانيتها وبالتالي امتصاصها وتوافرها الحيوي. أكسيتينيب هو مثبط لانزيم التايروسين كاينيز الذي يعمل عن طريق منع مستقبلات عامل نمو بطانة الأوعية الدموية مما يقلل بناء اوعية دموية جديدة وبالتالي يثبط نمو الورم. تم تصنيفه كدواء من الفئة الثانية والذي يتمتع بتوافر حيوي منخفض بسبب ضعف قابليته للذوبان والامتصاص. كبسولات الاكستنيب على أساس النايوسوم تم تصييغها بطريقة ترطيب الغشاء الرقيق كنايوسومات متشتتة على شكل سائل متشتت باستخدام سبان ٦٠: كوليسترول (نسبة وزن ١:١) , ٥ ملغ فوسفات ثنائي الأسيتيل ودواء ٥ ملغ (كفاءة الانحباس. ٩٦,٥٪ وحجم جسيم ٦٤,٥ نانومتر)، ثم تم تحويله إلى مسحوق باودر جاف عن طريق السليلوز البلوري (سيليسفايد مايكروكرستالاين سيليلوز) وتقييمه من حيث خصائص التدفق، وتحرر الدواء في المختبر، توافق الدواء مع بقية المكونات، نفاذية الدواء عبر الأمعاء (دراسة خارج الجسم) ودراسة السمية للخلايا السرطانية ودراسة التوافر الحيوي. أظهرت النتائج خصائص تدفق جيدة لمحتوى مسحوق النايوسومات المحضرة على شكل كبسولات حيث كانت زاوية السكون (٣٢,٣±١,١)، مؤشر كارس (١٢±٠,٥٨) ونسبة هاوزنر (١,١٢±٠,٠١). كان تحرر الدواء من الكبسولات سريعًا في الطور الأول حيث تم إطلاق ٤٠,٦١٪ من الدواء من النايوسومات في الوسط الحمضي (المعدة) يليه تحرر مستمر (في الأمعاء) حيث تم تحرر ١٠٠٪ من الدواء خلال ٤ ساعات، بينما تم تحرر الدواء بنسبة ١٠٠٪ من الحبوب المتوفرة تجاريا خلال ٢٠ دقيقة في الوسط الحمضي (المعدة). أظهرت نتائج التوافق بين الدواء وبقية المكونات نفاذ الدواء للامعاء عندما حضرناه على شكل جسيمات نانوية مقارنة بالحبوب التجارية حيث كانت النسبة المئوية للنفاذية تساوي ٧٨٪ للنيوسومات المحضرة على شكل كبسول بينما النسبة المئوية للنفاذية تساوي ٥٨٪بالنسبة للحبوب التجارية. كما اظهرت دراسة تأثير السمية الخلوية في المختبر أن التأثير السمي للنيوسومات المحضرة على شكل كبسولات أعلى بشكل ملحوظ حيث كانت قيمة (التركيز المثبط ٥٠) للدواء النقي وللنيوسومات المحضرة على شكل كبسول يساوي. ١٢٠ ميكروغرام/مل و٨٠,٢٦ ميكروغرام/مل على التوالي. تم الكشف عن دراسة التوافر الحيوي للنيوسومات المحضرة على شكل كبسولات مقارنة بالحبوب التجارية، حيث عززت النايوسومات من التوافر الحيوي للأكسيتينيب مقارنة بالأقراص التجارية، وأظهرت

نتائج الحركة الدوائية أن النيوسومات المحضرة على شكل كبسولات قد اظهرت تركيز أعلى للدواء حيث اعلى تركيز يساوي ٥,٢٣±٠,٧مايكروغرام / مل والحد الأقصى للوقت للوصل الى اعلى تركيز يساوي ٤ساعات بينما اعلى تركيز للحبوب التجارية يساوي ٤,٨±٠,٠٢٥ مايكرو غرام / مل والحد الأقصى للوقت للوصول لاعلى تركيز هو ست ساعات اما قياس المساحة تحت المنحني التي حققتها النايوسومات المحضرة على شكل كبسول تساوي ٧٠,٠٩٢مايكروغرام /مل* ساعة بينما قياس المساحة تحت المنحني التي حققتها الحبوب التجارية تساوي ٥٣,٧١٤مايكروغرام/مل*ساعة يشير هذا الاختلاف الكبير في النتائج دليل على تحسن في قابلية ذوبان الدواء، ، وامتصاصه عندما حضرناه على شكل حويصلات نانوية ، مما يؤدي في النهاية إلى تحسن في التوافر الحيوي الفموي كما بينت نتائج هذه الدراسة إلى أن النايوسومات تظهر إمكاناتها كحاملات نانوية لتعزيز التأثيرات السامة للخلايا للادوي المضادة للسرطان والنفاذية والتوافر الحيوي.

الكلمات المفتاحية: اكستنيب، مضاد سرطان، التوافر الحيوي، نايوسومات، كبسول فموي