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Research Article

Eudragit Gum Nanoparticles Encapsulating Moronic Acid: A Novel Approach to Cancer Treatment

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ABSTRACT

The employment of therapeutic techniques in the treatment of cancer is frequently associated with multidrug resistance or drug tolerance. The effectiveness of plant secondary metabolites in the fight against cancer can be significantly increased by synthesizing them at the nanometric scale. Moronic acid (MA) is one type of pentacyclic triterpenoid that inhibits the growth of cancer cells by blocking the regulation of cell growth. In this work, we used the oil-in-oil (O/O) emulsion solvent evaporation method to synthesize Eudragit nanoparticles (MENPs) loaded with MA. Enhancing the synergy and bioavailability of the nanoparticles was the aim of this intervention. The zeta potential of +28 mV observed in MENPs indicates the relative stability of the nanoformulations. MA was discovered to have 71.8% encapsulation efficiency. Transmission electron microscopy revealed that the MENPs' particle sizes ranged from 42 to 58 nm. The antioxidant and anticancer capabilities of the MENPs were significantly stronger and they showed a continuous release pattern when compared to the individual MA particles in their free state. The *in vitro* investigations showed that the combination of MA encapsulated in Eudragit had a greater inhibitory effect on the growth of A-549, MCF-7, and Hela cell lines as compared to the solo administration of MA. This finding confirms the potent anticancer properties of the encapsulated compounds.

Keywords: Eudragit, Moronic acid, Anti-cancer, Nanoparticles

Introduction

Oncology Nanotechnology is a swiftly growing field within the realm of medicine. Nanotechnology enables the creation of innovative nanoplatforms that are loaded with bioactive ingredients for combating cancer. Cytotoxic medicines and chemotherapy are commonly used together to treat several types of malignancies [1]. Nevertheless, there are adverse

with consequences associated these traditional therapeutic methods, such as the development of drug resistance or tolerance [2, 3]. Currently, the utilization of nanotechnology in the development of remedies for severe ailments is more prevalent. Nanometric bioactive compounds exhibit heightened antioxidant and anticancer properties, together with exceptional absorption capabilities at small doses. The United States National Institute of Cancer has advocated for the investigation of the possible anticancer properties of many secondary metabolites present in plants [4,5]. Several phytochemicals, such as boswellic acid, moronic acid, and ursolic acid, have been demonstrated to possess cytotoxic properties [6]. Recent research has demonstrated that optimizing the physical characteristics of nanoparticles is crucial for achieving effective anti-cancer effects while minimizing adverse reactions [7]. The size, shape, and surface characteristics of nanoparticles affect their pharmacokinetic and pharmacodynamic properties.

Moronic acid (MA) is a pentacyclic triterpenoid that can be found in two forms: either as the aglycone of saponins or as a free carboxylic acid [8]. Glucocorticoids lead to a modification of glucocorticoid receptors, which leads to a reduction in Bcl2 (proteins that prevent cell death) in human breast cancer cell lines (Michigan Cancer Foundation-7) [9, 10]. Methyl anthranilate (MA) is frequently included in pharmaceutical formulations for oral and topical delivery because of its minimal toxicity level [11]. The LD50 values for MA were determined to be 637 mg/kg (intraperitoneal treatment) and 8330 mg/kg (oral administration) in acute toxicity experiments done on mice [12].

When choosing an agent to encapsulate several drugs, it is crucial to consider the significant qualities of biodegradability and biocompatibility [13, 14]. Eudragit, a flexible group of synthetic polymers produced from methacrylic acid and acrylic acid esters, has garnered considerable interest in the realm of anticancer therapy. These polymers are extensively utilized in pharmaceutical formulations because of their exceptional biocompatibility, diversity in functional groups, and adjustable features such as solubility and release profiles. Eudragit polymers can be customized to alter the rates at which drugs are released, enhance drug stability, and specifically target certain locations in the body. This makes them highly valuable for developing sophisticated drug delivery systems for cancer therapy [15].

This work aimed to increase the bioavailability of moronic acid (MA) by

using a polymeric nanoplatforms. This strategy was also intended to reduce adverse effects and achieve synergistic results. The inclusion of MA within the polymeric nanoparticles leads to a regulated and extended-release, resulting in improved therapeutic efficacy even at lower doses.

Experimental Details

Materials

The Eudragit was acquired from MP Biomedicals, LLC, a company based in France. The purchase of moronic acid was made from Sigma Aldrich, an Indian-based company. However, Minimum Essential Eagle Medium, Foetal bovine serum (FBS), penicillin, and streptomycin were acquired from Hi-media Laboratories Pvt. Ltd. in Mumbai, India. The cell lines employed in this work, namely A-549 (human lung₂ adenocarcinoma epithelial cells), MCF-7 (human breast adenocarcinoma cells), and Hela (human cervical carcinoma cell line), were obtained from the National Centre for Cell Science (NCCS) in Pune. The chemicals utilized in the present investigation were of the analytical reagent grade.

Preparation of MA-loaded Eudragit nanoparticles (MENPs)

The production of the MENPs was achieved through the utilisation of the oilin-oil emulsion solvent evaporation process. A solution was made by dissolving

150 mg of Eudragit and 100 mg of moronic acid in 100 ml of n-propanol. The designated solution was treated with 25 mg of Calcium stearate, and the resulting mixture was stirred magnetically at a speed of 1200 rpm for a period of 1 hour. The mixture was continuously stirred at a rate of 1200 revolutions per minute, maintained at a temperature of 42 degrees Celsius, for a period of 60 minutes. Afterwards, the mixture was divided by spinning it in a centrifuge at a rate of 8000 revolutions per minute, at a temperature of 4 degrees Celsius, for a period of 20 minutes. Once the pellet was gathered, it underwent freeze-drying while being held in suspension with cryoprotectant (D-Mannitol, 5% w/v).

Characterization of synthesized MENPs

To ascertain the mean size and size distribution (polydispersity index) of the optimized nanoformulations of MENPs, we employed а Zetasizer Nano ZS-90 manufactured by Malvern Instruments in Malvern, United Kingdom. The unbound drug was separated from the mixture by centrifuging the solution at 9000 revolutions per minute at a temperature of four degrees Celsius for a duration of thirty minutes. The resulting supernatant was then collected and evaluated using highperformance liquid chromatography (HPLC). The subsequent equation was

employed to calculate the percentage of encapsulation efficiency:

The size and shape of the optimized batch of MENPs were examined using a transmission electron microscope (TEM-Hitachi-H-7501SSP/N-817-0520, Japan). The magnification factor was adjusted to 60,000, while the accelerating voltage was set to 80,000V. The TGA/DSC 3+ Star System, produced by Mettler Toledo AG, Analytical, Switzerland, is a device used for differential scanning calorimetrythermogravimetric analysis.

In vitro release profile of MENPs

The release profile was examined using the dialysis sac approach. A total of 10 milligrams (mg) of MA, which had been enclosed in Eudragit nanoparticles, were placed within a dialysis sac. Subsequently, the sac was placed in a solution consisting of 10 ml of water, 25% ethanol, and 0.1 M phosphate buffer saline with a pH of 7.4. The solution was stirred vigorously at a constant temperature of 37 °C with a continuous swirling action at a rate of 100 revolutions per minute. A 1 ml sample was collected at regular time intervals of 1, 2, 3, 6, 12, and 24 hours and analyzed using High Performance Liquid Chromatography (HPLC) on an Agilent 1200 Infinity Series instrument. The analysis was performed using a ZORBAX SB C-18 column measuring 150 x 4.6 mm and having a

particle size of 5 μ m. The mobile phase used consisted of a blend of Acetonitrile and Water at a volumetric ratio of 80:20. The compounds of interest, MA, were detected at wavelengths of 210 nm, respectively, and had retention durations of 6.17 minutes.

Antioxidant activity

The DPPH assay was used to evaluate the antioxidant ability of MA and MENPs. The molecule 1,1-diphenyl-2-picrylhydrazyl (DPPH), a free radical, was fully dissolved in methanol at a concentration of 3.9 mg per 100 ml. The pure samples of MA, Eudragit, and MENPs were combined with DPPH and incubated in darkness for a duration of 30 minutes. This was performed three times to guarantee precision. The UV spectrophotometer was used to measure the absorbance of the combination at a wavelength of 517 nm to determine the level of DPPH inhibition. Eudragit nanoparticles were used as a negative control, whereas pure moronic acid was used as a positive control.

In-vitro assay for cytotoxic activity (MTT assay)

Both normal and cancer cell lines were cultured in a medium containing inactivated fetal bovine serum (10%), streptomycin (100 μ l/ml), and penicillin (100 μ l/ml). The cells were subsequently placed in an incubator set at a temperature of 37°C and exposed to a carbon dioxide concentration of 5%. When the cells reached a point where they covered 75% of the surface, they were moved to a clean environment and further grown using a solution containing 0.25% trypsin. The chemicals and standards were prepared as stock solutions in DMSO with a concentration of micromoles per milliliter. Afterward, the medium was diluted at concentrations of 1µM, 10µM, 20µM, 50µM, and 100µM per ml. The cells were distributed in 96-well plates at a concentration of 5×103 cells per 100µl per well. The density of each cell line was evaluated according to their growth parameters. The wells were treated with different doses of MENPs (ranging from 0.1 to 1000µg/ml) and moronic acid for a duration of 3 days, with each treatment being performed in triplicate. This was done after an initial incubation period of 8 hours. After a three-day treatment period, the medium was replaced with 3µl of MTT solution (5mg/ml) and allowed to incubate for 3 hours. The percentage of cells displaying metabolic activity was assessed by comparing them to untreated control cells, using the conversion of 3-(4, 5dimethylthiazol-2-yl) 2. 5 diphenyltetrazolium bromide (MTT) into Formazan crystals by mitochondria. The formazan crystals were dissolved in dimethyl sulfoxide (DMSO) and the

absorbance was quantified at 570 nm using a microplate reader. The effectiveness of MENPs in treating cancer was evaluated using pure MA as a benchmark through the MTT test on three types of cells: A-549 (human lung adenocarcinoma epithelial cells), MCF-7 (human breast adenocarcinoma cells), and Hela (human cervical carcinoma cell line).

Results and Discussion

Particle size and Zeta Potential

A comprehensive examination was performed on the MENPs to ascertain their particle size and zeta potential. The dimensions of the MENPs were identified as 148 nm (Fig 1), and the zeta potential was assessed as +23 mV (Fig 2), suggesting the considerable stability of the produced nanoformulations

Percent encapsulation efficiency

The effectiveness of encapsulation depends on the particular molecule, technique, encapsulating materials, and media employed in the synthesis of nanoparticles [22]. The encapsulation effectiveness percentage was measured by evaluating the supernatant using **High-Performance** Chromatography (HPLC) Liquid to quantify the quantity of unbound medicine. demonstrated The results that the encapsulation efficiency of MA was 72.5%, as depicted in Figure 5. Both Moronic acids have hydrophobic characteristics. They exhibit exceptional solubility in a eudragit solution prepared with n-propanol. Due to its hydrophobic nature, Eudragit showed a strong affinity towards both MA. Consequently, the drug was encapsulated more efficiently in Eudragit due to this affinity.

Morphological characterization of MENPs by TEM

The Magnetic Nanoparticles (MENPs) were isolated and exhibited a uniform spherical morphology, as evidenced by Transmission Electron Microscopy analysis (Fig 3). Their dimensions varied between 45 and 55 nanometers. The size of the nanoparticles was shown to fluctuate when measured using Particle Size Analyzer (PSA) and Transmission Electron Microscopy (TEM). The PSA method considers the fluidized ionic environment around the nanoparticles, whereas TEM analyzes the size of the particles in a dehydrated and isolated atmosphere, leading to a reduction in particle size. The size of nanoparticles has an impact on their rate of release [23, 24]. Nanoparticles are dispersed throughout different organs in the body according to their shape and size. The of morphology and dimensions nanoparticles are essential factors in regulating their durability, biocompatibility, and ability to permeate cellular tissues [25]. Nanoparticles with

smaller dimensions have a longer duration of retention in the bloodstream compared to nanoparticles with bigger dimensions [26].

FTIR Analysis of Drug Samples

FTIR spectroscopy is used for studying the interaction studies [27] and evaluating the nanoencapsulation of bioactive substances [28]. Figure 4 exhibits the FTIR spectra of the separate constituents: unadulterated medicine MA, Eudragit, and MENPs. The MA FTIR spectrum displays absorption peaks at 3234 cm-1, which correspond to the -OH group, and at 2897.0 cm-1 and 2244 cm-1 for the terminal –CH3 groups. The wavenumber values of 1345 cm-1 and 3453 cm-1 correspond to the stretching of -NH2. The FTIR spectrum of Eudragit, shown in Figure 4 C, displays a peak at 3346 cm-1, which corresponds to the stretching of -OH groups, and a peak at 2845 cm-1, indicating the stretching of aliphatic –CH groups in Eudragit. The FTIR spectra of MENPs, as shown in Figure 4D, display alterations at wave numbers of 3400 cm-1, 1434 cm-1, 2453 cm-1, and 2871 cm-1. This region corresponds to the range of infrared stretching vibrations shown by functional groups such as hydroxyl (-OH), double bond (C=C) stretching, methyl group (-CH3) stretching, and carbon-hydrogen (C-H) stretching. The drug molecule exhibits weak intermolecular forces, specifically

dipole-dipole interactions, hydrogen bonding (with electronegative atoms like N, O, F), and weak Van der Waals forces (dependent on mass), with the OH groups in Eudragit. The drugs and inactive substances show identifiable peaks in the FTIR spectrum. The maximum intensity of the signal reduced and the positions of the bands moved, suggesting the occurrence of chemical interaction between moronic acid and Eudragit.

In-vitro drug release

The continuous release of bioactive chemicals from nanoparticles protects rapid metabolism and breakdown [29]. The in vitro drug release of MA and MENP is illustrated in Figure 6. The in-vitro drug release data demonstrates that 95% of the pure MA is released within a time frame of 4 hours. MENPs exhibit sustained drug of release as а result Eudragit emulsification. After one hour of treatment, Magnetic Nanoparticles (MENPs) release 20% of Methylamine (MA). Magnetic nanoparticles (MENPs) released 80% of the MA compound within 24 hours. Hydrophobic (nonpolar) maleic anhydride (MA) enables magnetic iron oxide nanoparticles (MENPs) to gradually release MA. Eudragit created a unified structure around MA, guaranteeing their extended distribution.

DSC Analysis

differential scanning calorimetry The (DSC) thermogram of free MA exhibits a less intense endothermic peak and advancing crests at 150°C, which suggests the presence of the medication. The DSC thermograms of moronic acid reveal two distinct endothermic peaks. The initial endothermic peak observed at 286°C, albeit it has a low strength, indicates the presence of moronic acid [30]. The sudden and strong climax verified its characteristic of being crystalline. The lack of prominent crests in all DSC graphs suggests that the nanoformulations are amorphous and that the medication is suitable for its amorphous state. Eudragit nanoparticles containing moronic acid display an endothermic peak with a low intensity of around 374°C, showing their amorphous nature. The initial decomposition of the nanoparticles starts at 318°C, while the secondary decomposition takes place at 445°C. The presence of a lowintensity, blunt peak hump serves as evidence of its amorphous nature. In comparison MENPs. fake to the nanoformulations exhibit a relatively little increase in temperature throughout the process of liquefaction, as seen in Figure 7B. The peak of the melting point of Eudragit nanoparticles loaded with MA higher changed to a temperature, suggesting the production of a coamorphous phase.

Thermo-gravimetric analytical studies

The highest weight reduction of the dummy Eudragit nanoformulations was observed at a temperature of 359°C. The melting endotherm movement of MENPs was significantly greater than that of the dummy Eudragit nanoformulations, with the maximum weight loss occurring at 412°C. The results suggest that the formation of the co-amorphous phase varied between the Eudragit nanoformulations containing MA and the dummy nanoformulations (Fig 7).

Anti-oxidant activity

The DPPH assay measures the level of activity of antioxidant encapsulated molecules [31]. DPPH, also known as 1,1diphenyl-2-picrylhydrazyl, is a stable free radical that contains unpaired electrons spread evenly across its structure, resulting in a distinct deep violet hue. Absorption takes place at a wavelength of around 517 nm [32]. The violet color of DPPH solutions is uniformly lost when they are combined with molecules that donate hydrogen. MA is a widely recognized antioxidant. The process of incubating the antioxidant molecules MA with DPPH, which acts as a hydrogen atom donor, resulted in the formation of a stable, nonradical form of DPPH. This transformation caused the color of the DPPH to change from violet to pale yellow. Therefore, the absorption band diminished. MA block DPPH undergoes hydrogen atom release, causing the DPPH to become labile. Because of their extremely small size, the nanometric MENPs showed a greater ability to block DPPH compared to MA alone during the period of dark incubation. This resulted in their protection from oxidation (Fig 8).

Anti-cancer activity

Nanoparticles exhibit cytotoxicity due to their extensive surface area, which enables efficient drug administration and potential for anticancer effects [33]. Nanoparticles of 100 nm in size can readily penetrate tumor cells by retention and vascular penetration [34]. The anticancer effects of NPs are sizedependent Microscopic [35,40]. nanoparticles exhibit enhanced tumor penetration capabilities. The enzyme mitochondrial dehydrogenase is present within live cells. The MTT dye is broken down, causing the pale-yellow tetrazolium ring structure to be disrupted. This results in the formation of dark purple formazan crystals, which then build up in cells [41-48]. The current study demonstrated that magnetic-engineered nanoparticles (MENPs) exhibited superior efficacy in inhibiting cancer growth compared to the pure active drug alone. In laboratory conditions, the of Eudragituse encapsulated MA was found to be more effective in inhibiting the growth of A-549,

MCF-7, and Hela cells compared to the unencapsulated medications. Table 1 displays the IC50 values (μ g/ml) of the nanoparticles that were produced, as well as the reference medicines moronic acid. When compared to regular pharmaceuticals MA alone, MENPs had strong anticancer effects with IC50s of 4.1, 3.8, and 4.2 μ g/ml against A-549, MCF-7, and Hela cell lines as observed through optical microscopy. The combined effect of MA in MENPs resulted in a potent anticancer effect.

Conclusions

Nanotechnology has greatly enhanced the process of formulating and developing treatments, hence enhancing the potential for efficiently treating and curing diseases. Advancements in nano-drug delivery enabled technology have oncology researchers to explore the synergistic effects of medications by utilizing nanoparticles with larger payloads, including dual drug delivery. Although numerous anticancer drugs have been created, the main obstacles to these treatments are drug tolerance/resistance, limited bioavailability, and short drug residence time. Novel nanoformulations containing more potent excipients can be utilized to improve solubility, bioavailability, therapeutic potential, and adverse effect profiles. According to the findings of this study, a novel formulation

of Eudragit nanoparticles loaded with moronic acid has great potential in combating cancer. This is because it possesses both antioxidant and anticancer capabilities that work together beneficially.

Conflict of Interest

There is no conflict of interest whatever.

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Figure 1. PSA image of MENPs



Figure 2. Zeta potential of MENPs



Figure 3: TEM image of MENPs



Figure 4. The FTIR spectra of MENPs



Figure 5. HPLC Chromatogram for supernatant



Figure 6. In-vitro release study



Figure 7. Differential Thermal Analysis (A) Blank Eudragit Nanoparticles (B) Drug Loaded Eudragit Nanoparticles



Figure 8. Antioxidant activity of MA, and MENPs

Table 1: IC50 value	s of MENPs along	with standard (drug moronic acid
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Sample	A-549		MCF-7		Hela	
code						
	IC50	pIC50	IC50	pIC50	IC50	pIC50
МА	26.30	-1.452732	25.52	-1.4503	29.12	-1.50187
MENPs	4.3	-0.643249	3.9	-0.58106	4.1	-0.618455