NANOPARTICLE FORMULATION OF ROSUVASTATIN: ENHANCING SOLUBILITY AND CLINICAL POTENTIAL

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Abstract

Rosuvastatin is a potent statin used in the management of dyslipidemia and the prevention of cardiovascular diseases. However, its low water solubility limits bioavailability and therapeutic outcomes. This study outlines a nanotechnology-based method to enhance its solubility and absorption of rosuvastatin. The process entails the formulation of rosuvastatin nanoparticles through a combination of precipitation and ultrasonic techniques. Rosuvastatin is initially dissolved in an organic solvent, such as acetone, and subsequently mixed with an aqueous phase containing stabilizers like Poloxamer 407 or PVP to induce precipitation. Ultrasound reduces particle size to 50-200 nm. Key parameters, including drug-to-stabilizer ratio, stirring speed, ultrasound duration, and temperature, were systematically optimized to archive uniform, nanoscale particles. The resulting nanoparticles exhibit improved stability and solubility, achieving a 6.8-fold increase compared to unmodified Rosuvastatin. Additionally, the application of nanotechnology safeguards the drug against gastric degradation, reduces the required dosage, and minimizes potential side effects. This approach offers promising applications for poorly soluble drugs and significantly improves therapeutic efficiency, marking a step forward in pharmaceutical development for better patient outcomes.

Keywords: *Rosuvastatin, cardiovascular, solubility, cancer, nanoparticle.*

Công thức hạt nano Rosuvastatin: Tăng cường độ hòa tan và tiềm năng lâm sàng

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Tóm tắt

Rosuvastatin là một statin mạnh để quản lý rối loạn lipid máu và phòng ngừa các bệnh tim mạch, nhưng độ hòa tan trong nước thấp của nó hạn chế sinh khả dụng và hiệu quả điều trị. Nghiên cứu này trình bày một phương pháp dựa trên công nghệ nano nhằm cải thiện độ hòa tan và hấp thu của thuốc.

Quy trình bao gồm việc tạo ra các hạt nano Rosuvastatin bằng cách kết hợp phương pháp kết

tủa và siêu âm. Rosuvastatin được hòa tan trong dung môi hữu cơ (ví dụ: acetone) và trộn với pha nước chứa các chất ổn định như Poloxamer 407 hoặc PVP để tạo kết tủa. Siêu âm được sử dụng để giảm kích thước hạt xuống còn 50-200 nm. Các thông số quan trọng, bao gồm tỷ lệ thuốc với chất ổn định, tốc độ khuấy, thời gian siêu âm và nhiệt độ, đã được tối ưu hóa để tạo ra các hạt đồng đều ở kích thước nano.

Các hạt nano thu được cho thấy tính ổn định và độ hòa tan được cải thiện, tăng 6,8 lần so với Rosuvastatin dạng thô. Công nghệ nano cũng bảo vệ thuốc khỏi sự phân hủy trong dạ dày, giảm liều lượng cần thiết và hạn chế tác dụng phụ.

Phương pháp này mang lại ứng dụng tiềm năng cho các loại thuốc có độ hòa tan kém, đồng thời cải thiện đáng kể hiệu quả điều trị, đánh dấu một bước tiến trong phát triển dược phẩm nhằm mang lại kết quả tốt hơn cho bệnh nhân.

Từ khóa: *Rosuvastatin, cardiovascular, solubility, cancer, nanoparticle.*

Introduction

Rosuvastatin, a member of the statin class of drugs, is widely used in conjunction with a suitable diet to lower low-density lipoprotein cholesterol (LDL-C) and triglycerides while increasing high-density lipoprotein cholesterol (HDL-C) in the blood. By inhibiting hepatic cholesterol synthesis, Rosuvastatin reduces the risk of cardiovascular diseases, including heart attacks and strokes. Among statins, Rosuvastatin calcium (ROSC) is particularly effective, earning its designation as a "superstatin" due to its ability to reduce LDL-C levels by up to 63% at a 40 mg dose.

Despite its efficacy, ROSC has poor water solubility, with oral bioavailability limited to approximately 20%, classifying it as a Biopharmaceutics Classification System (BCS) Class II drug. This low solubility constrains its dissolution rate and, consequently, its systemic absorption. Following oral administration, ROSC is rapidly absorbed, achieving peak plasma concentrations within 5 hours. It has a half-life of approximately 19 hours and is primarily eliminated via feces (~90%).

Rosuvastatin is indicated for the treatment of various lipid disorders, including primary hypercholesterolemia, hypertriglyceridemia, mixed dyslipidemia, and familial hypercholesterolemia. ROSCa has also demonstrated potential therapeutic effects in the management of osteoporosis, Alzheimer's disease, and benign prostatic hyperplasia. More recently, ROSCa has been investigated for its anti-cancer properties, with studies revealing its ability to inhibit the growth of Caco-2 cells, a human colorectal cancer cell line. Mechanistically, ROSCa acts as a selective and competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme in the biosynthesis of cholesterol. Specifically, ROSCa blocks the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate, a crucial precursor for cholesterol production (Bansal et al., 2024). This inhibition leads to: (i) an upregulation of low-density lipoprotein (LDL) receptors on hepatocytes, thereby enhancing the hepatic uptake and catabolism of LDL particles, and (ii) a reduction in the synthesis of very low-density lipoprotein (VLDL) in the liver, which subsequently lowers the overall circulating levels of both VLDL and LDL particles.

To address the challenge of poor solubility, various strategies have been investigated to enhance the dissolution and bioavailability of ROSC. These include techniques such as ß-cyclodextrin complexation, solid dispersion, hydrotropy, micellar solubilization, and nanoemulsion systems (Akbari et al., 2011; Nainwal et al., 2011). Among these,

nanotechnology-based approaches have gained particular attention, demonstrating significant potential to improve the solubility and oral bioavailability of ROSC (Alshora et al., 2018; Palani et al., 2015; Gabr etal, 2018; Krishnamoorthy et al., 2013).

In summary, while rosuvastatin calcium is highly effective for managing dyslipidemia and preventing cardiovascular events, enhancing its solubility remains critical for optimizing its therapeutic efficacy. The application of advanced formulation strategies continues to demonstrate p otential in overcoming this limitation.

This study introduces a method for the fabricating nanosized rosuvastatin calcium particles aimed at improving the drug's solubility and bioavailability through a ball milling technique, utilizing excipients and stabilizers such as Pluronic-F127 and Polyvinylpyrrolidone K-30 (PVP-K30).

The resulting nanorosuvastatin product was evaluated using the MTS assay, a widely used method for assessing metabolic activity and cell viability in biological research. The MTS (3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay involves the conversion of a tetrazolium salt into a measurable colored product through the action of reducing enzymes in living cells. During the assay, MTS is added to the cell culture medium, where it reacts with NADPH-dependent dehydrogenase enzymes present in metabolically active cells. When the cells are metabolically active, MTS is reduced to a colored compound, and the intensity of this color can be measured using a spectrophotometer. The color intensity is proportional to the level of metabolic activity of the cells, thereby allowing the growth rate or survival of the cells in the test medium to be determined (Wang et al., 2020).

2. Materials and Methods

2.1. Materials

Rosuvastatin calcium (GR) supplied by SPM, excipients PVP (Sigma), Tween-80 (Sigma), Pluronic F-127 (Sigma) and other excipients. Equipment: Rotary evaporator, freeze dryer, ball mill, magnetic stirrer, and other equipment. Cell line human gastric cancer cells (AGS), liver cancer cells HepG2, leukemia cells HL-60; RPMI medium, DMEM medium.

2.2. Methods

- Development of the Rosuvastatin Calcium Standard Curve

The standard curve for rosuvastatin calcium concentration is constructed by dissolving rosuvastatin calcium (GR) in methanol at concentrations of 20, 30, 40, 50, and 100 µg/mL. The The optical density OD is measured at a wavelength of 244 nm. A linear equation is then derived to represent the correlation between concentration and OD244.

- Determination of the Product Solubility

Dissolve 50 mg of the lyophilized product (NR3) and 50 mg of Rosuvastatin calcium (GR) in 1 mL of H2O to saturation. Centrifuge at 3000 rpm for 5 minutes and collect the supernatant from the samples. Repeat the centrifugation step 3 times.

Lyophilize the centrifuged supernatant, then dissolve in Methanol and determine the OD at 244 nm. Develop a standard equation to determine the solubility of nanoRosuvastatin calcium.

- Preparation of Rosuvastatin Calcium Nanoparticles (NR)

The ball milling technique was used to produce nanorosuvastatin to enhance the solubility and bioavailability of rosuvastatin calcium using Rosuvastatin calcium, Pluronic F127, polyvinylpyrrolidone (PVP), Tween 80, and water at a weight ratio of 1:1.5:0.5:0.3. The mixture of components was milled for 90 minutes, with a cycle of 15 minutes of operation and 5 minutes of rest, at a speed of 250 rpm. After milling, the sample was freeze-dried at -50°C to obtain the final product. The product was stored at 4oC and store at 4°C until use.

- Determination of Product Size

The particle size and zeta potential of the

obtained product are determined using dynamic light scattering (DLS) and laser Doppler anemometry (LDA), respectively, with a Zetasizer 3000 (Malvern Instruments, UK). The nanoparticle suspension was filtered through a 0.45 µm filter, and each sample was analyzed three times.

- Cultivation of Cancerous and Non-Cancerous Cell Lines

The human gastric cancer cell line (AGS) is cultured in RPMI medium, while the HepG2 liver cancer cell line and leukemia cell lines HL-60 (acute promyelocytic leukemia) are cultured in DMEM medium at 37°C with 5% CO2. Both culture media contain 10% FBS and 1% penicillin-streptomycin. To maintain the cells in their exponential growth phase, they are passaged twice a week.

- Drug Testing on Cell Lines

MTT and MTS assays exhibit several similarities, primarily in their application for measuring cell viability in vitro. Both assays evaluate the impact of various compounds on cell proliferation and cytotoxicity and are classified as colorimetric assays. Another similarity is both assays measure the metabolic activity of cells based on their ability to create formazan crystals, utilizing NADPH as the reduction agent. Additionally, both assays must be conducted in the dark due to the light sensitivity of the MTT reagent. Finally, the incubation periods for both assays are consistent, lasting between 1 to 4 hours at 37 degrees Celsius. The IC50 values were determined based on the percentage of cell growth inhibition:

$$IC_{50} = High_{conc} = (High_{Inh\%}-50)\ x\ (High_{Conc}-Low_{Conc})/(High_{Inh\%}-Low_{Inh\%})$$

$$\%\ cell\ inhibition = (OD_{Control\ (+)} - OD_{Test}) / (OD_{Control}\ (+) - OD_{Test}\ (-)$$

In which: HighConc/LowConc: Test substance at high concentration/Test substance at low concentration; $High_{Inh\%}/Low_{Inh\%}$: % inhibitory at high concentrations / % inhibitory at low concentrations.

3. Results and Discussion

Solubility of Nanorosuvastatin

The results of constructing the standard curve of rosuvastatin calcium concentration (GR) are presented in Figure 1 and the linear equation between the optical density (OD244) and rosuvastain calcium concentration is expressed by the equation: Y= 0.0056X + 0.0415, with an R2 value of 0.9992. The concentration of Rosuvastatin calcium in the sample was determined using the titration equation, which subsequently allowed for the determination of the solubility of the resulting product compared to the original sample as detailed above (Table 1).

Fig1. The Standard Curve of Rosuvastatin calcium Concentration with R2 value of 0.9992

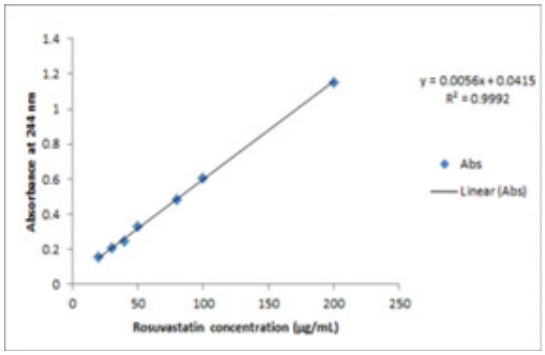


Table 1. Solubility of Rosuvastatin calcium (GR) and Nanorosuvastatin (NR3)

Name	Dilution	OD 244	Average value	Concentration after dilution (µg/mL)	Initial concentration (µg/mL)
Rosuvastatin cacium (GR)	36x	0.801	0.811666667	137.5297619	4951.071429
		0.823			
		0.811			
Nanorosuvastatin (NR3)	300x	0.653	0.678433333	113.738095232	34121.428569
		0.676			
		0.694			

The solubility of Nanorosuvastatin calcium (NR3) is 6.89 times higher than that of crude Rosuvastatin calcium (GR).

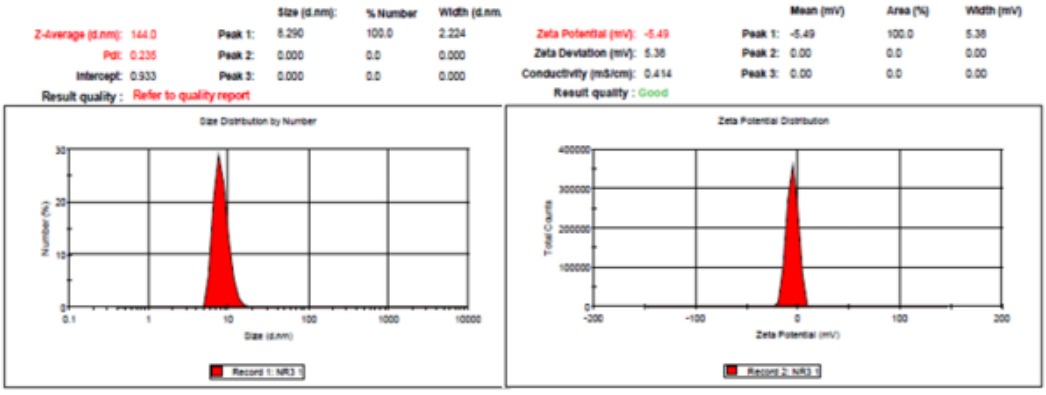
Product Size

The particle size and zeta potential of the nano rosuvastatin (NR3) were determined using a Zetasizer 3000 (Malvern Instruments, UK) as shown in figures 1.

Figure 1. The particle size and zeta potential of the nano rosuvastatin (NR3).

The obtained product has a Rosuvastain calcium content of 68.02%.

The average particle size is 144 nm. Zeta potential: -5.49 mV



The Toxicity of Nano rosuvastatin (NR3)

The toxicity of nano rosuvastatin (NR3) was determined on the gastric carcinoma cell line AGS, hepatocellular carcinoma HepG-2 and leukemia cell lines HL-60 (acute promyelocytic leukemia). Cells were seeded at a density of 5,000 cells/well in a 96-well plate and maintained in 5% CO_2 at 37°C. After 24 hours, NR3 and GR were treated with a range of rosuvastatin concentrations (0–100 µM). Control cells were not supplemented with the test substance. Evaluation of the inhibition and cell death levels by MTT method of NR3, GR and the excipients used to create NR3 on tested cell lines is shown in Figure 2, 3 ,4, 5.

After 72 hours of treatment, cell viability was assessed using the MTT assay. The differences in survival and inhibition rates between control and excipient-supplemented samples at different concentrations were not statistically significant each sample was conducted in triplicate.

Fig2. Effect of excipients to create NR3 on the viability and death of AGS

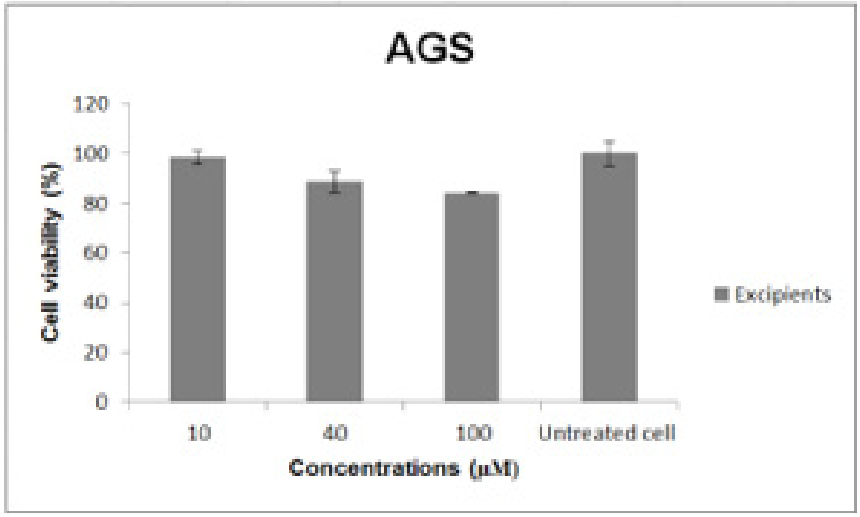
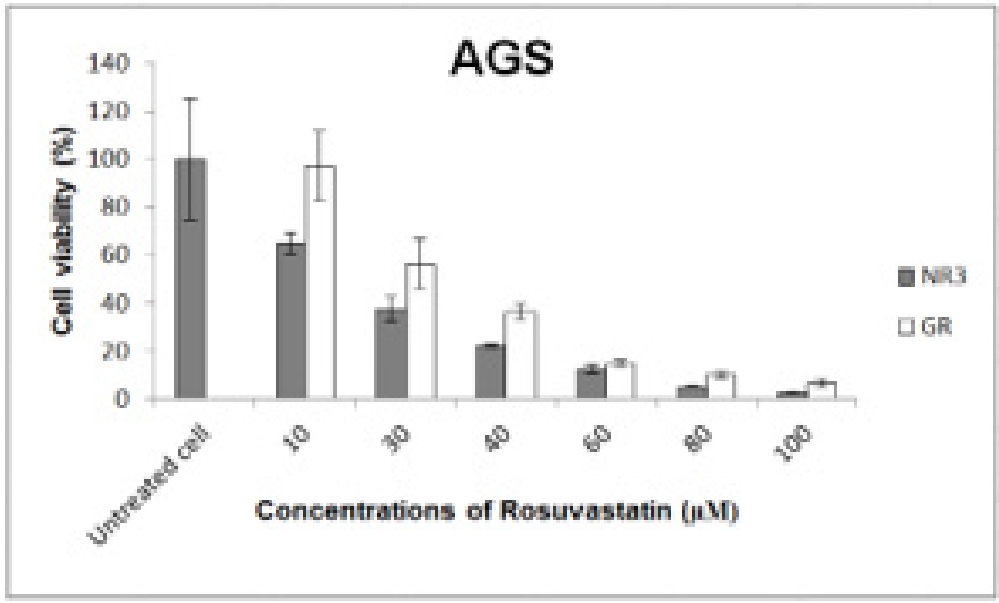


Fig3. The effect of NR3 and GR on the viability of AGS cells

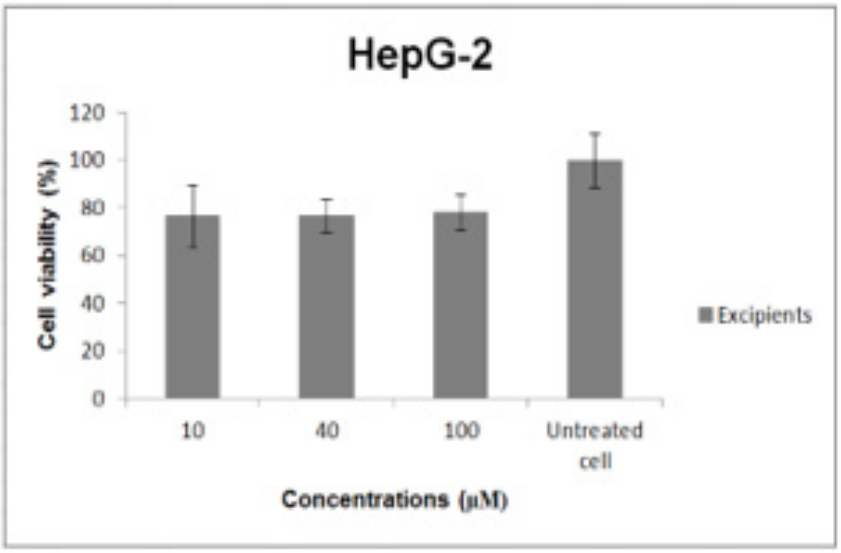


After 72 hours of treatment, cell viability was assessed using the MTT assay. IC50 of NR3 and GR on AGS line were 16.91µM and 32.71µM, respectively each sample was conducted in triplicate.

AGS cells treated with NR3 showed a significantly lower survival rate compared to those treated with GR at all tested concentrations. At a concentration of 10 µM, NR3 and GR induced cell death of approximately 35% and 3%, respectively, compared to the control. At 40 µM, NR3 and GR caused AGS cell death rates of 78% and 63%, respectively, compared to the control.

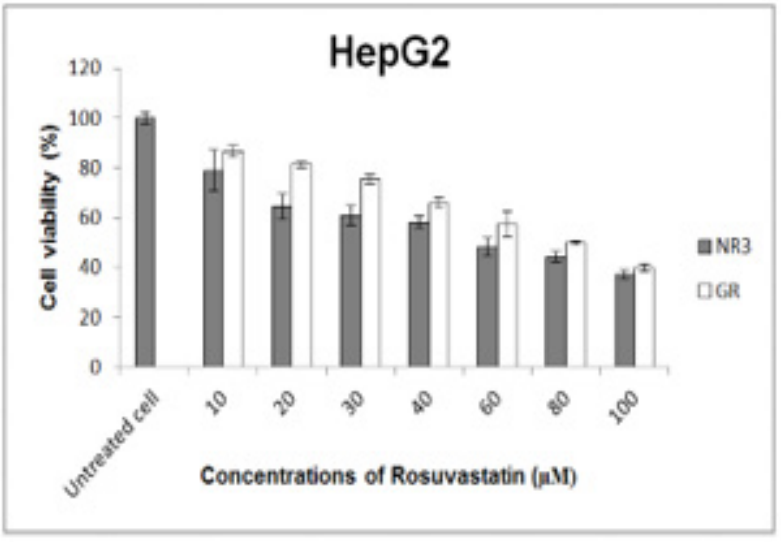
HepG-2 cells treated with NR3 showed a lower survival rate compared to those treated.

Fig4. Effect of excipients to create NR3 on the viability and death of HepG-2



After 72 hours of treatment, cell viability was assessed using the MTT assay. The differences in survival and inhibition rates between control and excipient-supplemented samples at different concentrations were not statistically significant Each sample was conducted in triplicate.

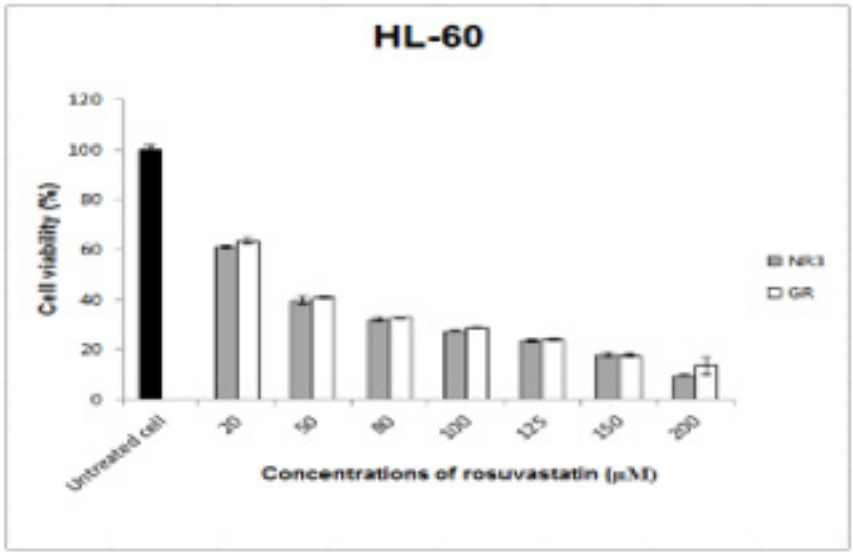
Fig5. Effect of NR3 and GR on the viability and death of HepG-2



After 72 hours of treatment, cell viability was assessed using the MTT assay. Each sample was performed in triplicate. The IC50 of NR3 and GR on HepG-2 line were 54.22 µM and 75.34µM, respectively.

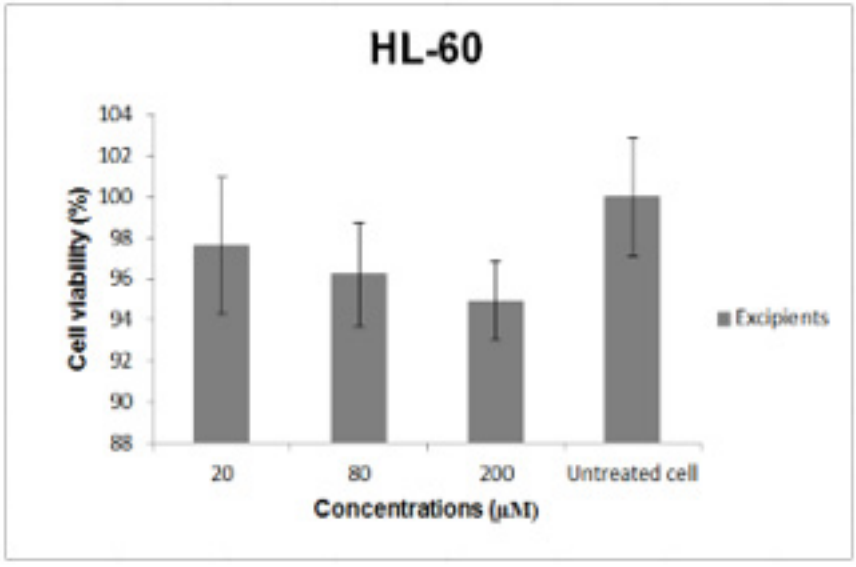
HepG-2 cells treated with NR3 showed a lower survival rate compared to those treated with GR at most of the tested concentrations. At a concentration of 10 µM, NR3 and GR induced cell death of approximately 21% and 13%, respectively, compared to the control. At 40 µM, NR3 and GR caused HepG-2 cell death rates of 41% and 33%, respectively, compared to the control.

Fig6. Effect of NR3 and GR on the viability and death of HL-6



After 72 hours of treatment, cell viability was assessed using the MTT assay. The IC50 of NR3 and GR on HL-60 strain were 33.38 µM and 35.98µM, respectively. Each sample was conducted in triplicate.

Fig7. Effect of excipients to create NR3 on the viability and death of HL-6



After 24 hours, NR3 and GR were treated with a range of rosuvastatin concentrations (0–100 µM). Control cells were not supplemented with the test substance. After 72 hours of treatment, cell viability was assessed using the MTT assay. Each sample was performed in triplicate. The IC50 of NR3 and GR on HL-60 are 33.38 µM and 35.98µM, respectively.

HL-60 cells treated with NR3 and GR showed similar survival rates at the tested concentrations. At a concentration of 20 µM, NR3 and GR caused cell death of approximately 39% and 36%, respectively, compared to the control. At a concentration of 80 µM, NR3 and GR induced HL-60 cell death rates of 68% and 67%, respectively, compared to the control.

4. Discussion

The study results demonstrate the superior properties of Nanorosuvastatin calcium (NR3) compared to crude Rosuvastatin calcium (GR) in terms of solubility and anticancer activity. NR3 exhibited an average particle size of 144 nm, a zeta potential of -5.49 mV, and a Rosuvastatin calcium content of 68.02%. These improvements resulted in a solubility enhancement of 6.89-fold compared to GR, which likely contributed to the increased biological efficacy observed in cancer cell line tests.

In terms of anticancer activity, NR3 consistently outperformed GR in inhibiting AGS and HepG-2 cell growth across all tested concentrations. At 10 µM, NR3 induced 35% AGS cell death compared to 3% by GR. This difference became more pronounced at 40 µM, where NR3 and GR induced 78% and 63% AGS cell death, respectively. The IC50 value of NR3 (16.91 µM) was nearly half that of GR (32.71 µM), demonstrating its significant potential in suppressing gastric cancer cell survival. For HepG-2 cells, NR3 also exhibited superior cytotoxicity, causing 21% and 41% cell death at 10 µM and 40 µM, respectively, compared to 13% and 33% for GR. The IC50 value of NR3 (54.22 µM) was lower than that of GR (75.34 µM), further confirming its enhanced anticancer effects through nano-dosing.

Interestingly, NR3 and GR showed comparable cytotoxicity against HL-60 cells, with no significant differences in cell death rates at any concentration. However, NR3 demonstrated a slightly lower IC50 value (33.38 µM) than GR (35.98 µM), indicating modest improvement. This cell-line-specific response may reflect differences in cellular pathways or drug uptake mechanisms influencing Rosuvastatin's efficacy.

The enhanced anticancer activity of NR3 could be attributed to its improved solubility and intracellular delivery, which likely amplify Rosuvastatin's known mechanisms of action. Previous studies have shown that Rosuvastatin inhibits HMG-CoA reductase, disrupting the mevalonate pathway, which is critical for tumor cell proliferation and invasion. Additionally, Rosuvastatin suppresses oncogenic signaling pathways such as Ras/Rho and induces apoptosis in cancer cells, as demonstrated by Göbel et al. (2020) and Shin et al. (2017). The nanoformulation of NR3 enhances these processes, resulting in improved anticancer efficacy.

Furthermore, the potential of statins as adjunctive cancer therapies is increasingly recognized. Kettana et al. (2024) highlighted Rosuvastatin's role in enhancing chemotherapy efficacy and reducing cardiotoxicity in HER2-positive breast cancer patients. This suggests that nanoformulations like NR3 could not only improve standalone anticancer activity but also enhance the effectiveness of combination therapies.

In summary, this study highlights the advantages of Nanorosuvastatin calcium (NR3) in improving solubility and anticancer efficacy, particularly against AGS and HepG-2 cells. These findings, along with prior evidence, position NR3 as a promising therapeutic candidate for cancer treatment. Further in vivo and clinical investigations are essential to validate its efficacy and safety.

Reference

Akbari BV, Valaki BP, Mardiya VH, Akbari AK, Vidyasagar G. (2011). Enhancement of solubility and dissolution rate of rosuvastatin calcium by complexation with β cyclodextrin. *Int J Pharm Biol Arch*, 2(1):511–20.

Alshora DH, Ibrahim MA, Elzayat E, Almeanazel OT, Alanazi F. (2018). Rosuvastatin calcium

nanoparticles: Improving bioavailability by formulation and stabilization codesign. *PLoS ONE* 13(7), e0200218.

Bansal AB, Cassagnol M. (2024). HMG-CoA Reductase Inhibitors. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available from: https://www.ncbi.nlm.nih.gov/books/NBK542212.

Gabr MM, Mortada SM, Sallam MA. (2018). Carboxylate cross-linked cyclodextrin: A nanoporous scaffold for enhancement of rosuvastatin oral bioavailability. *Eur. J. Pharm. Sci.* 111, 1–12;

Göbel A, Zinna VM, Dell'Endice S, Jaschke N, Kuhlmann JD, Wimberger P, Rachner TD. (2020). Anti-tumor effects of mevalonate pathway inhibition in ovarian cancer. BMC *Cancer*. 20(1):703. doi: 10.1186/s12885-020-07164-x.

Kettana KM, El-Haggar SM, Alm El-Din MA, El-Afify DR. (2024). Possible protective effect of rosuvastatin in chemotherapy-induced cardiotoxicity in HER2 positive breast cancer patients: a randomized controlled trial. *Med Oncol*. 41(8):196. doi: 10.1007/s12032-024-02426-1.

Krishnamoorthy B, Chellan VR, Natarajan TS, Ranganathan H, Siyad A. (2013). Self nano emulsifying drug delivery system (SNEDDS) of Rosuvastatin calcium: design, formulation, bioavailability and pharmacokinetic evaluation, *Colloids Surf*. B 112, 337–343.

Nainwal P, Sinha P, Singh A, et al. (2011). A comparative solubility enhancement study of rosuvastatin using solubilization technique. *Int J Appl Biol Pharm Technol.* 2(4):14–8.

Palani K, Christoper Gv P, Kesavan SK. (2015). Enhancement of rosuvastatin calcium bioavailability applying nanocrystal technology and in-vitro, in-vivo evaluations. *Asian J Pharm Clin Res*. 8: 88–92.

Shin SK, Cho JH, Kim EJ, et al.. (2017). Anti-inflammatory and anti-apoptotic effects of rosuvastatin by regulation of oxidative stress in a dextran sulfate sodium-induced colitis model. *World J Gastroenterol.* 23(25):4559-4568. doi: 10.3748/wjg.v23.i25.4559.

Wang Y, Nguyen DT, Yang G, et al. (2020). An Improved 3-(4,5-Dimethylthiazol-2-yl)-5-(3-Carboxymethoxyphenyl)-2-(4-Sulfophenyl)-2H-Tetrazolium Proliferation Assay to Overcome the Interference of Hydralazine. *Assay Drug Dev Technol*. 18(8):379-384. doi: 10.1089/adt.2020.1004.