RESEARCH ARTICLE

Editorial Process: Submission:03/22/2025 Acceptance:08/08/2025 Published:08/22/2025

In Vitro and *In Vivo* Evaluation of Rosuvastatin and *Momordica charantia* (*Bitter Melon*) Extract: Pharmacokinetic Interactions and Anticancer Potential

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Abstract

Objective: This study investigates the pharmacokinetic interactions and anticancer potential for rosuvastatin in combination with *Momordica charantia* (*M. charantia*) extract through both in vitro and in vivo models. **Methods:** A validated high-performance liquid chromatography (HPLC) method ($R^2 = 0.9983$) was used to quantify rosuvastatin plasma levels. In vivo pharmacokinetic parameters were assessed in Wistar rats following oral administration of rosuvastatin, *M. charantia* extract, and their combination. In vitro, HepG2 liver cancer cells were treated with rosuvastatin, *M. charantia*, and their combination to evaluate anticancer activity. The half-maximal inhibitory concentration (IC_{50}) and fractional inhibitory concentration (FIC) were calculated to assess cytotoxic effects and interaction profiles. **Results:** The IC_{50} values were 231.7 µg/mL for *M. charantia*, 59.1 µg/mL for rosuvastatin, and 48.7 µg/mL for the combination, with an FIC index of 1.03, indicating additive effects. The combination enhanced anticancer efficacy by promoting apoptosis, modulating oxidative stress, and altering lipid and cholesterol metabolism in HepG2 cells. In vivo, co-administration of *M. charantia* significantly shortened rosuvastatin's half-life and increased its elimination rate, without affecting the maximum plasma concentration (Cmax) or the area under the plasma concentration-time curve (AUC). **Conclusion:** These findings suggest that *M. charantia* may influence rosuvastatin pharmacokinetics and enhance its anticancer effects. The study supports the potential of combining natural products with conventional pharmaceuticals as adjunctive therapies in cancer treatment while highlighting the need for further clinical investigation.

Keywords: *Momordica charantia*- rosuvastatin- anticancer- HepG2 cells- Hepatoprotection

Asian Pac J Cancer Prev, **26 (8)**, 3065-3073

Introduction

Rosuvastatin, a widely prescribed statin, effectively lowers cholesterol levels and manages cardiovascular diseases by inhibiting HMG-CoA reductase, a key enzyme in cholesterol biosynthesis [1]. By reducing low-density lipoprotein (LDL) cholesterol, a critical marker for cardiovascular risk, rosuvastatin is particularly beneficial for patients requiring intensive lipid management [2, 3]. Its unique pharmacokinetic properties further enhance its clinical value [4].

Due to extensive first-pass metabolism, rosuvastatin's oral bioavailability is notably low, at approximately 20% [5]. This characteristic localizes its primary action to the liver, the central site of cholesterol synthesis [6]. Unlike many statins, rosuvastatin undergoes minimal metabolism via the cytochrome P450 pathway, primarily involving CYP2C9. This reduces the risk of interactions with substances that affect CYP3A4, making it especially pertinent to study its interactions with natural products like *M. charantia*, which can modulate CYP2C9 activity and influence rosuvastatin metabolism [7].

Furthermore, most rosuvastatin is excreted unchanged through bile and feces, with minimal renal clearance. Its elimination of a half-life of approximately 19 hours allows for once-daily dosing, ensuring consistent blood levels and sustained LDL cholesterol reduction [8, 9]. These features make the drug convenient for patients and enhance treatment adherence.

Given its distinctive pharmacokinetics, understanding rosuvastatin's interaction with natural products is crucial, as M. Charantia extract may influence rosuvastatin's absorption, metabolism, and elimination. *M. charantia* contains bioactive compounds that can affect enzymes

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and transporters involved in drug metabolism, potentially impacting rosuvastatin levels in both laboratory studies (*in vitro*) and living organisms (in vivo) [10]. Investigating these interactions is crucial to ensuring the drug remains effective and safe for patients.

Literature reviews

The pharmacological action of rosuvastatin and its pharmacokinetic properties have been widely explored in the context of lipid-lowering therapy. Figure 1 illustrates rosuvastatin's mechanism of action, including its ability to reduce cholesterol synthesis by inhibiting HMG-CoA reductase and increasing LDL receptor activity. This dual mechanism enhances the clearance of LDL cholesterol from the bloodstream, making rosuvastatin a potent option for managing hypercholesterolemia and reducing cardiovascular risks [11, 12]. Its hydrophilic nature focuses its activity on the liver, minimizing side effects in other tissues and improving its overall safety profile compared to other statins [13].

M. charantia is a well-established medicinal plant recognized for its antidiabetic, anti-inflammatory, and antioxidant effects [14, 15]. Its pharmacological activity is linked to bioactive constituents such as charantin, polypeptide-p, and momordicin [16, 17]. While several studies have shown its efficacy in glycemic control—primarily through insulin-mimetic activity and inhibition of gluconeogenesis—these investigations often rely on unstandardized extracts or animal models [18, 19].

The variability in extract composition and lack of pharmacokinetic validation hinder reproducibility and limit their clinical relevance. Furthermore, few studies have evaluated its interaction with synthetic drugs such as statins, a gap this study aims to address. In addition to its therapeutic effects, *M. charantia* has been reported to modulate key metabolic enzymes, including various cytochrome P450 isoforms [20]. These interactions suggest a potential to alter drug pharmacokinetics when co-administered with conventional therapies. However, most existing studies are either predictive (in silico) or limited to animal data, lacking confirmation in controlled pharmacokinetic models. Moreover, the pharmacodynamic impact of such interactions, particularly with drugs metabolized via CYP-independent pathways like rosuvastatin, remains unclear [21, 17]. This highlights a critical need for experimental validation, which the current study attempts to fulfill by integrating both *in vivo* and *in vitro* approaches.

Understanding potential herb-drug interactions is essential, particularly with widely used medications like rosuvastatin whose therapeutic index is narrow [22]. Although *M. charantia* is believed to affect lipid metabolism, prior studies have not provided conclusive evidence of how these effects translate in co-administration scenarios with statins [23]. Furthermore, many of these claims are derived from observational or mechanistic studies lacking quantitative pharmacokinetic assessments. This study is distinct in combining HPLC-based quantification with pharmacokinetic and cytotoxicity analyses to provide more definitive insights. Investigating these interactions is essential for ensuring patient safety, determining optimal drug dosages, and minimizing potential adverse pharmacokinetic interactions.

Rosuvastatin, a widely prescribed statin, is well-established for its effectiveness and safety in managing cardiovascular diseases [24]. However, co-administration with natural products like *M. charantia* could influence its pharmacokinetics, potentially altering the drug's therapeutic outcomes.

This study explores the interaction between *M. charantia* extract and rosuvastatin through both *in vitro* and *in vivo* experiments. High-performance liquid chromatography (HPLC) will accurately quantify rosuvastatin levels in rat plasma. The research focuses on the pharmacokinetics of rosuvastatin, examining its absorption, metabolism, and elimination in the presence of *M. charantia*. In vitro studies, such as enzyme inhibition assays and drug solubility tests, will be compared with in vivo pharmacokinetic data, including parameters like maximum plasma concentration (Cmax), time to reach

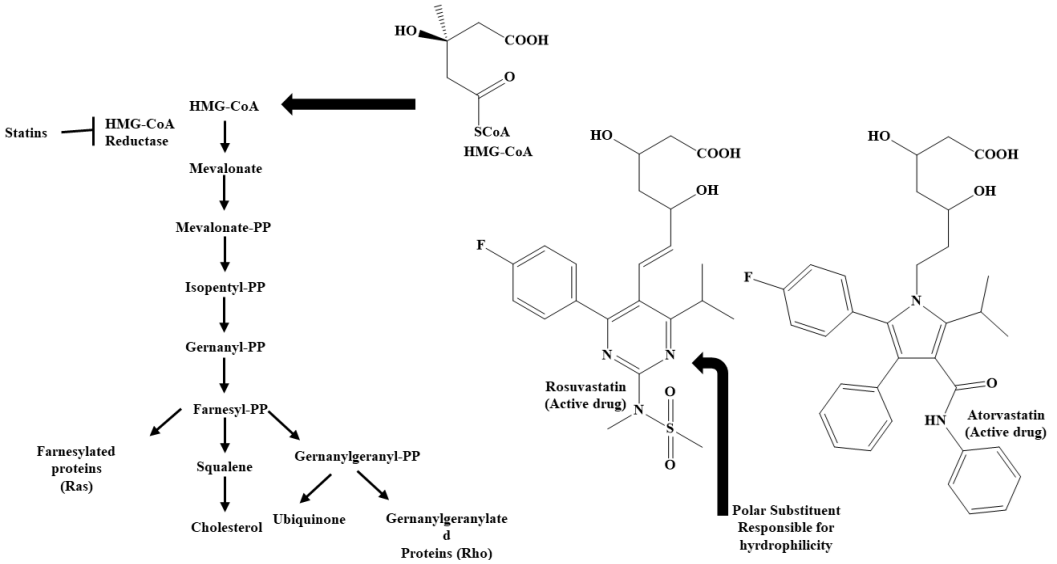

Figure 1. Rosuvastatin's Mechanism of Action: Inhibition of HMG-CoA Reductase and Enhanced LDL Clearance

maximum concentration (Tmax), and area under the curve (AUC). The broader objective is to improve drug safety and optimize dosing regimens when natural products are used alongside prescription medications. Additionally, the study investigates the anticancer effects of *M. charantia* and rosuvastatin, both individually and in combination, against HepG2 liver cancer cells. These findings may provide valuable insights into their potential therapeutic applications.

Materials and Methods

Chemicals and reagents

High-purity chemicals and reagents were utilized to ensure precision and reliability in the analysis. Rosuvastatin calcium was sourced from JOSWE Medical (Amman, Jordan), and standardized *M. charantia* extract was obtained from ZN Bio (China) under batch number SPZD220908. Analytical-grade acetic acid (Medex), high-performance liquid chromatography (HPLC)--grade acetonitrile (MERCK), and sodium acetate trihydrate (China) were employed in the preparation of mobile phases and buffer solutions.

HPLC Analysis and Laboratory Equipment

High-performance liquid chromatography (HPLC) was performed using a Finnigan Surveyor System (Thermo Electron Corporation, San Jose, CA, USA), equipped with a Hypersil BDS C-18 column (250 mm × 4.6 mm; 5 µm particle size). Chromatographic data were analyzed using ChromQuest Software (version 4.2.34). Detection was achieved at a wavelength of 254 nm using a UV-VIS Plus Detector, ensuring optimal sensitivity and specificity.

Additional laboratory equipment included a JENWAY pH meter for precise pH adjustments, a STUART Scientific magnetic stirrer for solution preparation, an Elmasonic S100 Elma ultrasonic cleaner for degassing and cleaning, an Eppendorf centrifuge (model M-24A) for sample processing, and a Phoenix Instrument AS-224 analytical balance for accurate weighing.

All experimental procedures were performed at the Petra University Pharmaceutical Center Instrumental Laboratory, ensuring controlled conditions and adherence to standardized analytical practices.

Chromatographic Conditions

The chromatographic analysis was performed using a mobile phase composition that was selected to optimize resolution and sensitivity for rosuvastatin detection of 70% acetonitrile and 30% sodium acetate buffer (6.1 g/L in distilled water) with the pH adjusted to 3.5 using acetic acid. Separation was achieved on a Hypersil BDS C-18 column (250 mm × 4.6 mm, 5 µm) with a 1.0 mL/min flow rate. The system was maintained at a column oven temperature of 25°C to optimize separation, while the autosampler was set at 5°C to preserve sample integrity. A sample volume of 20 µL was injected into the system, and detection was carried out at 254 nm.

Rosuvastatin Stock Solutions

A stock solution of rosuvastatin was prepared at a 200 µg/mL concentration by dissolving 20 mg of rosuvastatin calcium in 100 mL of acetonitrile. This stock solution was diluted to prepare calibration standards with concentrations ranging from 40 to 2,000 ng/mL. The details of these calibration standards are summarized in Table 1.

HPLC Method Validation

The HPLC method was validated by the guidelines established by the International Council for Harmonisation (ICH) and the European Medicines Agency (EMA). Seven calibration points were employed to assess linearity, and the calibration curve was rigorously analyzed for accuracy and precision. The method exhibited exceptional reliability, with a coefficient of determination (R^2) consistently exceeding 0.998, confirming its robustness and suitability for precise quantifying rosuvastatin levels in plasma.

Quality Control Samples

Quality control (QC) samples were prepared at three concentration levels low (120 ng/mL), medium (1000 ng/mL), and high (1,600 ng/mL) by EMA guidelines to ensure reliable method performance across the calibration range. Each QC sample was analyzed in triplicate to confirm consistency and reproducibility. To maintain accuracy, recalibration was conducted whenever new batches of reagents or samples were introduced, ensuring the method's robustness and reliability throughout the study.

Animal Housing and Ethical Approval

The study was conducted by ethical guidelines approved by the Faculty of Pharmacy and Medical Sciences, University of Petra (Amman, Jordan), under approval number A1/6/2021. Male and female Wistar albino rats weighing approximately 200 grams each were

Table 1. Rosuvastatin Calibration Points

Calibration Point	Concentration (ng/mL)	Volume of Stock Solution (µL)	Volume of Plasma (µL)	Total Volume (µL)
Cal 1	40	10	990	1000
Cal 2	100	25	975	1000
Cal 3	145	35	965	1000
Cal 4	250	62.5	937.5	1000
Cal 5	500	125	875	1000
Cal 6	1000	250	750	1000
Cal 7	2000	500	500	1000

obtained from the animal facility at the Applied Science Private University (Amman, Jordan).

The animals were housed in controlled environmental conditions, including a 12-hour light/dark cycle, a 22–24 °C temperature range, and relative humidity between 55% and 65%. They were given unrestricted water access and fasted overnight before the experimental procedures to standardize metabolic conditions.

Study Design and Treatment Regimens

The rats were randomly divided into four groups, with eight animals in each treatment group and six in the control group, as detailed in Table 2. Group 1 (Control) received no treatment and had unrestricted access to feed and water throughout the experiment. Group 2 (rosuvastatin) was administered a single oral dose of rosuvastatin solution (20 mg/kg) via gavage on the 14th day of the experiment. Group 3 (*M. charantia*) received *M. charantia* extract (4 mg per rat per day) administered by oral gavage for 14 consecutive days. Group 4 (Combination) was treated with *M. charantia* extract (4 mg per rat per day) by oral gavage for 14 days, followed by a single oral dose of rosuvastatin solution (20 mg/kg) on the 14th day.

Sample Collection and Processing

Blood samples were collected from the rats' ocular veins following a fasting period of 12–14 hours. Samples were obtained at specific time intervals: 0.0, 0.5, 1.0, 2.0, 2.5, 3.0, 5.5, 8.0, 24.0, and 48.0 hours post-treatment. The blood was immediately transferred into microtubes coated with EDTA to prevent coagulation and then centrifuged at 5,000 RPM for 10 minutes. The plasma was carefully separated, transferred into labeled Eppendorf tubes, and stored at -80°C to preserve its integrity until analysis.

Post-Experiment Procedures and Tissue Collection

After the experiment, all animals were euthanized via cervical dislocation by ethical guidelines. Liver samples were immediately collected for histopathological evaluation. The livers were examined for visible abnormalities, cleaned, and weighed before being preserved in 10% neutral buffered formalin. The preserved samples were subsequently processed at ALPHA Medical Laboratory (Amman, Jordan) for detailed histological analysis to identify any tissue alterations resulting from the treatments.

In Vitro Study Overview and Materials

The *in vitro* study evaluated the effects of *M. charantia* extract and rosuvastatin, both individually and in combination, on HepG2 human liver cancer cells. The following sections detail the materials used, cell culture methods, experimental treatment protocols, and viability assay setup employed in the study.

HepG2 human liver cancer cells (ATCC, HB-8065) were obtained from the American Type Culture Collection. The cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) (HyClone, USA). Additional supplements included penicillin-streptomycin, amphotericin B (Fungizone), L-glutamine, sodium pyruvate, and non-essential amino acids (NEAA), all sourced from PAN Biotech and Capricorn Scientific.

Dimethyl sulfoxide (DMSO) was used as the solvent to prepare stock solutions for *M. charantia* extract and rosuvastatin. Reagents for viability assays, including MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), were purchased from TCI, Japan. Sterile culture flasks and 96-well plates, essential for the experiments, were procured from SPL Life Sciences, Korea. Using high-quality materials ensured the reliability and reproducibility of the experimental results.

Cell Culture

HepG2 cells were cultured in 75 cm^3 sterile flasks containing Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS), penicillin-streptomycin, 2.5 mg/L amphotericin B, 2 mM L-glutamine, 1 mM sodium pyruvate, and non-essential amino acids (NEAA). The flasks were incubated at 37°C in a humidified atmosphere with 5% CO_2 to ensure optimal growth conditions.

Cell cultures were monitored daily under a microscope, and the medium was replaced every three days to maintain cell health. Upon reaching 70–80% confluence, the cells were detached using trypsin-EDTA, centrifuged, and resuspended in a fresh medium. Cell viability was determined using the Trypan blue exclusion method, and viable cell counts were performed with a hemocytometer to ensure an accurate experimental setup.

Cell Seeding and Treatments

HepG2 cells were seeded into 96-well plates at a density of approximately 10,000 cells per well in 200 µL of MEM and incubated at 37 °C with 5% CO_2 for 24 hours to allow cell adhesion. Following incubation, treatments were applied, including serial dilutions of *M. charantia* extract, rosuvastatin, and their combination. Stock solutions of *M. charantia* extract and rosuvastatin

Table 2. Study Groups and Treatment Protocols

Category	Group 1 (Control)	Group 2 (Rosuvastatin)	Group 3 (M Charantia)	Group 4 (Combination)
Total number of rats	6	8	8	8
Treatment settings	No treatment	Single dose of 20 mg/kg rosuvastatin	M. Charantia extract (4 mg/day)	M. Charantia (4 mg/day) + Single dose rosuvastatin (20 mg/kg)
Duration	14 days	Day 14 of the experiment	14 days	M. Charantia for 14 days + single rosuvastatin dose on day 14

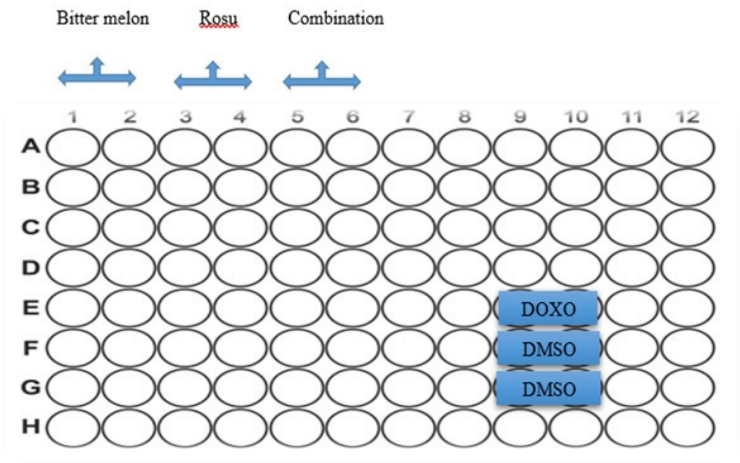

Figure 2. The 96-well Plate Setup Included Duplicate Wells for Each Treatment Group. Abbreviations: Rosu, Rosuvastatin; DOXO, Doxorubicin; DMSO, Dimethyl sulfoxide.

were prepared at 10 mg/mL in DMSO and diluted to final concentrations ranging from 1.171 µg/mL to 150 µg/mL, with combination treatments prepared at equimolar ratios starting at 75 µg/mL. Doxorubicin (10 µg/mL) was the positive control, while 1.5% DMSO was used as the negative control. The experimental layout on the 96-well plate included serial dilutions of *M. charantia* extract (Columns 1 and 2), rosuvastatin (Columns 3 and 4), combination treatments (Columns 5 and 6), the negative control (Column 9), and the positive control (Column 10). The arrangement of treatments is illustrated in Figure 2, highlighting the positions of controls and test samples. The plate was then incubated for 24 hours under standard conditions to ensure sufficient treatment exposure.

MTT Cell Viability Assay

The MTT assay was employed to evaluate the viability of HepG2 cells following treatment with *M. charantia* extract, rosuvastatin, and their combination. MTT powder was dissolved in phosphate-buffered saline (PBS) to prepare a 5 mg/mL stock solution, which was sterilized using a 0.2 µm filter and stored at 4°C until use.

For the assay, 200 µL of the treatment solution was carefully aspirated from each well of the 96-well plate. A working solution was prepared by mixing 15 µL of MTT stock with 100 µL of media, and 115 µL of this solution was added to each well. The plate was incubated at 37 °C for four hours, allowing metabolically active cells to convert MTT into insoluble formazan crystals. After incubation, the MTT solution was gently removed, and 150 µL of 100% DMSO was added to each well to dissolve the formazan crystals. The plate was left at room temperature for 15 minutes to ensure complete dissolution.

Absorbance was measured at 570 nm using a Thermo MultiSkan spectrophotometer. Cell viability was quantified by comparing treated wells' optical density (OD) to untreated control wells, providing a percentage of viable cells for each treatment group.

All treatment conditions were tested in duplicate wells per concentration on a 96-well plate, and the experiment was independently repeated at least twice. Cell viability values represent the mean ± standard deviation of these replicates, and IC_{50} values were derived using non-linear regression analysis in GraphPad Prism software.

Data Analysis

The relative viability of treated cells was calculated using the following formula:

Viability (%)= (Treated Cells OD-Blank OD)/(1.5% DMSO Medium control OD-Blank OD)×100

Logarithmic regression analysis was performed using GraphPad Prism software (GraphPad, Boston, USA) to determine the IC_{50} value for each treatment, representing the concentration required to inhibit 50% of cell growth. These IC_{50} values were subsequently used to evaluate the interactions between *M. charantia* extract and rosuvastatin through the fraction inhibitory concentration (FIC) index, calculated as:

FIC= A/(IC50(A))+B/(IC50(B))

Here, AAA and BBB denote the concentrations of the two compounds in combination, while IC_{50} (A) and IC_{50} (B) represent the individual IC_{50} values of each compound. The FIC index was employed to classify interactions into synergy (FIC < 0.5), additive effects (0.5 ≤ FIC ≤ 4), or antagonism (FIC > 4), as detailed in Table 3.

The FIC index was used to classify drug interactions as follows: FIC < 0.5 indicates synergistic interaction, 0.5 ≤ FIC ≤ 4.0 indicates additive interaction, and FIC > 4.0 indicates antagonistic interaction. Among these, synergy (FIC < 0.5) is considered the most favorable outcome, as it indicates enhanced combined efficacy at lower individual

Table 3. FIC Index Classification

Interaction Type	FIC Value
Synergy	< 0.5
Additive	0.5−4.0
Antagonism	> 4

concentrations.

All statistical analyses were conducted using GraphPad Prism software and Microsoft Excel 365. A p-value of ≤ 0.05 was considered statistically significant. This rigorous analytical approach thoroughly evaluated the tested compounds' cytotoxic effects and potential interactions.

Results

Effect of M. Charantia Extract on Rats
Impact on Mobility, Appearance, and Urine Output

Rats treated with *M. charantia* extract exhibited increased urine output compared to the control group (p = 0.058), indicating a trend toward statistical significance. However, no noticeable changes were observed in the rats' physical appearance or mobility. Activity levels, skin, fur, posture, and body size remained consistent across all groups, suggesting that *M. charantia* extract does not adversely impact the rats' physical behavior or external characteristics. Table S1 summarizes these observations.

Effect on Liver Weight During the Study Period

The group treated with *M. charantia* extract alone experienced a slight but statistically significant reduction in liver weight (~20%) (mean liver weight: 6.89 g, p = 0.037). In contrast, the combination group (*M. charantia* + rosuvastatin) showed no significant difference in liver weight compared to the control group (p = 0.177), see Table 4.

Quantitative Analysis of Rosuvastatin
Calibration Method

The calibration for rosuvastatin plasma concentrations (40–2,000 ng/mL) demonstrated excellent linearity (R^2 = 0.9983), confirming the reliability of the HPLC method (Table S2).

The high correlation coefficient (R^2 = 0.9983) highlights the sensitivity and reliability of the HPLC method for measuring rosuvastatin concentrations. This validation meets the standards of the European Medicines Agency (EMA) and the International Council for Harmonisation (ICH) guidelines. These findings are consistent with previous research that established robust reversed-phase HPLC (RP-HPLC) methods for analyzing rosuvastatin, achieving similar linearity and confirming the method's suitability for routine pharmaceutical quality control.

Quality Control

Quality control samples tested at three concentration levels (low, medium, high) yielded accuracy values ranging from 96.9% to 105.7% and low coefficients of variation (CVs) between 1.6% and 3.1%, ensuring precision and reproducibility as shown in Table S3.

Pharmacokinetics of Rosuvastatin and M. Charantia Plasma Concentrations

The plasma concentration of rosuvastatin, measured in the presence and absence of *M. charantia* extract, revealed no statistically significant differences (p = 0.950). Despite this, minor variations were observed in the pharmacokinetic profile (Table S4, Figure 3).

Pharmacokinetic Parameters

The half-life ($T_{0.5}$) of rosuvastatin was significantly shorter in the combination group (3.39 ± 0.82 hours) compared to rosuvastatin alone (15.27 hours, p = 0.045). Similarly, the elimination rate constant (Kel) was significantly higher in the combination group (0.22 h^{-1}) than in the rosuvastatin-alone group (0.07 h^{-1}, p = 0.013). The Tmax was also delayed in the combination group (5.5 hours vs. 3.4 hours, p = 0.026), while Cmax and AUC last showed no significant differences between the groups (p = 0.31 and p = 0.85, Table S5).

Liver Histopathology
Histological Analysis

Liver tissues from all groups showed intact architecture, with mild congestion observed in the control and

Table 4. Effect of M. Charantia Extract on Liver Weight

Group	Mean (g)	S.D	P-value
Control	8.5	±1.19	N/A
Rosuvastatin	7.6	±1.45	0.289
M. Charantia extract	6.89	±0.53	0.037
M. Charantia + Rosuvastatin	7.46	±0.91	0.177

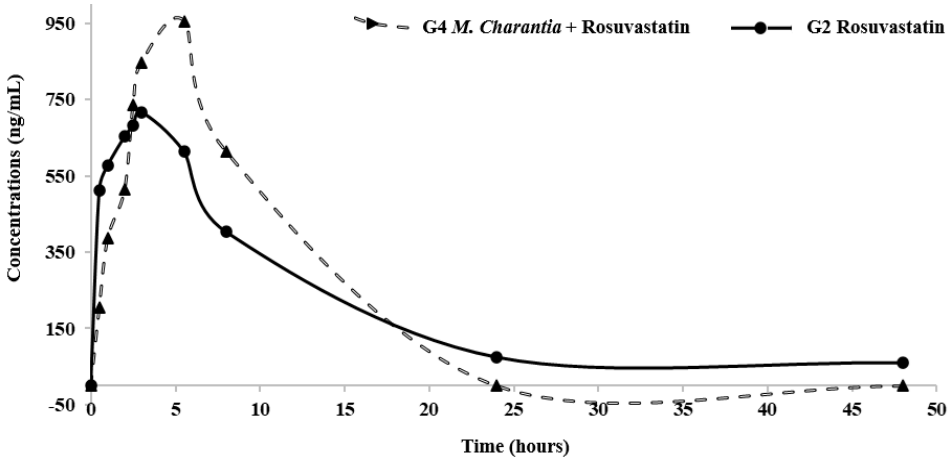

Figure 3. Represent M. Charantia + rosuvastatin Concentrations after HPLC Analysis.

Table 5. Serum Concentration of ALT and AST of Control and M. Charantia Groups.

	ALT (IU/L)	AST(IU/L)
Control group	64.1	153.7
M. Charantia group	60.7	140.7
P-value	0.017	0.028

rosuvastatin-treated groups. Plasma cells were present in the *M. charantia*-treated group, suggesting a mild immune response. No fibrosis or significant inflammation was observed in any group.

Serum ALT and AST Levels

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) levels were significantly lower in the M. charantia group (ALT: 60.7 IU/L, AST: 140.7 IU/L) compared to the control group (ALT: 64.1 IU/L, AST: 153.7 IU/L, p = 0.017 and p = 0.028, respectively, Table 5).

Anticancer Activity

MTT Cell Viability Assay

Rosuvastatin exhibited potent anticancer activity, achieving 103.2% inhibition at 300 µg/mL, while M. charantia extract showed limited cytotoxicity (21.9% inhibition at 300 µg/mL, Table S6 and S7). The combination treatment demonstrated enhanced anticancer effects, reaching 135.9% inhibition at 150 µg/mL (Table S8).

In contrast, rosuvastatin alone exhibited potent anticancer activity, with 103.2% inhibition at the highest concentration (300 µg/mL). The cytotoxic effect decreased dose-dependent at lower concentrations, as shown in Table S8. These results highlight rosuvastatin's potential efficacy against HepG2 liver cancer cells.

The combination of rosuvastatin and *M. charantia* showed an enhanced anticancer effect, reaching 135.86% inhibition at a concentration of 150 µg/mL. Even at lower concentrations, the combination achieved higher inhibition percentages than either treatment alone, indicating a possible synergistic or additive interaction. These results are presented in Table S8.

IC_{50} and FIC Analysis

The IC_{50} values for *M. charantia*, rosuvastatin, and their combination were 231.7 µg/mL, 59.1 µg/mL, and 48.7 µg/mL, respectively (Table 6). The FIC index for the combination treatment was 1.03, indicating additive effects.

Table 6. IC_{50} Values of the Tested Compounds

Compound	IC_{50} (µg/mL on HepG2)
M. Charantia	231.7
Rosuvastatin	59.1
M. Charantia + rosuvastatin	48.7

Discussion

For the effect of *M. charantia* extracts on rats, the observed increase in urine output aligns with previous studies highlighting its diuretic properties. This is likely mediated by increased nitric oxide (NO) production, which promotes smooth muscle relaxation and reduces antidiuretic hormone activity. The lack of changes in physical appearance or mobility supports its safety profile.

The significant reduction in liver weight observed with *M. charantia* treatment alone may result from its effects on lipid metabolism, including reduced fat accumulation in the liver. These findings align with earlier studies suggesting *M. charantia*'s potential role in supporting liver health and metabolic regulation. However, the absence of significant liver weight changes in the combination group indicates that rosuvastatin may counterbalance this effect.

The quantitative analysis of rosuvastatin showed strong linearity in the calibration curve, and reliable QC data confirmed the robustness of the HPLC method for rosuvastatin quantification. These findings are consistent with previous research validating HPLC methods for pharmaceutical analysis.

The pharmacokinetics of rosuvastatin and *M. charantia* presented a shortened half-life and increased elimination rate constant in the combination group, suggesting enhanced clearance of rosuvastatin, potentially mediated by *M. charantia*'s bioactive compounds influencing hepatic metabolism. Delayed Tmax in the combination group could result from *M. charantia*'s effects on gastrointestinal motility and enzyme activity. However, the lack of significant changes in Cmax and AUClast indicates that systemic exposure to rosuvastatin remains unaffected, suggesting compensatory mechanisms in absorption and metabolism.

The liver histological findings and reductions in serum ALT and AST levels suggest that *M. charantia* may exert hepatoprotective effects by mitigating cellular damage and reducing inflammation. These results align with studies demonstrating *M. charantia*'s role in regulating liver enzymes and supporting hepatic health. The enhanced anticancer activity in the combination treatment highlights a potential additive interaction between rosuvastatin and *M. charantia*. *M. Charantia's* bioactive compounds, including flavonoids and saponins, may complement rosuvastatin's inhibition of the mevalonate pathway, disrupting cancer cell growth. These findings align with broader research supporting the therapeutic potential of combining natural products with pharmaceutical agents for cancer treatment.

The IC_{50} and FIC index substantiate the combination's additive effects, emphasizing its therapeutic potential. Further studies are needed to elucidate the underlying molecular mechanisms and evaluate the clinical relevance of these findings.

The absence of molecular or biochemical assessment of glycolysis- and lipid metabolism–related enzymes in the *in vitro* setting. Given that both *M. charantia* and rosuvastatin are known to modulate key metabolic pathways including cholesterol biosynthesis and energy metabolism quantifying the expression or activity of enzymes such as HMG-CoA reductase, fatty acid synthase

(FAS), or glycolytic enzymes (e.g., hexokinase, pyruvate kinase) would have provided mechanistic insights into the observed additive anticancer effects. However, due to resource and scope constraints, this analysis was not included. Future studies are planned to employ proteomic and transcriptomic techniques (e.g., Western blotting, RT-qPCR) to characterize the molecular basis of drug interactions in hepatic cancer models more comprehensively.

One limitation of the present study is the use of a single human liver cancer cell line (HepG2) for evaluating the anticancer effects of *M. charantia* and rosuvastatin. While HepG2 cells are a well-established model for hepatocellular carcinoma and offer valuable insights due to their hepatic origin, the inclusion of additional cell lines with varying genetic and phenotypic profiles, such as Huh7 or Hep3B, would enhance the robustness and generalizability of the findings. Employing multiple cell lines would allow for a broader assessment of cellular responses, accounting for differences in receptor expression, signaling pathways, and drug metabolism. Future studies should therefore consider incorporating diverse hepatic and non-hepatic cancer cell lines to validate the consistency of the observed cytotoxic and synergistic effects across different biological models. This approach would further strengthen the translational relevance of the findings for clinical applications.

In conclusion, this study evaluated the pharmacokinetic interactions and anticancer potential of rosuvastatin and *M. charantia* extract using validated *in vitro* methods. The findings demonstrated that *M. charantia* altered the pharmacokinetics of rosuvastatin, significantly influencing parameters such as the drug's half-life, elimination rate constant, and time to maximum concentration. These results suggest that *M. charantia*'s bioactive compounds modulate hepatic metabolism and gastrointestinal motility, impacting drug clearance and absorption.

Moreover, the study highlighted *M. charantia*'s hepatoprotective effects, evidenced by reduced liver enzyme levels and preserved liver histology. The combination therapy exhibited enhanced anticancer activity against HepG2 liver cancer cells, indicating additive cytotoxic effects. This synergy is attributed to the complementary mechanisms of *M. charantia*'s bioactive compounds and rosuvastatin's mevalonate pathway inhibition.

The findings underscore the potential of integrating natural products like M. charantia with pharmaceutical agents to optimize therapeutic outcomes. However, further research, including clinical trials, is warranted to validate these interactions in vivo and ensure safety and efficacy in human populations. These insights pave the way for novel adjunctive therapeutic strategies that harness the benefits of natural and synthetic agents in managing complex diseases.

Author Contribution Statement

All authors contributed to the interpretation of the findings, critically revised the manuscript, and approved the final version for publication. Formal analysis and validation: Rania Akeel (RA), Soha Yahya Rahhal (SYR), Jamile Bashir Al-Saqqa (JBA). Conceptualization, methodology, and Project administration: Kenza Mansoor (KM), Eyad Mallah (EM). Provision of animals and experimental materials: Luay Abu-Qatouseh (LA), Feras Darwish El-Hajji (FDE). Investigation and primary manuscript drafting: Khaled W. Omari (KWO). Data and statistical analysis: Nasir Idkaidek (NI).

Acknowledgements

General

The authors gratefully acknowledge the support provided by the University of Petra and the Applied Science Private University, which were essential for this research.

Funding Statement

The authors would like to acknowledge the deanship of Scientific Research at the University of Petra for financial support.

Ethical Declaration

The study was conducted by ethical guidelines approved by the Faculty of Pharmacy and Medical Sciences, University of Petra (Amman, Jordan), under approval number A1/6/2021.

Data Availability

Research data is accessible upon request to the corresponding author.

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