International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 17, Issue 1, 2025

Original Article

A STUDY OF PHARMACOKINETIC INTERACTION BETWEEN MANGIFERIN AND ATORVASTATIN IN HYPERLIPIDEMIC RATS

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Received: 05 Aug 2024, Revised and Accepted: 16 Nov 2024

ABSTRACT

Objective: The main aim of this study is to determine the effect of Mangiferin on the pharmacokinetics of Atorvastatin in hyperlipidemic rats.

Methods: The plasma concentration of Atorvastatin (ATR) to the time profile was calculated using High Performance Liquid Chromatography (HPLC). The experiment was carried out in normal and High Fat Diet (HFD) induced hyperlipidemic rats in single and multiple-dose treated rats for pharmacokinetic study. HFD was administered to the rats for 8 w to induce hyperlipidemia. Each group contains six animals, Group I received ATR alone (20 mg/kg), Group II received Mangiferin (MGF) (40 mg/kg) followed by ATR for Single-Dose Interaction(SDI), Group III received MGF for 7 days, followed by ATR on 8th d for Multiple Dose Interaction (MDI). Blood samples were collected and estimated for pharmacokinetic parameters such as the Area under Curve (AUC), Maximum Plasma Concentration (C_{max}), Time to achieve Cmax (Tmax), Biological Half-Life ($t_{1/2}$), mean Residence Time (MRT) and Volume of distribution (V_d), Clearance (CL) and Elimination Rate Constant (K_e).

Results: In normal and disease-induced rats of SDI and MDI studies it has shown increased plasma concentration of ATR in the presence of MGF. MGF significantly increased the following pharmacokinetic parameters: C_{max} (83.56±5.94 ng/ml) in SDI, (100.28±6.37 ng/ml) in MDI, AUC (265.25±14.73ng. h/ml) in SDI, (299.04±14.11ng. h/ml) in MDI. $t_{1/2}$, V_d, and MRT were also enhanced in both studies. However, CL and K_e were reduced significantly.

Conclusion: This study revealed that MGF altered the ATR pharmacokinetics. This could be due to MGF Cytochrome P450 (CYP3A) enzyme inhibition.

Keywords: Atorvastatin, Mangiferin, Hyperlipidemia, Pharmacokinetics

DOI: https://dx.doi.org/10.22159/ijap.2025v17i1.52248 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Hyperlipidemia is characterized by elevated levels of lipids such as cholesterol, Triglycerides (TG), Low-Density Lipoproteins (LDL) and Very Low-density Lipoproteins (VLDL). High levels of cholesterol and TG lead to aggrandizement risk of Cardio Vascular Stroke (CVS). Pharmacological and non-pharmacological approaches are used to reduce the hyperlipidemia. In pharmacological approaches-hypolipidemic drugs are used to prevent the development of cardiovascular risk, atherosclerosis, coronary heart disease, and peripheral artery diseases. Non-pharmacological approaches include regular exercise, and avoiding high-fat foods, trans fats, and saturated fat. Specific nutritional food intake such as soya protein, tree nuts, omega-3 fatty acids and sterols improves lipid profile [1].

Our daily diet consists of fruits, vegetables, and grains that contain active phytochemicals like polyphenols, terpenoids, steroids, alkaloids, tannins, saponins, phytosterols and flavonoids. These phytochemicals play a key role in the reduction of cholesterol and the prevention of Cardio Vascular Diseases (CVD) [2]. ATR is a lipid-lowering drug, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor used in the prevention of Coronary Heart Disease (CHD), and hypertriglyceridemia. ATR is rapidly absorbed after oral administration, it has low bioavailability because of first-pass metabolism and high plasma protein-binding property. CYP3A is involved in the metabolism of ATR. Adverse effects include arthralgia, myopathy, and rhabdomyolysis [3].

MGF is a C-glycosylxanthone natural polyphenol obtained from the *mango* tree *Mangifera indica*, *Iris unguicularis*, tubers of *Pueraria tuberosa* and *Anemarrhena asphodeloides* [4]. It has shown tremendous pharmacological activities such as neuroprotective, antioxidant, anti-inflammatory, immunomodulatory, anticancer, antidiabetic and analgesic [5-8].

Nowadays, phytochemicals showing various pharmacological effects with fewer side effects have been used in combination with modern drugs to reduce the adverse effects and enhance the potentiality of the drug. Still, such a combination of herbs/phytochemicals/diet containing rich in phytochemicals leads to herb-drug interaction by interfering with drug absorption, metabolism and elimination. This study is mainly designed to observe the pharmacokinetic interaction between MGF and ATR.

MATERIALS AND METHODS

ATR and amlodipine were procured as gift samples from Dr. Reddy's Laboratory, Hyderabad. Acetonitrile, methanol, and orthophosphoric acid of HPLC grade were purchased from Taranath Chemicals, Hanumakonda. MGF was purchased from SRL laboratories, Mumbai. HFD purchased from Biotechnolabs, Hyderabad.

HPLC analysis of ATR

Mobile phase

The mobile phase was prepared from 20 mmol phosphate: acetonitrile (60:40 v/v) (pH 4.5) adjusted with 1% orthophosphoric acid with absorbance recorded at 248 nm.

Preparations of standard and stock solutions

1 mg/ml of stock solutions of ATR, and amlodipine was dissolved in HPLC-grade methanol. Then, stock solutions were further diluted to get working standard and internal standard solutions in a concentration range of 10 µg/ml and 1µg/ml. All solutions were stored at-20 °C. From this, different concentrations (80, 100, 200, 400, 600, 800, 1000 ng/ml) (300 ng/ml) of standard and internal standard solutions were used for the standard graph calibration of the drug.

Extraction of plasma samples

In a 2 ml centrifuge tube 500 µl plasma, 100 µl of different concentrations (80-1000 ng/ml) of standard and 200 µl internal standard (300 ng/ml) solutions were added, then 2 ml of acetonitrile was added, and vortexed for 2 min, centrifuged at 8000 rpm for 3 min. The resultant organic layer was separated. After separating, drying, and reconstituting the residue with mobile phase, a 20 µl

aliquot was injected into the HPLC system to perform chromatographic analysis. Peak area ratios were calculated [9-11].

HFD induced hyperlipidaemia

Wistar rats were fed with HFD for 8 w, hyperlipidemia was confirmed by measuring the lipid levels, then animals were divided into various treatment groups for study [12].

Experimental animals

Adult male Wistar rats weighing between 180 and 200 g, were obtained from Vyas Enterprises in Hyderabad, India. The study adhered to CPCSEA guidelines (01/IAEC/UCPSc/K U/2022: CPCSEA), as approved by Kakatiya University, Warangal. The animals were housed in cages and maintained under standard laboratory conditions, including a 12 h light/dark cycle, an ambient temperature of 25±5 °C, and relative humidity of 35-60%. They were fed a standard rat pellet diet and had access to water ad libitum.

Grouting of normal and HFD-induced hyperlipidemic rats

Healthy wistar rats were divided into 3 groups for normal study and 3 groups for HFD-induced hyperlipidemic study, each group containing six rats.

Group I: treated with ATR (20 mg/kg) [13].

Group II: treated with MGF (40 mg/kg) followed by ATR for an SDI study [14].

Group III: treated with MGF for 7 d, followed by ATR on 8th d for MDI study.

Blood samples were drawn from the retro-orbital plexus using heparinized capillaries at 0.5h, 1h, 2h, 4h, 8h, 12h, and 24h intervals into a microcentrifuge tube consisting of anticoagulant (sodium citrate). After completion of centrifugation, plasma samples were separated and stored at-20°C until HPLC sample analysis.

Data and statistical analysis

Data is expressed as mean±Standard Deviation (SD). *p<0.05, **p<0.01, **p<0.001 considered as statically significant in comparison to Atorvastatin alone group. One-way ANOVA using Dennett's test was applied to determine the significance. Kinetica software (version 5.0, Thermo Fisher Scientific, Inc., USA) was used for plasma concentration-time data analysis. Pharmacokinetic parameters were also expressed in terms of fold changes which helps to determine the enhanced or decreased values when compared to the ATR alone.

RESULTS AND DISCUSSION

ATR-MGF interactions

Pharmacokinetics of ATR with pre-treated MGF in normal rats

Pharmacokinetic parameters of ATR alone and in combination with MGF following single and multiple dose administration were represented in table 1. In both SDI and MDI, C_{max} was increased to (0.2 and 0.44) fold, (0.09 and 0.23) fold in AUC_{0-n}, (0.06 and 0.16) fold in AUC_{total}, (0.2 and 0.65) fold in $t_{1/2}$, (0.16 and 0.58) fold in MRT, (0.1 and 0.25) fold in V_d. CL was decreased to (0.06 and 0.1) fold, (0.15 and 0.47) fold in the K_e in combination of ATR with MGF-treated rats when compared to the ATR alone group. In both SDI and MDI, significant changes were observed in ATR with MGF combined rats when compared to the ATR alone group. Plasma concentration of ATR was higher in combination than ATR alone which was shown in fig. 1.

Table 1: Pharmacokinetic parameters of ATR alone and ATR in the presence of MGF in normal rats

PK parameters in normal rats	ATR (Group-I)	ATR+MGF(SDI) (Group-II)	ATR+MGF(MDI) (Group-III)
C_{max}(ng/ml)	69.21±6.98	83.56±5.94*	100.28±6.37***
T_{max}(h)	1	1	1
AUC_{0-n} (ng. h/ml)	242.43±11.90	265.25±14.73*	299.04±14.11***
AUC_{total}(ng. h/ml)	349.22±10.52	371.27±14.83*	406.39±15.36***
$t_{1/2}$ (h)	4.17±1.36	5.04±1.94*	6.92±1.57*
MRT (h)	6.79±1.67	7.89±2.89*	10.74±1.56**
V_d(ml/kg)	75.37±5.28	83.37±5.55*	94.36±4.90**
CL (ml/h/kg)	229.12±9.17	213.74±9.47*	195.47±10.03***
k_{el} (h−1)	3.05±1.02	2.56±1.19	2.07±1.06*

Value are mean±SD n = 6/group ATR: Atorvastatin; MGF: Mangiferin; SDI: Single dose interaction; MDI: Multiple dose interaction; ∗ p<0.05, **p<0.01; *** p<0.001 vs ATR alone group (One-way ANOVA followed by Dunnett's test).

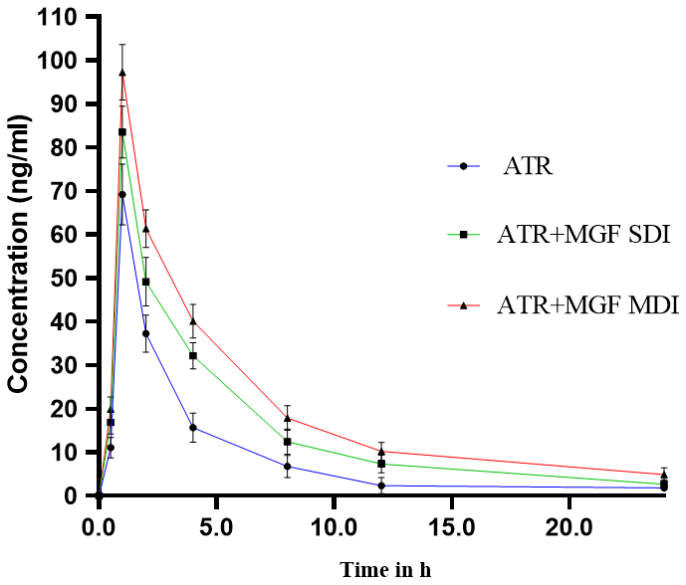

Fig. 1: Mean plasma concentration of ATR alone and ATR in the presence of MGF in normal rats, data expressed as (mean±SD) N = 6

Pharmacokinetic parameters of ATR alone and ATR in the presence of MGF in HFD-induced hyperlipidemic rats

Pharmacokinetic parameters were represented in table 2. C_{max} was increased to (0.23 and 0.54) fold, AUC_{0-n} increased to (0.09 and 0.15) fold, AUC_{total} increased to (0.07 and 0.13) fold, $t_{1/2}$increased to (0.21 and 0.40) fold, MRT increased to (0.35 and 1.01) fold, V_d increased to (0.13 and 0.35) fold, CL and K_e decreased to (0.07 and 0.32) fold and (0.15 and 0.5) fold in both SDI and MDI of the combined group when compared to ATR alone group. The mean plasma concentration of ATR alone and in combination with MGF is represented in fig. 2.

Table 2: Pharmacokinetics of ATR alone and ATR in the presence of MGF in HFD-induced hyperlipidemic rats

PK parameters in HFD-induced hyperlipidemic rats	ATR (Group-I)	ATR+MGF(SDI) (Group-II)	ATR+MGF(MDI) (Group-III)
C_{max}(µg/ml)	84.27±5.82	115.82±6.06*	152.12±.5.83***
T_{max}(h)	1	1	1
AUC_{0-n} (µg. h/ml)	278.26±10.58	305±12.65*	321.21±12.53***
AUC_{total}(µg. h/ml)	384.74±14.72	411.57±14.39*	437.49±14.28***
$t_{1/2}$ (h)	5.15±2.47	6.26±1.06	7.45±2.72*
MRT (h)	7.93±2.17	10.73±4.26*	15.94±3.55**
V_d(ml/kg)	104.15±8.69	118.17±7.88*	141.50±7.10***
k_{el} (h−1)	2.30±1.22	1.94±0.93	1.15±0.87*
CL (ml/h/kg)	240.85±10.05	222.91±8.04*	163.56±8.88***

Value are mean±SD n = 6/group ATR: Atorvastatin; MGF: Mangiferin; SDI: Single dose interaction; MDI: Multiple dose interaction; * p<0.05, **p<0.01; *** p<0.001 vs ATR alone group (One-way ANOVA followed by Dunnett's test).

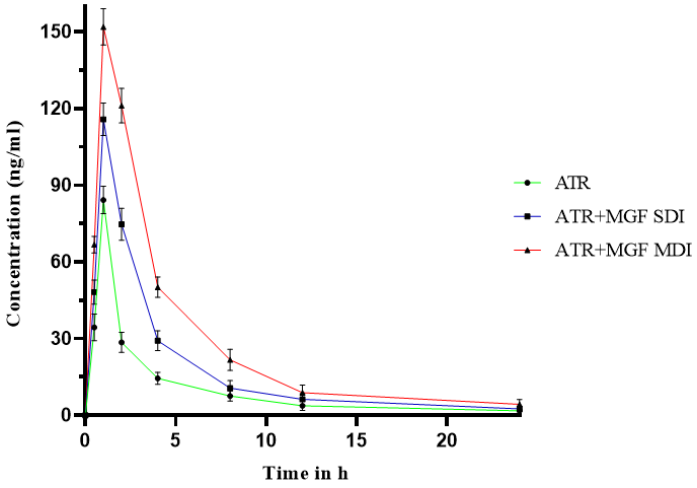

Fig. 2: Mean plasma concentration of ATR alone and ATR in the presence of MGF in hyperlipidemic rats, data expressed as (mean±SD) (N=6)

ATR is the most commonly used statin drug to treat hyperlipidemia which reduces the risk of CHD. It is metabolized via hepatic CYP3A/CYP3A4 in rats/humans [13]. MGF a xanthone derivative found in various parts of mango fruit shown to have anticancer, antialzhemers, antiviral, antioxidant, hepatoprotective, immunomodulatory, antidiabetic, and antihyperlipidemic activities [15-17].

Researchers reported that the extract of *Mangifera indica* and MGF in an *in vitro* study reduced the activity of some cytochrome P-450 enzymes in rat hepatocytes and human liver microsomes. They also revealed these two reduced the UGT1A1, UGT1A9, UGT2B7, CYP3A4, CYP3A5 activity and increased the CYP1A1, CYP1A2 activity [18]. Phytochemical supplementation along with prescription drugs becoming more and more popular but usage of phytochemicals/herbs with prescribed drugs leads to interaction, which results in therapeutic failure or increased toxicity. Based on this view, we have designed the work to observe that MGF administration along with ATR produces no interaction.

Within this research, significant changes in pharmacokinetic parameters were observed in both SDI and MDI studies of pre-treated rats of MGF with ATR when compared to the ATR alone treated group. These values coincided with the results reported by Reddy *et al.*, 2011 [19]. MGF increased the AUC, C_{max}, MRT, and $t_{1/2}$ but the CL and elimination rate of ATR was reduced, whereas T_{max} of ATR was not altered. MGF, which has been reported for its inhibitory activity on CYP3A4 in *vitro* and *silico* studies strongly correlated with our study [20, 21].

Enhanced C_{max}, AUC, and reduced CL indicates that MGF increased the ATR plasma levels. In a previous study authors reported the synergistic interaction between MGF and gabapentin. These observations agree with our studies showing that MGF produces interaction with drugs [22]. Kirankumar *et al.*, 2013 found in their research that MGF does not counteract the positive benefits of Repaglinide when given in combination with it, which concludes combination of MGF and Repaglinide was safely preferred in diabetes [23]. An elevation in the values of AUC and C_{max} signifies the enhanced bioavailability of ATR when co-administered with MGF. This phenomenon could be attributed to the potential interaction between MGF and ATR metabolism, where MGF is believed to suppress hepatic metabolism by acting as an inhibitor. Consequently, the efficacy of ATR is potentiated in the presence of MGF.

CONCLUSION

In this research, MGF changed the pharmacokinetics of ATR in both single and multiple-dose studies when compared to the ATR alone group. More significant changes were observed in the combination study of MDI when compared to the ATR alone group. Thus the

present study revealed phytochemical and drug interaction in which MGF enhanced the plasma levels of ATR could be due to MGF inhibiting the metabolism of ATR.

ACKNOWLEDGEMENT

The funds for this study were provided by the AICTE, New Delhi, for which the authors are grateful. The authors would also like to thank Kakatiya University and Vaagdevi College of Pharmacy in Warangal for providing the facilities needed for the completion of the study.

AUTHORS CONTRIBUTIONS

Study design, methodology, data collection, analysis, interpretation, and manuscript draft were prepared by Mrs. Meesa Madhavi. Prof. Yellu Narsimha Reddy supervised the study design, methodology, and interpretation of results and reviewed the final manuscript.

CONFLICT OF INTERESTS

There is no conflict of interest, according to the authors.

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