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Synthesis, characterization, and pharmacological evaluation of some metal complexes of quercetin as P-gp inhibitors

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Abstract

Background: Six different metal complexes of quercetin (Cu, Zn, Co, Vd, Mo, Ni) were synthesized, purified, and characterized by their physical and spectral (UV, IR) data. They were evaluated for their P-gp (permeability glycoprotein) inhibitory activity by in vitro everted sac method in rats. The apparent permeability of atorvastatin (P-gp substrate) from everted sac of the rat intestine was determined in control, standard (verapamil), and groups treated with quercetin-metal complexes. The drug contents were analyzed by validated RP-HPLC method using a mixture of acetonitrile and water (60:40 v/v) adjusted to pH 2.8 with phosphate buffer as mobile phase.

Results: In vitro studies revealed that the apparent permeability of atorvastatin (P-gp substrate) across the small intestine is much affected by the treatment with Cu/Co/Ni complexes of quercetin. The mean ± SD and apparent permeability of atorvastatin decreased after pre-treatment with these metal complexes.

Conclusions: The quercetin Cu/Co/Ni complexes could inhibit P-gp and increase the atorvastatin absorption. Hence, they could be considered P-gp inhibitors.

Keywords: Quercetin, Metal complexes, Atorvastatin, P-gp, Inhibitors, P-glycoprotein

Background

Cancer is a dreadful disease, killing a large number of the population worldwide. More than 100 different types of cancer are reported to affect humans [1, 2]. Chemotherapy is widely used for cancer treatment but it is hindered mostly due to the resistance of tumor cells to anticancer drugs [3, 4]. Several mechanisms underlying drug resistance were identified. Increased efflux of drugs by cancerous cells, due to over expression of membrane transporter proteins (efflux pumps) is one of the major mechanisms documented. P-glycoprotein (P-gp) is the first discovered multidrug transporter that pumps drugs out of tumor cells, resulting in decreased intracellular drug concentrations and thus reducing the efficacy of drugs [5]. It is present in several normal tissues like intestinal lining epithelium, endothelial cells, and bone marrow.

Quercetin (Q) is a major naturally occurring flavonoid, belonging to the class of flavonols. It is ubiquitously found in a wide variety of plant products like coffee, tea, dyes, vegetables, and fruits [6]. The beneficial effects of quercetin are mostly due to its free radical scavenging or antioxidant property and its ability to chelate metal ions (Fe^{2+} and Fe^{3+}, Cu^{2+}, Ni^{2+}) [7–12]. Quercetin and some of its metal complexes displayed various biological actions such as antimicrobial, antiulcer, antiallergic, anti-Alzheimer's, and anticancer [13–18]. It was reported that quercetin could competitively inhibit the members of MDR family, P-gp, MRP1, and BCRP [19–23]. But, hitherto, there are no reports on the P-gp inhibitory activity of quercetin-metal complexes. In this regard, the present

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work was aimed to synthesize, characterize, and pharmacologically evaluate the metal (M) complexes of quercetin (M=Cu, Zn, Mo, VO, Ni, Co) for their P-gp (P-glycoprotein) inhibition activity by in vitro rat everted sac method in Wistar albino rats [24, 25], as this model despite a few limitations is an economic, simple, and highly efficient tool in investigating the effect of P-gp inhibitors across the small intestine of rats and serves to predict the drug absorption in humans.

Methods
Drugs and chemicals
The drug atorvastatin was purchased from Stallion Laboratories Pvt. Ltd., Ahmedabad; verapamil was purchased from Ranbaxy Laboratories Ltd., Hyderabad; and quercetin was obtained from Sigma Aldrich Chemicals, Bangalore. HPLC-grade methanol and acetonitrile and BDH-grade of the chemicals copper chloride dihydrate, zinc chloride hexahydrate, ammonium molybdate,

vanadyl sulphate monohydrate, nickel chloride hexahydrate, cobaltous chloride hexahydrate, and hydrochloric acid were purchased from Qualigens Fine Chemicals, Mumbai. Dimethyl sulfoxide, sodium hydroxide, and hexane were obtained from Finar Chemicals, Mumbai. Dulbecco's phosphate buffer pH of 7.4 was purchased from Hi Media Ltd., Mumbai, and HPLC-grade water was procured from S.R. Enterprises, Hanamkonda.

Instruments
UV-visible double-beam spectrophotometer (Shimadzu UV-1800, 240V, 2010), FT-IR spectrophotometer (Bruker ALPHA, 2011), HPLC (Cyberlab, SPD-10A UV-Vis detector, LC 10AD pump, 2011), refrigerator (LG 360L, 2008), electronic weight balance (Shimadzu AUY 220, 2011), sonicator (Electric supply 1.5L50, 2010), electro-magnetic stirrer (Remi G3M5-3600, 2008), and vacuum desiccators were used.
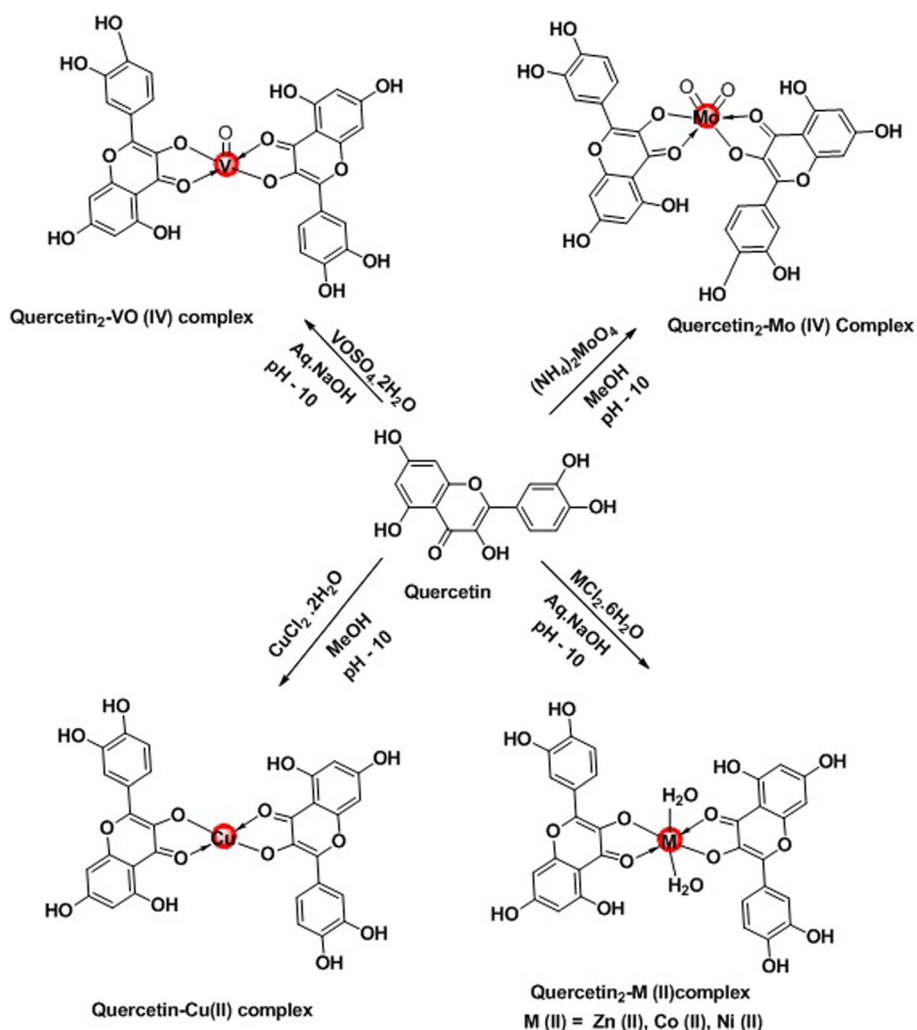
Scheme 1 Synthesis of quercetin-metal (Q-M) complexes

Table 1 Physical data of quercetin-metal complexes

S. no.	Metal complexes	Color	% Yield
1	Quercetin-copper	Brownish yellow	83.07
2	Quercetin-zinc	Dark brown	82.63
3	Quercetin-molybdenum	Dark red	80.80
4	Quercetin-vanadyl	Brownish yellow	82.90
5	Quercetin-nickel	Pale yellow	80.39
6	Quercetin-cobalt	Dark brown	84.64

Animals

Twenty-four healthy Wistar albino adult male rats 7–9 weeks of age, weighing between 200 and 250 g, procured from Mahaveer Enterprises (reg no. 146/1999 CPCSEA) Hyderabad, Telangana, were used in this research work. The animals were accommodated at the animal house of the institute approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (reg. no. 1047/ac/07 CPCSEA). They were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2 °C and $50 \pm 15\%$ relative humidity, with a 12-h light/dark cycle. Rats were fed with a commercial pellet (Hindustan lever Pvt. Ltd. Mumbai, India) diet and water ad libitum. They were overnight fasted prior to the experiment. The animal experiments were performed in accordance with the CPCSEA guidelines after prior approval of the study protocol by the Institutional Animal Ethics Committee (IAEC).

Spectral analyses of quercetin complexes

UV-visible study of quercetin and its metal complexes (Cu, Zn, Mo, VO, Ni, Co) were carried out in the wavelength range of 200 to 500 nm by using UV-visible spectrophotometer. Infrared spectral study of these complexes was performed using IR spectrophotometer and the obtained spectral data were analyzed [9, 26, 27].

Preparation of quercetin-metal complexes

The quercetin-metal complexes have been synthesized and prepared according to previously reported method [28].The Cu(II), Zn(II), Ni(II), and Co(II) complexes were prepared using respective metal chlorides; VO(IV) complex was prepared using vanadyl sulphate; and Mo(IV) complex was prepared using its ammonium salt.

In the preparation of all the metal complexes, the metal and the quercetin compounds were combined in 1:2 mole ratio (the metal being in slight excess of the desired ratio) using appropriate quantities of aqueous methanolic quercetin and methanolic or aqueous metal salt solutions, respectively. The pH of the solutions was adjusted to 10 by adding sodium hydroxide solution.

General procedure for the preparation of quercetin-metal complexes

To a hot methanolic solution of quercetin taken in a 50-ml two-necked round-bottomed flask equipped with magnetic stirrer, a solution of an appropriate metal salt (copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$), zinc chloride hexahydrate ($ZnCl_2 \cdot 6H_2O$), nickel chloride hexahydrate ($NiCl_2 \cdot 6H_2O$), cobaltous chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), vanadyl sulphate monohydrate ($VOSO_4 \cdot H_2O$), ammonium molybdate (($(NH_4)_2MoO_4$)) in methanol was added dropwise with stirring and the resultant mixture was heated under reflux for 4–8 h. Obtained solids were filtered off and washed with diethyl ether and dried under vacuum (Scheme 1).

Pharmacological evaluation

Everted rat sac method

The in vitro everted sac technique was introduced by Wilson and Wiseman [29] and later modified into modern everted sac technique. It is a simple, renowned tool to study various pharmacokinetic phenomena like absorption, metabolism, and interaction of drugs and to carry out studies related to multidrug resistance (MDR), role of efflux transporters, and the effect of MDR modulators on drug absorption in the gut. This model involves the gentle eversion of a freshly excised portion of rat small intestine using a glass rod, clamping, and tying one end of the segment and filling it with appropriate solution, sealing the other end tightly, immersing the obtained sac in physiological solution maintained at 37 °C, collecting the samples at different time intervals, and analyzing the results obtained. The everted sac technique measures the serosal-to-mucosal transport of drug substrates [30, 31] unlike the non-everted sac technique which measures the mucosal to serosal transport of drug substrates [32].

The animals were overnight fasted and anaesthetized using isoflurane (2–3% by drop jar method). After anaesthetizing the animals, they were euthanized with cervical decapitation and the whole small intestine of each animal was isolated and flushed with 50 ml of ice-cold saline. The small intestine was divided into duodenum, jejunum, and ileum ($n = 3$). Segments were everted, and 10-cm-long sacs were prepared. About 1 ml of atorvastatin

Table 2 UV-spectral data of quercetin-metal complexes

Bands	Quercetin (Q) (nm)	Cu(II)-Q complex (nm)	Zn(II)-Q complex (nm)	Mo(II)-Q complex (nm)	VO(IV)-Q complex (nm)	Ni(II)-Q complex (nm)	Co(II)-Q complex (nm)
I	369	401	403	410	404	415	424
II	252	263	253	339	257	257	263

Table 3 HPLC profile of atorvastatin

Concentration (µg/ml)	Peak height (mAU)
0	0
0.05	61
0.1	116
0.25	150
0.5	285
1	500
2.5	1268
5	2588

solution (1 µg/ml) in DMSO was introduced into the everted sacs (serosal side) and both ends of the sacs were ligated tightly. The sacs (n = 3) containing atorvastatin solution were immersed on their mucosal side into 30 ml of Dulbecco's phosphate buffer solution (D-PBS) containing 30 µM concentration of glucose and 30 µM concentration of quercetin-metal complexes (Q-M; M=Cu, Co, Ni, Zn, Mo, VO) in DMSO. The buffer solution was prewarmed and oxygenated throughout the experiment. Similarly, the sacs (n = 3) filled with atorvastatin without any inhibitor on mucosal side served as control and the sacs (n = 3) filled with the atorvastatin and verapamil (300 µM) on mucosal side served as standard [33, 34].

The Everted sac model, i.e., intestinal serosal-to-mucosal transport of atorvastatin was measured by the periodic collection of samples from the mucosal medium at different time intervals (0, 10, 30, 45, 60 min). All the samples were mixed with acetonitrile in 2:1 ratio, vortexed (2 min), and centrifuged at 3000 rpm (15 min). Aliquots of supernatant were separated, filtered through membrane filter (0.22-µm pore size) and preserved at − 20 °C until analysis. Twenty microliters of it was injected into the HPLC column using a Hamilton syringe. The transport of atorvastatin in the absence of P-gp inhibitor (control), in the presence of Q-M complexes (test) or verapamil (standard) was measured by

validated reverse-phase high-performance liquid chromatography (RP-HPLC) method [35]. Apparent permeability coefficients (P_{app}) of atorvastatin were calculated from the equation [36] given:

$$P_{app} = \frac{dQ}{dt} \cdot \frac{1}{AC_0}$$

where dQ/dt is the steady-state appearance rate on the acceptor solution

A is the surface area of the intestinal sacs (7.85 cm^2/sac)

C_0 is the initial concentration of drug (inside the sac)

Sensitive RP-HPLC method
Chromatographic conditions
Acetonitrile and water in the proportion of 60:40 (v/v) adjusted to pH 2.8 with phosphate buffer was used as mobile phase, stationary phase was a C-18 reverse-phase column of 250 × 4.6 mm dimension, and 5 µ particle size, flow rate was 1 ml/min at λ max of 247 nm, run time was < 15 min, injection volume was 20 µl, and AUFS (absorbance units full scale) was 0.005.
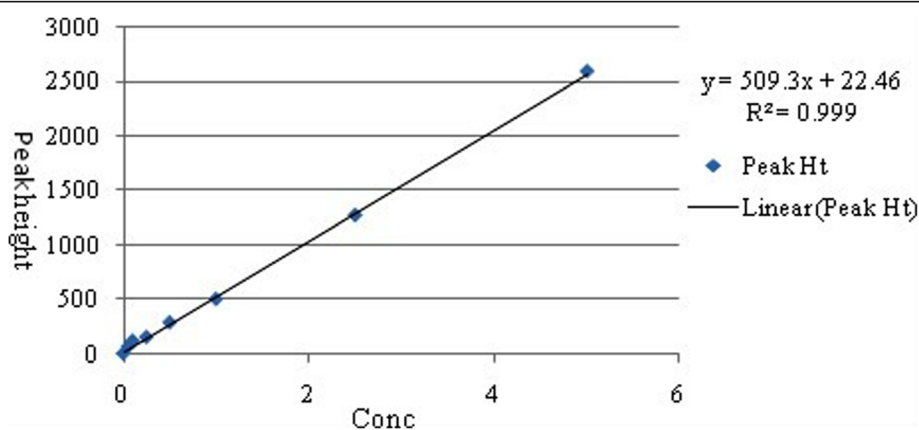
Preparation of calibration curve of atorvastatin
Preparation of stock solutions
A stock solution representing 1 mg/ml of atorvastatin was prepared in methanol. The working standard solutions were prepared prior to use from stock solution by sequential dilution with methanol to yield final concentrations of 0.05, 0.1, 0.25, 0.5, 1, 2.5, and 5µg/ml of atorvastatin.

Construction of calibration curve
The calibration curve was constructed using peak height ratios of the drug versus concentration [37]. The slope of the plot determined by the method of linear regression analysis was used to calculate the atorvastatin

y= 509.3x + 22.46
R²= 0.999
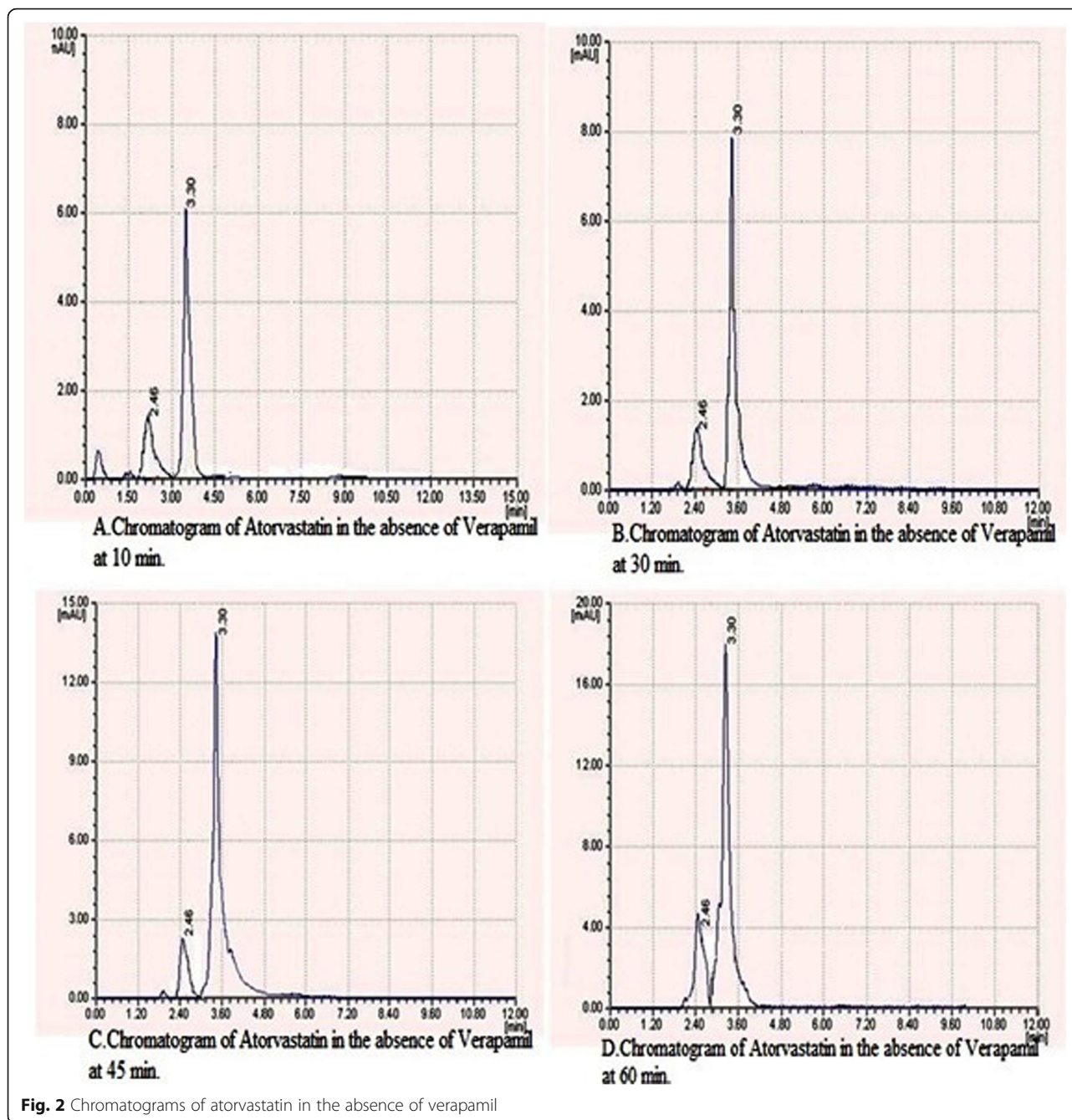
Fig. 1 Calibration curve of atorvastatin

Fig. 2 Chromatograms of atorvastatin in the absence of verapamil

concentration in the unknown sample. A linear regression curve in the range of 0.05 to 5 μg/ml was established.
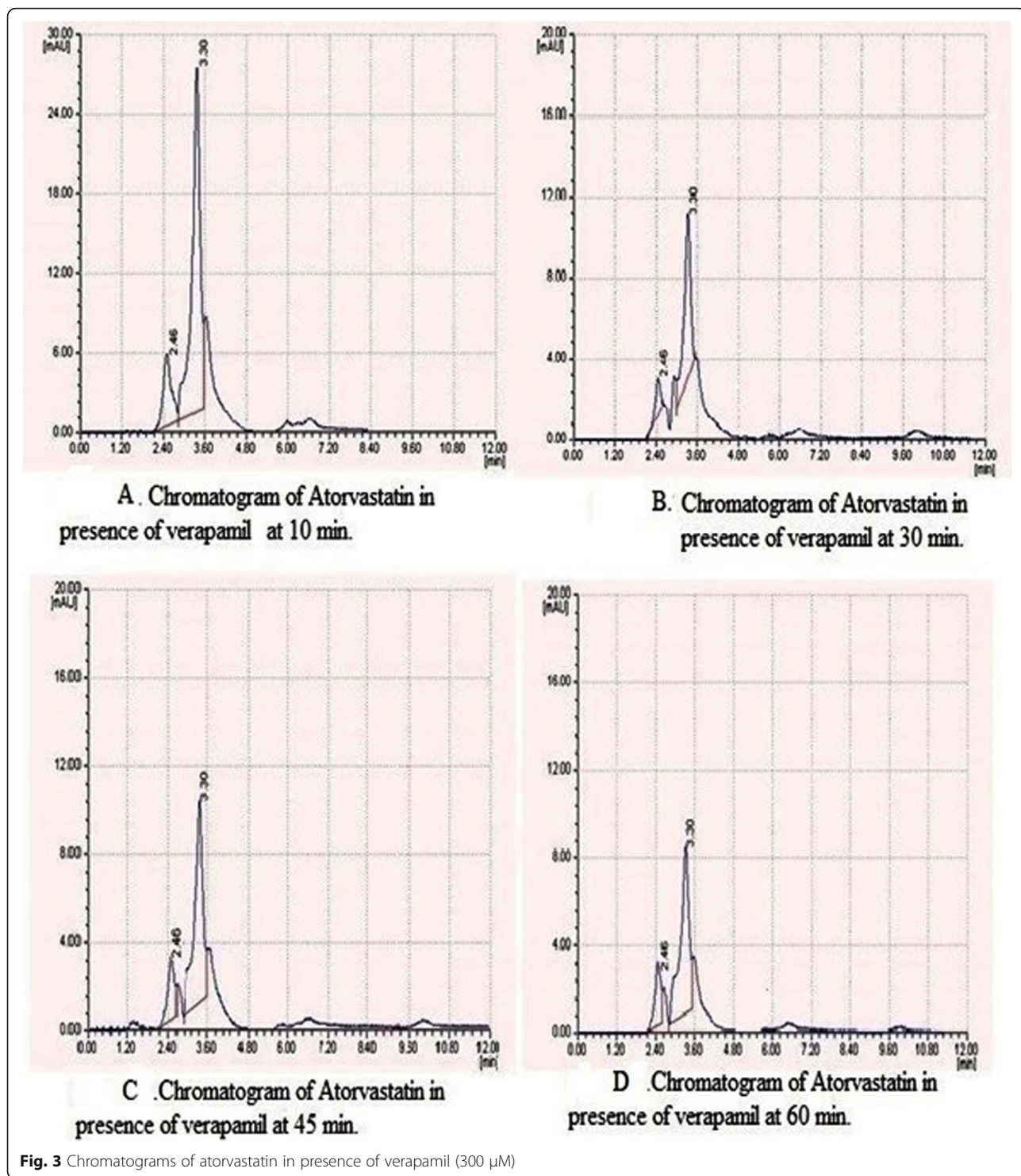
Statistical analysis

The in vitro results were compared by Student's paired t-test using Graph Pad Instant Software version-5.0. A value of $p < 0.05$ was considered statistically significant.
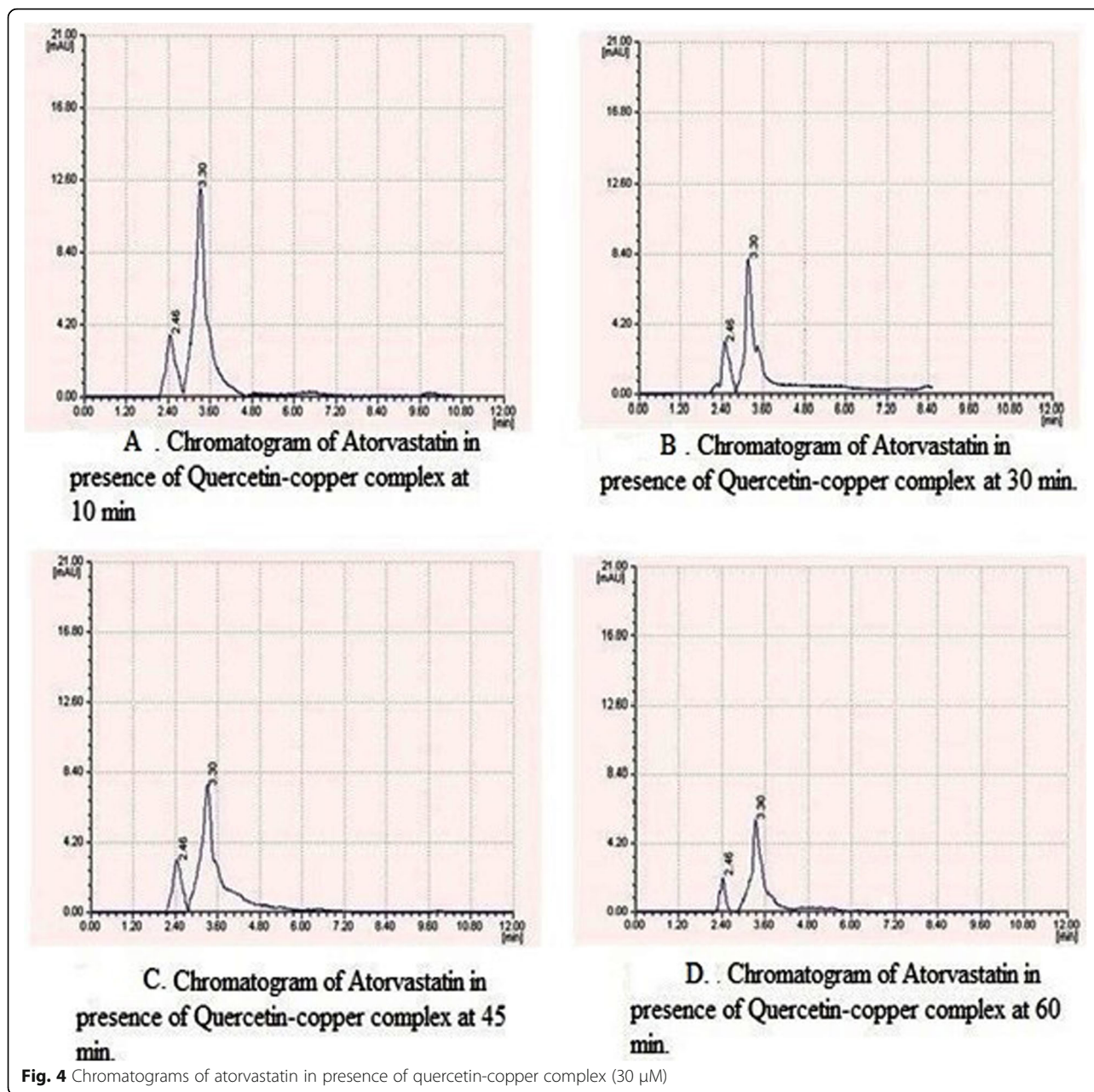
Results

Chemistry

Addition of aqueous sodium hydroxide during the synthesis of metal complexes resulted in the deprotonation of the OH groups and thus favored the stability of quercetin at pH 10. All the metallic salts were soluble in the reaction mixture except cobaltous chloride hexahydrate which was dissolved using dimethyl formamide. All the quercetin-metal complexes were obtained in good yields ranging from 80.39 to 84.64% as indicated in Table 1.

A. Chromatogram of Atorvastatin in presence of verapamil at 10 min.

B. Chromatogram of Atorvastatin in presence of verapamil at 30 min.

C. Chromatogram of Atorvastatin in presence of verapamil at 45 min.

D. Chromatogram of Atorvastatin in presence of verapamil at 60 min.

Fig. 3 Chromatograms of atorvastatin in presence of verapamil (300 μM)

UV-visible study of the quercetin-metal complexes

The UV-visible spectrum of quercetin in DMSO and phosphate buffer shows two major absorption bands due to π-π^* transitions at 369 (band-I) and 252 nm (band-II) (Table 2). When the solution of a metallic salt was added, bands I and II gradually shifted to longer wavelengths,

accompanied with slight decrease in absorption. The results indicated formation of a complex between quercetin and respective metal ions. Band-I bathochromic shift (401 nm) is a common feature observed with all the quercetin-metal complexes. It could be due to the interaction of metal ions (Cu, Zn, Co, VO, Mo, and Ni) with the 3-hydroxy and 4-

Fig. 4 Chromatograms of atorvastatin in presence of quercetin-copper complex (30 µM)

carbonyl groups, the two possible chelating sites on quercetin resulting in electronic redistribution between the quercetin molecule and metal ion.

Infrared spectral study of the quercetin-metal complexes

Quercetin showed peaks at 3417 cm^{-1}(broad) for O-H stretch, 1665 cm^{-1}for C=O stretch, 1510 cm^{-1}for C=C stretch, 1316 cm^{-1}for OH bend, 1243 cm^{-1} for C-O stretch, and 1162 cm^{-1} and 997 cm^{-1} for CH bend. The presence of peaks at 3355, 3370, 3398, 3325, 3379, and 3395 cm^{-1} in IR spectra of the quercetin-metal complexes indicate the formation of O-Cu, O-Zn, O-Mo, O-VO, O-Ni, and O-Co bonds,

respectively, through the complexes [27, 38]. The C=O stretching mode of the free ligand (quercetin) occurs at 1665 cm^{-1}. By the interaction of ligand with copper acetate/zinc chloride/ammonium molybdate/vanadyl sulphate monohydrate/ammonium nickel sulphate hexahydrate/cobaltous chloride hexahydrate, it has been shifted to 1655, 1658, 1633, 1637, 1627, and 1637 cm^{-1}, respectively, which can be explained by coordination of carbonyl oxygen with Cu^{2+}, Zn^{2+}, Mo^{2-}, vo^{2+}, Ni^{2+}, and Co^{2+} ions, respectively. Moreover, an increase in bond order from 1316 cm^{-1}in ligand to 1346, 1342, 1325, 1342, 1352, and 1371 cm^{-1}, respectively, after complexation with copper,

Fig. 5 Chromatograms of atorvastatin in presence of quercetin-cobalt complex (30 μM)
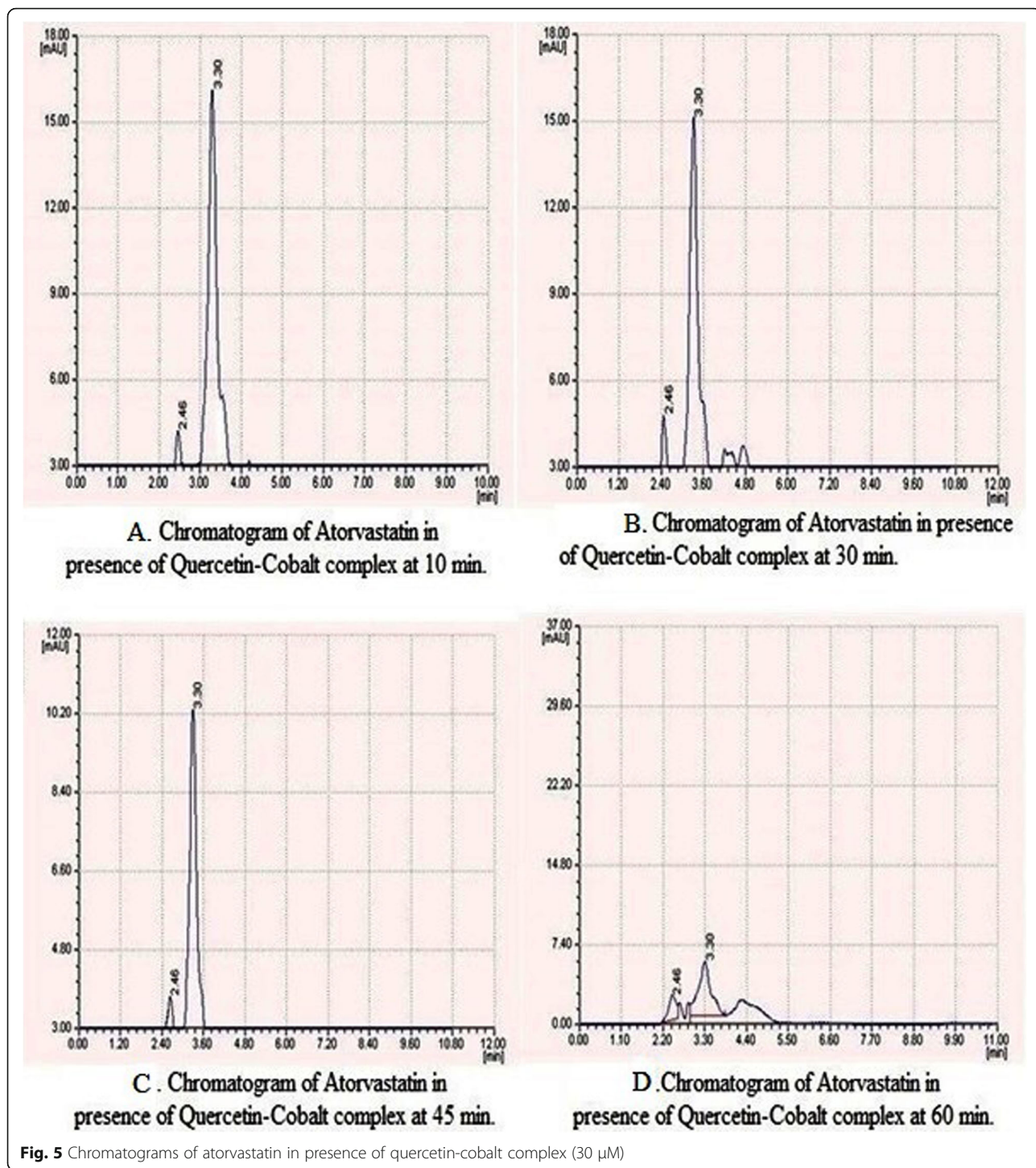
zinc, molybdenum, vanadium, nickel, and cobalt ions, respectively, indicates the involvement of O-H deformation vibration which co-ordinates in metal chelation.

Everted rat sac method
In the present study, the amount of atorvastatin transported from everted sacs (serosal-to-mucosal surface) was determined in different regions of the rat intestine. Control was compared with the standard P-gp inhibitor (verapamil) and quercetin-metal (Q-M) complexes (M=Cu, Zn, Mo, VO, Ni, Co). The transport of atorvastatin across the everted intestinal sacs was determined and expressed as mean ± SD (standard deviation).
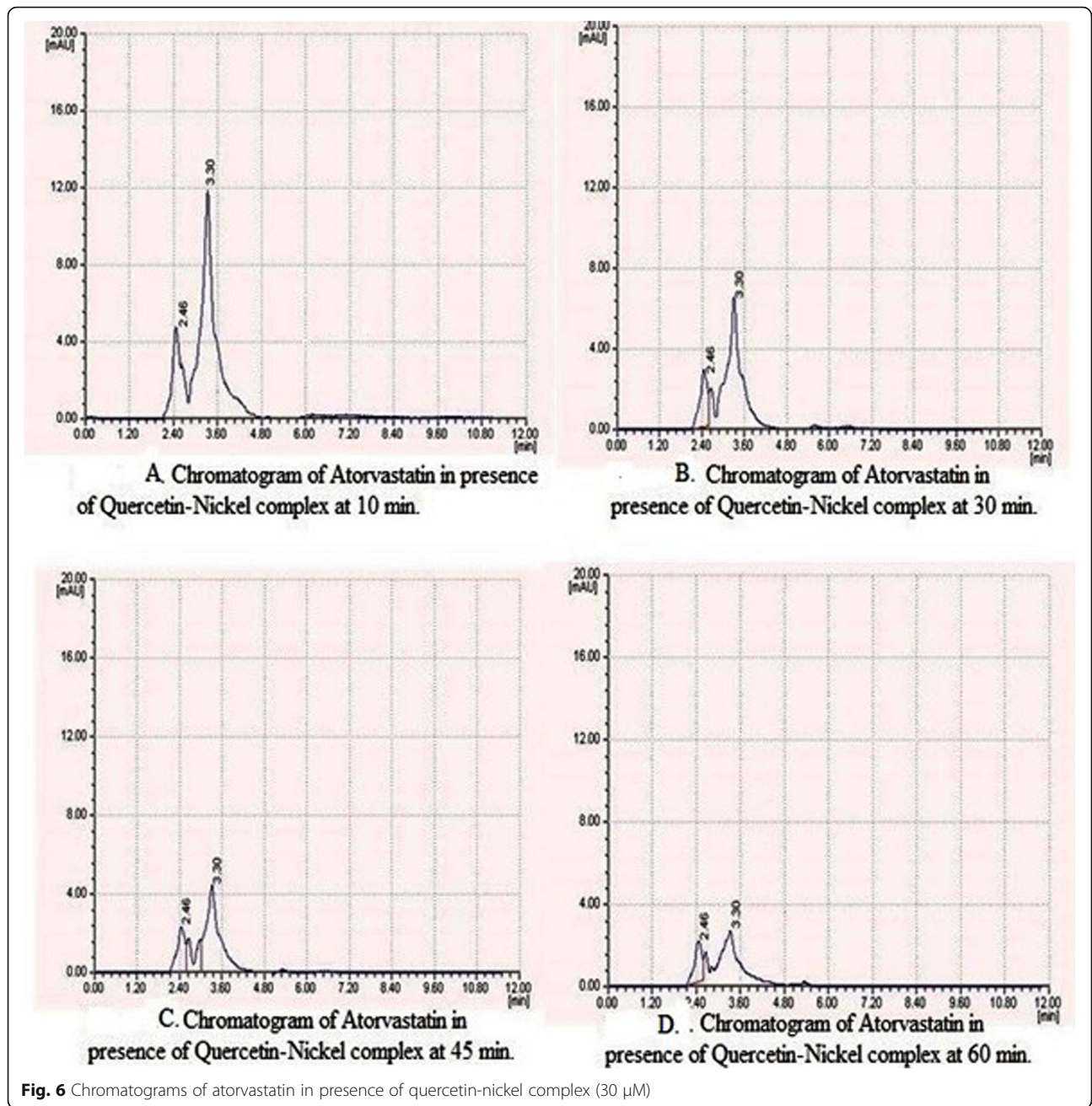
A. Chromatogram of Atorvastatin in presence of Quercetin-Nickel complex at 10 min.

B. Chromatogram of Atorvastatin in presence of Quercetin-Nickel complex at 30 min.

C. Chromatogram of Atorvastatin in presence of Quercetin-Nickel complex at 45 min.

D. . Chromatogram of Atorvastatin in presence of Quercetin-Nickel complex at 60 min.

Fig. 6 Chromatograms of atorvastatin in presence of quercetin-nickel complex (30 µM)

Table 4 Apparent permeability coefficients (P_{app}) ($\times 10^{-6}$ cm/s) of atorvastatin in the everted duodenum of healthy rat small intestine ($n = 3$)

Time (min)	Duodenum				
	Atorvastatin-treated rats	Atorvastatin + verapamil-treated rats	Atorvastatin + Q-Cu complex-treated rats	Atorvastatin + Q-Co complex-treated rats	Atorvastatin + Q-Ni complex-treated rats
10	0.030 ± 0.003	0.028 ± 0.001	0.035 ± 0.001	0.035 ± 0.001	0.042 ± 0.001
30	0.036 ± 0.003	0.026 ± 0.002	0.024 ± 0.001	0.019 ± 0.001	0.027 ± 0.000
45	0.073 ± 0.019	0.022 ± 0.002*	0.023 ± 0.001*	0.012 ± 0.001**	0.020 ± 0.001*
60	0.119 ± 0.060	0.010 ± 0.002***	0.021 ± 0.001***	0.010 ± 0.0004***	0.009 ± 0.0001***

All values expressed as mean ± SD. *$p < 0.05$ significant, **$p < 0.01$ highly significant, ***$p < 0.001$ very highly significant, compared to atorvastatin control

Table 5 Apparent permeability coefficients (P_{app}) ($\times 10^{-6}$ cm/s) of atorvastatin in the everted jejunum of healthy rat small intestine ($n = 3$)

Time (min)	Jejunum				
	Atorvastatin-treated rats	Atorvastatin + verapamil-treated rats	Atorvastatin + Q-Cu complex-treated rats	Atorvastatin + Q-Co complex-treated rats	Atorvastatin + Q-Ni complex-treated rats
10	0.034 ± 0.004	0.032 ± 0.002	0.027 ± 0.001	0.028 ± 0.001	0.031 ± 0.001
30	0.042 ± 0.004	0.028 ± 0.002	0.022 ± 0.002	0.017 ± 0.002	0.022 ± 0.001
45	0.078 ± 0.019	0.026 ± 0.003	0.020 ± 0.002*	0.012 ± 0.001*	0.016 ± 0.001*
60	0.131 ± 0.074	0.023 ± 0.004**	0.019 ± 0.002**	0.009 ± 0.0005**	0.007 ± 0.0002**

All values expressed as mean ± SD. *$p < 0.05$ significant, **$p < 0.001$ very highly significant, compared to atorvastatin control

RP-HPLC method of analysis

In the present study, the atorvastatin concentrations were estimated by a sensitive RP-HPLC method (Table 3). The calibration curve of atorvastatin was plotted with concentration versus peak height as shown in Fig. 1. The typical chromatograms corresponding to solvent buffer and atorvastatin in the absence and presence of verapamil and in the presence of quercetin-metal complexes at different time points of the experimental study are depicted in Figs. 2, 3, 4, 5, and 6. The separation of both peaks (solvent and atorvastatin) was satisfactory, with retention times of 2.46 min and 3.30 min, respectively.

Tables 4, 5, and 6 reveal the apparent permeability coefficients (P_{app}) of atorvastatin alone (control) and in presence of verapamil (standard) and the quercetin-metal complexes (test) in the everted duodenum, jejunum, and ileum, respectively.

Discussion

Clinically, multidrug resistance (MDR) is one of the major causes for chemotherapeutic treatment failure in cancer patients. Several strategies for overcoming drug resistance have been studied, including (1) developing reversal agents which target drug resistance markers such as P-gp and MRP1, (2) developing new agents to which drug-resistant cancer cells are sensitive, (3) employing agents to which drug-resistant cancer cells show collateral sensitivity, and (4) developing antiangiogenesis therapy and gene therapy for drug-resistant cancer cells [39]. Among them,

developing reversal agents which target P-gp has been a principal strategy.

Multidrug resistance of cancer cells is often associated with over expression of P-gp, a plasma membrane transporter that extrudes chemotherapeutic drugs by using ATP hydrolysis as the energy source. P-gp is encoded by the MDR1 gene and functions as an energy-dependent multidrug membrane transporter that rapidly extrudes a variety of hydrophobic anticancer drugs from target cancer cells and thereby prevents the drugs from exerting cytotoxic effects. Since the first P-gp inhibitor, verapamil, was found in 1981, a variety of agents including cyclosporin A (CysA) and others have been reported as agents for overcoming MDR [40].

However, the clinical trial results of these drugs are very disappointing so far, due to dose-limiting toxicity. Therefore, it is necessary to find new reversing agents with lesser side effects. Natural sources come under this category of having no dose-limiting toxicity and lesser side effects [41, 42]. Several members of flavonoids were found to be able to modulate drug efflux in MDR cancer cells, such as genistein, quercetin, and naringenin [43, 44]. The flavonoid quercetin was shown to inhibit P-gp-mediated drug transport by inhibiting the ATP binding site of P-gp. It was reported to decrease the expression of P-gp significantly in multidrug-resistant human cervical carcinoma (KB-V1) cells at the concentration of 30 μM [45].

Experimental studies proved that quercetin exhibits both P-gp inhibitory and antitumor activities and it also

Table 6 Apparent permeability coefficients (P_{app}) ($\times 10^{-6}$ cm/s) of atorvastatin in the everted ileum of healthy rat small intestine ($n = 3$)

Time (min)	Ileum				
	Atorvastatin-treated rats	Atorvastatin + verapamil-treated rats	Atorvastatin + Q-Cu complex-treated rats	Atorvastatin + Q-Co complex-treated rats	Atorvastatin + Q-Ni complex-treated rats
10	0.040 ± 0.003	0.041 ± 0.002	0.023 ± 0.001	0.022 ± 0.002	0.023 ± 0.0006
30	0.046 ± 0.004	0.036 ± 0.003	0.019 ± 0.001	0.015 ± 0.001	0.018 ± 0.001
45	0.085 ± 0.021	0.033 ± 0.002	0.018 ± 0.001	0.009 ± 0.001	0.013 ± 0.001
60	0.169 ± 0.122	0.032 ± 0.003*	0.016 ± 0.001**	0.007 ± 0.0001**	0.005 ± 0.0002**

All values expressed as mean ± SD. *$p < 0.01$ highly significant, **$p < 0.001$ very highly significant, compared to atorvastatin control

has the ability to chelate metal ions [16]. Further, some metal complexes show P-gp inhibitory activity [46–48]. But till now, there are no reports on P-gp inhibitory activity of quercetin-metal complexes.

In the present study, we have synthesized metal (Cu, Co, Ni, Zn, Mo, VO) complexes of quercetin in high yields and purity and characterized them by using UV-visible and IR spectral data. From spectroscopic studies, quercetin was found to chelate metal ions (Cu, Co, Ni, Zn, Mo, VO) at the 3-hydroxy and 4-carbonyl groups. They were pharmacologically evaluated for their P-gp inhibitory activity by in vitro everted sac method in rats by comparing the apparent permeability of P-gp substrate (atorvastatin) with that of standard P-gp inhibitor, verapamil.

Results of this in vitro study revealed that the transport of atorvastatin (P-gp substrate) across the small intestine is much affected by the Q-Cu, Q-Co, and Q-Ni complex treatment. The mean ± SD and apparent permeability of atorvastatin decreased in the presence of the Q-Cu, Q-Co, and Q-Ni complexes. A time-dependent effect was observed. Also, a significant reduction in the Papp values was observed in the everted sacs treated with Q-Cu, Q-Co, and Q-Ni complexes as compared with controls. All the three metal complexes were found to be more potent than the standard verapamil at all three sites of small intestine (i.e., duodenum, jejunum, and ileum) at only one-tenth dose of verapamil. Among the three complexes, Q-Ni complex exhibited superior inhibitory activity on the permeability glycoprotein (P-gp). Next to it, Q-Co complex significantly reduced the transport of atorvastatin across the intestinal membranes. The rest of the complexes (Q-Mo, Q-Zn, Q-VO) did not show any effect on the transport of atorvastatin and hence considered inactive. The study revealed that Q-Cu, Q-Co, and Q-Ni metal complexes could inhibit P-gp (at much lesser concentrations than verapamil) and increase the absorption of atorvastatin.

Conclusion
The transport of atorvastatin (P-gp substrate) across the small intestine is much affected by the Q-Cu, Q-Co, and Q-Ni complexes treatment. The treatment of sacs with these metal complexes decreased the intestinal permeation of atorvastatin (P-gp substrate) when compared with that of standard P-gp inhibitor and control, which may be due to inhibition of P-glycoprotein. Hence, they could be considered P-gp inhibitors and studied further.

Abbreviations
RP-HPLC: Reverse-phase high-performance liquid chromatography; MDR: Multidrug resistance; M: Metal; Q: Quercetin; Q-Cu: Quercetin-copper complex; Q-Zn: Quercetin-zinc complex; Q-Mo: Quercetin-molybdenum complex; Q-Vo: Quercetin-vanadyl complex; Q-Ni: Quercetin-nickel complex; Q-Co: Quercetin-cobalt complex; P-gp: P-glycoprotein (permeability glycoprotein); SD: Standard deviation; UV: Ultraviolet spectrophotometer; IR: Infrared spectrophotometer; CPCSEA: Committee for the Purpose of Control and Supervision of Experiments on Animals; IAEC: Institutional Animal Ethics Committee

Declarations

Ethics approval and consent to participate
The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee (application no. 44/IAEC/VCOP/2013).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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