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Potential herb-drug interaction of a flavone glycoside from *Cuminum cyminum*: Possible pathway for bioenhancement of rifampicin

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Traditional knowledge on classical herbal based *Ayurvedic* formulation namely '*Trikatu*' in the Indian system of medicine has led to the discovery of 'Risorine', a world's first boosted rifampicin in combination with piperine (one of ingredient in *Trikatu*) as bioenhancer for the treatment of tuberculosis. This encourages us to combine rifampicin with a flavone glycoside (CC-I), one of ingredient of *Cuminum cyminum* which found its application in culinary purposes and immensely widespread in diverse ethnomedical systems worldwide as an integral part of folklore therapy. Therefore, aim of the study is to explore the reason for bioenhancement of rifampicin by CC-I using a panel of *in vitro* and *in vivo* experimentations for the first time. Plasma concentration of rifampicin was markedly enhanced by CC-I orally in Wistar rats. Mechanistic studies showed that CC-I have action on efflux transporters based on rhodamine transport and P-glycoprotein dependent ATPase assay but no alteration of *in vitro* transcellular diffusion and plasma protein binding of rifampicin. Intestinal transit of rat was not affected upon treatment with CC-I whereas inhibition of CYP3A4 in rat and human liver microsomes was occurred to a little extent. Bioenhancer effect of CC-I was mainly through improving absorption by down regulation of efflux transporters.

Keywords: *Cuminum cyminum*, Flavone glycoside, Pharmacokinetic interaction, Bioenhancer, Herb-drug interaction

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The use of bioenhancer is a promising pharmacological approach for enhancing bioavailability and consequent bioefficacy of a drug in combination that can lead to lower toxicity, reduce wastage and minimize resistance of drug[1]. Piperine, quercetin, lysergol, niaziridin, glycerrhizin are the classical examples as these have gained significant importance in the era of bioenhancer[2]. In this context, 'Risorine', a world's first boosted rifampicin in combination with piperine as bioenhancer has successfully lowered the dose of rifampicin from 450 mg to 200 mg per tablet and already been marketed in India[3]. This success story has encouraged further research on superior bioenhancer of such a life saving drug.

Cuminum cyminum (family: apiaceae), commonly known as cumin is a popular traditional medicinal plant and its seeds are used regularly as spices/food additive[4-5]. This medicinal plant has potential for bioenhancement of antibiotics[2,5]. Particularly, a flavone glycoside chemically, 3′,5–dihydroxyflavone-7–O–β–D–galacturonide-4′-O–β–D-glucopyranoside (CC-I) has been isolated from *Cuminum cyminum* showed its prospect as bioenhancer of rifampicin based on snap pharmacokinetic study in rat model[6]. Although *Cuminum cyminum* extract and CC-I in pure form showed a lot of initial promise as bioenhancer for the above mentioned drugs and rifampicin, respectively but the major bottleneck is the lack of proper understanding of its mechanism of interaction with the drug molecules responsible for improved plasma concentration. This understanding may unravel bioenhancer effect of CC-I for other poor/variable oral bioavailable drugs. Thus, investigation of overall bioenhancer effect based on detailed pharmacokinetic study and most importantly, understanding of pharmacological mechanism (s) has utmost importance.

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Hence, it has taken up as objectives of the present study using a panel of *in vitro* and *in vivo* experimentations (Fig. 1).

Materials and methods

Materials

Rifampicin (purity ≥ 98 %), verapamil (purity ≥ 98 %), atropine sulphate (purity ≥ 97 %), testosterone (purity ≥ 98 %), ketoconazole (purity ≥ 98 %), piperine (purity ≥ 97 %), NADPH and phosphate buffer saline, *p*H 7.4 (PBS) were purchased from Sigma-Aldrich. DMSO, formic acid, acetonitrile and methanol (HPLC grade) were procured from Rankem. Other chemicals or reagents used were of analytical grade. Water having resistivity of 18.2 MΩ.cm was used throughout the analysis (Millipore water purification system, Millipore).

Plant based natural product aspects

Seeds of *Cuminum cyminum* were collected from Gujarat, India followed by identification and authentication of sample by Dr BK Kapahi, taxonomist and voucher specimen (RJM 0200/P06) was submitted to our institute herbarium. CC-I, a flavones glycoside (Purity ≥ 99 %) was isolated from *Cuminum cyminum* in pure form and was characterised before use[4,6].

Animals and ethical prerequisites

All *in vivo*/*ex vivo* experiments was carried out using healthy adult Wistar rats which were housed in polypropylene cages, kept at standard laboratory conditions (25±2 °C, 50±20 % relative humidity, 12h light /12 h dark cycle), fed with standard pellet diet with water *ad libitum*. All animal experimentation was performed with 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA) guidelines after obtaining necessary approval from Institutional Animal Ethics Committee of CSIR-Indian Institute of Integrative Medicine, Jammu, India.

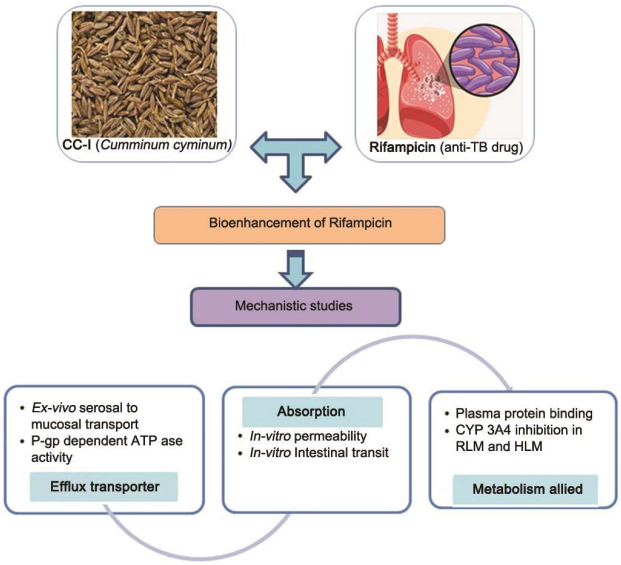

Fig. 1 — Overview of the present pharmacological investigations

Pharmacokinetic studies for rifampicin bioenhancement using CC-I

Study design

Pharmacokinetic studies were performed following single dose oral administration of rifampicin (40 mg/kg) alone as well as in combination with CC-I (5 mg/kg) using Wistar rats divided into two groups. Each group contained total fifteen animals which were divided into three subgroups (n=5) for sparse sampling. Animals were fasted for 10 h prior to experiment with free access to water. Dose formulation was prepared using 0.5 % DMSO, 50 % PEG-400 and 49.5 % water (v/v) for rifampicin and 5 % DMSO, 5 % polyethylene glycol, 10 % solutol, 10 % PEG-400 and 70 % water (v/v) for CC-I which was administered 0.5 h prior to rifampicin dosing to evaluate bioenhancer effect. Blood samples were collected at a predetermined time interval of 0 h, 0.25 h, 0.5 h 1 h, 2 h, 4 h, 8 h, 10 h and 24 h. Plasma was separated and stored frozen at -20 °C until analysis.

Bioanalysis

Stock solutions of rifampicin and CC-I were prepared individually in DMSO and diluted with methanol accordingly to prepare calibration curve (1.9-4000 ng/mL) for rifampicin by spiking into blank plasma. Plasma sample (50 µL) was processed by using methanol (200 µL) as plasma protein precipitation agent and injected onto the LC-MS/MS system (Shimadzu, Japan) for quantitation of rifampicin in plasma. Chromatographic separation was achieved by Chromolith High Resolution RP18e (100 x 4.6) mm column (Merck) using 0.1 % (v/v) formic acid in water (A) and acetonitrile (B) as mobile phase. Gradient program for elution was: 0.01–2 min, 10 % B; 2–4 min, 10–60 % B; 4–6 min, 60 % B; 6–8 min, 60–10 % B, and 8–12 min, 10 % B. Flow rate was kept at 0.6 mL/min and column oven temperature was maintained at 30 °C. Triple-quadrupole tandem mass spectrometer was operating in positive ion mode where Q1 and Q3 were at unit resolution. Quantification was done in multiple reaction monitoring mode using precursor/product ion transition pair at m/z 823.565/791.60 (Fig. 2) and

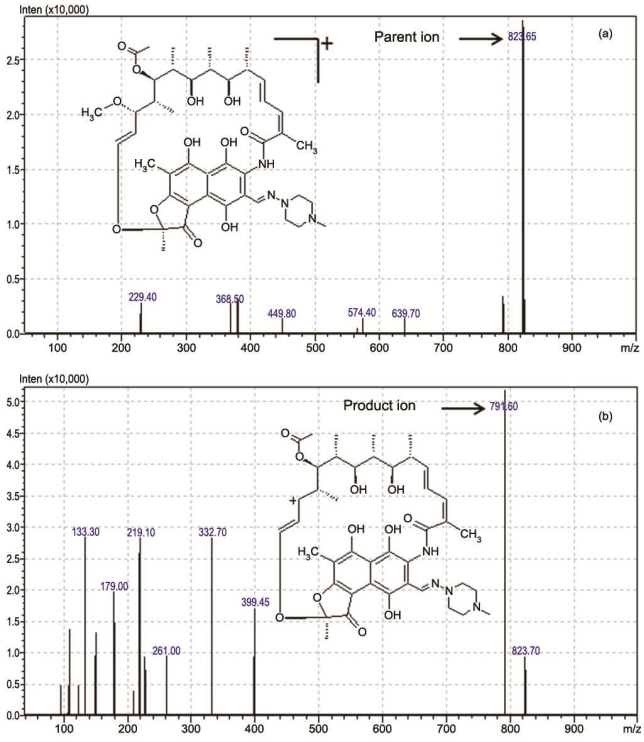

Fig. 2 — Mass spectra of rifampicin with possible chemical structures: (A) parent ion at m/z 823.65 and (B) product ion at m/z 791.60

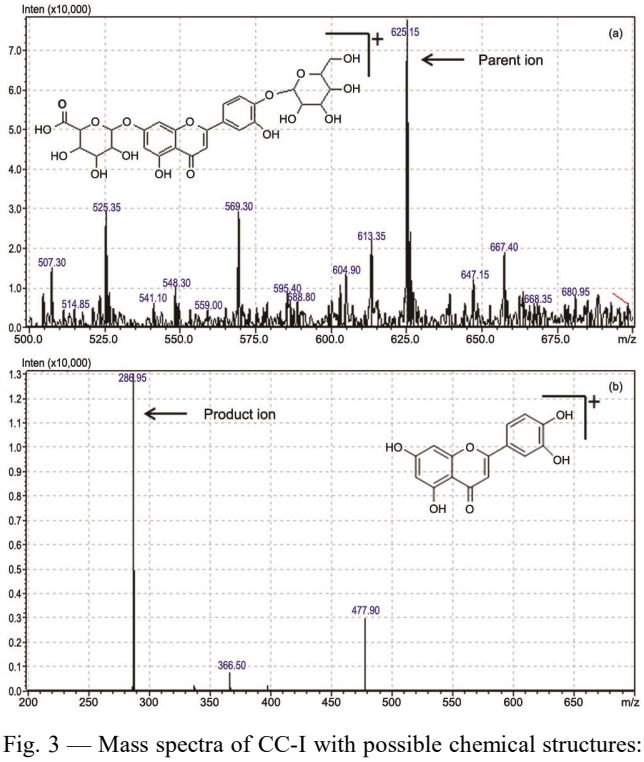

Fig. 3 — Mass spectra of CC-I with possible chemical structures: (A) parent ion at m/z 625.15 and (B) product ion at m/z 286.95

625.15/286.95 (Fig. 3) for rifampicin and CC-I, respectively. Data were monitored by using Lab Solutions software. Mean plasma concentrations of rifampicin at respective time point were evaluated for the different pharmacokinetic parameters (C_{max}, Maximum plasma concentration; T_{max}, Time to reach maximum plasma concentration; $T_{1/2}$, Elimination half-life; AUC_{0-t}, Area under the curve for plasma concentration from zero to the last measurable plasma sample time; $AUC_{0-\infty}$, Area under the curve for plasma concentration from zero to time infinity; V_d, Volume of distribution; Cl, Clearance; MRT, Mean residence time) by using PK solution software (Summit Research Services, USA).

Mechanistic studies for bioenhancer activity of CC-I

Ex vivo everted jejunal sac assay

Effect of CC-I on the efflux transporter was performed based on Rhodamine 123 (Rh123) transportation from serosal to mucosal layer. Everted sacs (6-8 cm) of rat jejunum were prepared from untreated animals, filled with Krebs-Ringer buffer containing Rh123 (10 µM) and incubated at 37 ºC under constant motion in oxygenated Krebs-Ringer buffer (50 mL). Mucosal samples collected at 10, 20 and 30 min during the incubation period. Rh123 concentration was measured based on fluorescence at 504/525 nm. Apparent permeability coefficient (P_{app}) was calculated based on transport rate, surface area of the everted sac and mucosal load of Rh123. Study was carried out in triplicate. Rats were treated orally with CC-I (5 mg/kg) and the above experiment was repeated after 3 h of treatment. Verapamil, a known p-glycoprotein inhibitor (25 µM) was added to both serosal and mucosal sides of untreated sacs and same experimental procedure was repeated[7-8].

P-gp dependent ATPase assay

P-gp dependent ATPase assay was carried out using crude jejunal mucosa fractions from rats[7,9]. Vanadate sensitive release of inorganic phosphate (Pi) from ATP in presence and absence of CC-I (100 µM) was calculated as the difference between ATPase activity obtained in the presence and absence of sodium ortho vanadate. Each study was performed in triplicate using verapamil (100 µM) as positive control.

In vitro permeability assay

Effect of CC-I on *in vitro* permeability of rifampicin was investigated using parallel artificial membrane permeability assay (PAMPA) model. Concisely, experimentation was carried out using PAMPA assembly (Millipore) where acceptor plate

containing PBS (300 µL) and donor plate containing rifampicin (50 µM) with or without CC-I at same concentration level in PBS (150 µL). These were separated by microfilter disc, coated with 5 % (v/v) hexadecane in hexane. Then, donor plate and acceptor plate were sandwiched, covered and incubated for 5 h. After that, samples from each well was analysed by LC-MS/MS after diluted accordingly with methanol. Then, permeability (P_e) was calculated. Each study was performed in triplicate and rifampicin without CC-I was used as self control[10].

Intestinal transit test

Effect of CC-I on intestinal transit was investigated based on extent of charcoal suspension passage through small intestine (SI). Briefly, rats were fasted for 18 h and randomly divided into three groups namely vehicle control, treatment with CC-I (5 mg/kg) and treatment with atropine sulphate (2 mg/kg) as positive control containing three numbers of animals per group. 0.5 %, w/v CMC in water was used as vehicle. Charcoal meal was prepared as suspension containing 5 %, w/v charcoal powder and 10 %, w/v gum acacia in water. CC-I or positive control was given orally and after 45 min of administration, charcoal meal was administered orally. After 20 min, animals were sacrificed followed by removal of segment from the duodenum to the caecum. Then, distance covered by charcoal in SI and total length of the SI was measured. Finally, % intestinal transit in SI were calculated and compared with control data[11].

Plasma protein binding assay

Effect of CC-I on protein binding of rifampicin in rat plasma was carried out by rapid equilibrium dialysis method (Pierce, Thermo-Fisher Scientific). Briefly, study was carried out using inserts in base plate with the addition of PBS (350 µL) to buffer chamber and sample in sample chamber (200 µL) which were separated via dialysis membrane and was incubated in shaking water bath having the speed of 120 rpm at 37 ^{0}C for 4 h. After that, plasma (100 µL) was taken out and mixed with equal volume of PBS and vice versa. Then, sample was vortex mixed with methanol, incubated for 30 min in ice bath and centrifuged at 14000 rpm for 10 min. Supernatant was collected to quantitate rifampicin by LC-MS/MS and calculated % plasma protein binding. Study was performed in triplicate for rifampicin (50 µM) in presence or absence CC-I at same concentration level where rifampicin without CC-I was used as self control[10].

CYP3A4 inhibition assay

Investigations were carried out using testosterone as probe substrate in rat liver microsomes (RLM) and human liver microsomes (HLM). The reaction conditions were optimized for proper conversion and its final form is represented here. In brief, testosterone (500 µM) was pre-incubated with the presence and absence of CC-I (25/50/100 µM) in the reaction mixture containing phosphate buffer (0.1M, *p*H 7.4), $MgCl_2$ (6 mM), EDTA (1 mM) and RLM (1 mg) at 37 °C for 5 min. Then, reaction was initiated by addition of NADPH (1 mM). Final incubation volume was 500 µL. After 20 min of incubation, reaction was stopped by using ice cold methanol. Sample was vortex mixed and centrifuged followed by supernatant was transferred into vial for quantitation of testosterone using HPLC[12]. Each study was performed in triplicate using piperine (25 µM) as positive control. The above experiment was repeated using HLM except changes in testosterone concentration (25 µM), HLM (0.25 mg) and incubation time (2 h) and ketoconazole (1 µM) as positive control.

Statistical evaluation

Statistical analysis was performed using Student's t-test in online Graphpad Prism software by comparing the data of treatment group with control/self control group. Data were expressed as mean ± SEM and considered to be significant if $p < 0.05$.

Results

To assess the oral pharmacokinetics of rifampicin, quantitation of rifampicin in rat plasma was carried out by LC-MS/MS (Fig. 4) at first and then data were analysed by non-compartmental method. Mean plasma concentrations of rifampicin vs. time profiles obtained are represented in Fig. 5. It was found that peak plasma concentration (C_{max}) was increased from 4050 ng/mL to 5784 ng/mL upon treatment with CC-I (Table 1). Area under the plasma concentration-time curve from zero to time infinity ($AUC_{0-\infty}$) was raised significantly from 46124 ng.h/mL to 79834 ng.h/mL in presence of CC-I (Table 1). To explore the reason for improvement in rifampicin pharmacokinetics by CC-I, the effect of CC-I on efflux transporter was evaluated at first. Upon treatment with CC-I, serosal to mucosal transport of Rh123 was decreased significantly in comparison to the control value

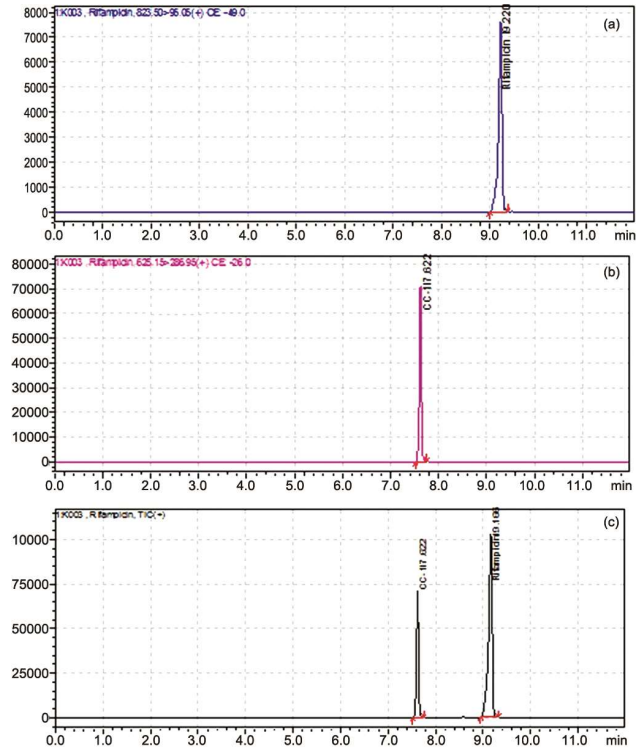

Fig. 4 — Representative MRM chromatograms in LC-MS/MS analysis of plasma sample for pharmacokinetic studies: (A) plasma sample spiked with rifampicin, (B) plasma sample spiked with CC-I and (C) plasma sample of pharmacokinetic study

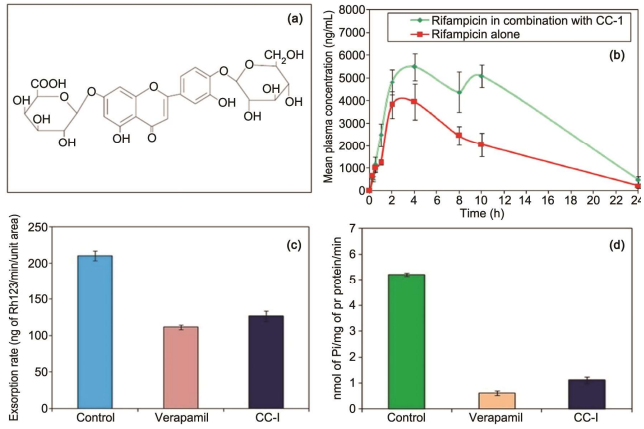

Fig. 5 — Chemical structure of CC-I (a); Mean plasma concentration versus time profiles of rifampicin as alone and in combination with CC-I after oral administration in Wistar rats (b); Effect of CC-I on serosal to mucosal transfer of Rh123 in jejuna sac of Wistar rats (c); Effect of CC-I on P-gp ATPase activity (d)

(Fig. 5). Therefore, $P_{app} \times 10^{-5}$ (cm/s) for control, CC-I and verapamil were found to be 1.84, 1.10 and 0.86. Basal P-gp dependent ATPase activity in freshly prepared rat jejunal membranes was found to be 5.21±0.13 nmol of Pi released/min/mg protein. Results demonstrated that enzyme activity was

Table 1 — Pharmacokinetic parameters of rifampicin (mean ± SEM) after oral administration as alone and in combination with CC-I in Wistar rats

Pharmacokinetic parameter	Rifampicin (40 mg/kg)	Rifampicin (40 mg/kg) + CC-I (5 mg/kg)
C_{max} (ng/mL)	4050 ± 829	5784 ± 592*
T_{max} (h)	3.5 ± 0.5	4.5 ± 1.9*
$T_{1/2}$ (h)	4.3 ± 0.5	5.4 ± 1.6*
AUC_{0-t} (ng.h/mL)	44511 ± 8189	75248 ± 10581**
$AUC_{0-\infty}$ (ng.h/mL)	46124 ± 8783	79834 ± 9300***
V_d (L/kg)	6.2 ± 1.7	4.6 ± 2.0*
Cl (L/h/kg)	1.0 ± 0.3	0.5 ± 0.1*
MRT(h)	7.9 ± 0.7	9.8± 1.0*

Statistical evaluation in comparison to rifampicin alone group as per online Graphpad Prism software at $p < 0.05$ level of significance: *Not statistically significant; **Not quite statistically significant; ***Statistically significant. Pharmacokinetic parameters: C_{max}, Maximum plasma concentration; T_{max}, Time to reach maximum plasma concentration; $T_{1/2}$, Elimination half-life; AUC_{0-t}, Area under the curve for plasma concentration from zero to the last measurable plasma sample time; $AUC_{0-\infty}$, Area under the curve for plasma concentration from zero to time infinity; V_d, Volume of distribution; Cl, Clearance; MRT, Mean residence time.

inhibited around 78 % at the concentration of 100 µM by CC-I (Fig. 5). On the other hand, verapamil as positive control was able to inhibit ATPase activity around 88 % at the same concentration level. Results of CC-I and verapamil were statistically significant. Mean permeability ($logP_e$) of rifampicin was found to be unchanged due to the treatment of CC-I in the PAMPA model (Fig. 6). It was observed that there was no effect of CC-I on intestinal transit in Wistar rats whereas atropine sulphate showed significant reduction (Fig. 6). It was observed that CC-I have no effect on plasma protein binding of rifampicin with rat plasma (Fig. 6). CYP3A4 inhibition study in RLM was performed and CC-I displayed inhibition (21-34 %) of CYP3A4 enzymes at the concentration range of 25-100 µM in concentration independent manner (Fig. 6). Piperine, a positive control showed about 77 % inhibitions at the concentration of 25 µM. CYP3A4 inhibition in HLM was also carried out and CC-I showed 7-10 % inhibition at 25-100 µM. Ketoconazole could able to inhibit about 80 % at 1 µM concentration level.

Discussion

Rifampicin is the most potent antitubercular drug for conventional oral therapy but variable oral bioavailability, autoinduction of hepatic enzymes for metabolism and dose dependent side effects take the shine away and lead to patients drop out in the

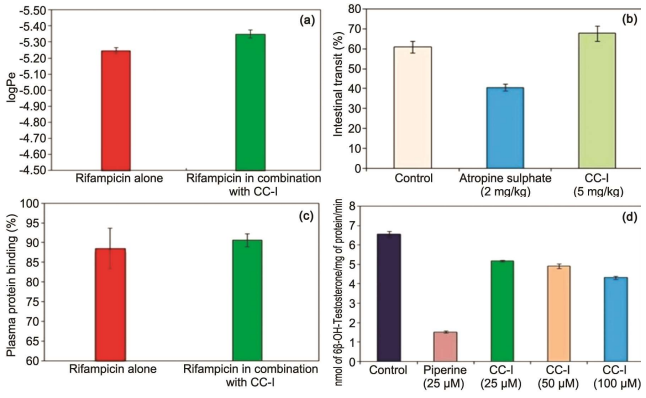

Fig. 6 — Effect of CC-I on in-vitro permeability of rifampicin (a); Effect of CC-I on intestinal transit of charcoal meal in Wistar rats (b); Effect of CC-I on rat plasma protein binding of rifampicin (c); Effect of CC-I on CYP3A4 inhibition in rat liver microsomes (d)

prolonged therapy regime of tuberculosis and subsequent developing resistance[13-15]. Therefore, use of bioenhancer for such a life saving drug is a very effective way to combat this deadly disease[16]. Detailed pharmacokinetic study of rifampicin in presence and absence of CC-I for upto atleast 3-4 half lives of rifampicin may provide an accurate and reliable estimate of the pharmacokinetic parameters[17]. Results of pharmacokinetic studies illustrated that AUCs were markedly higher (69-73 %) in CC-I treated group of rats compared to rifampicin alone group. These improvements in extent of plasma drug concentration have ability to decrease the dose of rifampicin. Result of this improvement is promising as the effect is better than rifampicin in comparison to piperine which was around 60 %[3]. Overall, CC-I at 5 mg/kg has been shown its potential to act as bioenhancer. But the mechanistic investigations of bioenhancement by CC-I as well as *Cuminum cyminum* extract for rifampicin[5] as well as other antibiotics[2] are not reported till date.

We designed the mechanistic investigations based on hypothesis for bioenhancer mechanism and rifampicin pharmacokinetics[2]. Rifampicin is a substrate for ATP-binding cassette transporters such as P-gp and its absorption from gastrointestinal tract is affected by efflux pumps[3]. Results depicted that CC-I have the ability to inhibit efflux pump through reducing the serosol to mucosal transport and P-gp dependent ATPase activity for better absorption of rifampicin and subsequent improved bioavailability. Allicin from *Allium sativum*, catechins from *Camellia sinensis*, 6-gingerol from *Zingiber officinale,* etc., are the classical examples reported to have effect on P-gp modulation to improve bioavailability of drugs[18-20]. PAMPA, a simple and swift *in vitro* method can provide information for permeation of a compound correlated to passive transcellular diffusion of rifampicin. No significant effect of CC-I on permeation of rifampicin confirm the role of P-gp inhibition in absorption enhancement of rifampicin. Alteration of plasma protein binding from its binding site leads to drug interactions in several instances[21] but CC-I have no effect on plasma protein binding of rifampicin. Modification of gastrointestinal tract physiology plays an important role in the oral absorption characteristics of a drug and so that charcoal meal transit test was performed to find out the effect of CC-I on delaying of intestinal transit which can effect on extent of drug absorption[11]. Results confirm that CC-I have no role on intestinal transit of rifampicin in rats. Rifampicin metabolism is mainly catalysed by CYP3A4 hepatic isoenzymes[3,7] and therefore, CYP3A4 inhibition by CC-I was the integral part of mechanistic investigations[22]. Results of CYP3A4 inhibition by CC-I in RLM and HLM reveal that CC-I have little ability to delay the metabolism of rifampicin and consequent increase the extent of plasma concentration as inhibition achieved at much higher concentration. Naringenin from grape fruit juice, ginkgolide A/B from *Ginkgo biloba*, hyperforin from *Hypericum perforatum,* etc., are the typical examples that can interact with the drug metabolizing enzymes to effect on bioavailability of drugs[18,23].

Conclusion

It can be concluded that CC-I, a natural bioenhancer can enhance the plasma concentration of rifampicin by around 1.7 fold mainly through down regulation of efflux transporters and through inhibition of CYP3A4 to a little extent. Further development of this effective combination for translation from bench to bedside is warranted.

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References

1 Randhawa GK, Kullar JS & Rajkumar, Bioenhancers from mother nature and their applicability in modern medicine, *Int J Appl Basic Med Res*, 1 (1) (2011) 5-10.

2 Tatiraju DV, Bagade VB, Karambelkar PJ, Jadhav VM & Kadam V, Natural bioenhancers: an overview, *J Pharmacogn Phytochem,* 2 (3) (2013) 55-60.

3 Atal N & Bedi KL, Bioenhancers: revolutionary concept to market, *J Ayurveda Integr Med*, 1(2) (2010) 96-99.

4 Satti NK, Amina M, Dutt P, Sharma VK, Sharma P, Khan I, Gupta BD, Suri KA, Sharma SC, Johri RK & Sharma SN, A simple and reliable preparative high- performance liquid chromatographic technique for isolation of a bioactive flavone diglycoside from and extract of *Cuminum cyminum* seeds, *Acta Chromatogr*, 21 (3) (2009) 499-512.

5 Deepak, Importance of *Cuminum cyminum* L. and *Carum carvi* L. in traditional medicaments-a review, *Indian J Tradit Knowle*, 12 (2) (2013) 300-307.

6 Bhusari SS, Sharma SC, Sethi S, Tasduq SA, Tikoo MK, Tikoo AK, Satti NK, Gupta BD, Suri KA, Johri RK & Qazi GN, Herbal modulation of drug bioavailability: enhancement of rifampicin levels in plasma by herbal products and a flavonoid glycoside derived from *Cuminum cyminum*, *Phytother Res*, 21 (2) (2007) 157-163.

7 Najar IA, Sharma SC, Singh GD, Koul S, Gupta PN, Javed S & Johri RK, Involvement of P-glycoprotein and CYP 3A4 in the enhancement of etoposide bioavailability by a piperine analogue, *Chem Biol Interact*, 190 (2-3) (2011) 84-90.

8 Veau C, Leroy C, Banidel H, Daniel A, Tardivell S, Farinotti R & Lacour B, Effect of chronic renal failure on the expression and function of rat intestinal P-glycoprotein in drug excretion, *Nephrol Dial Transplant*, 16 (8) (2001) 1607-1664.

9 Yumoto R, Murakami T, Sanimasa M, Nasu R, Nagai J & Takano M, Pharmacokinetic interaction of cytochrome P450 3A related compounds with rhodamine 123, a P-glycoprotein substrate in rats pretreated with dexamethasone, *Drug Metab Dispos*, 29 (2) (2001) 145-151.

10 Sharma A, Magotra A, Dogra A, Kumar SK, Rayees S, Wazir P, Sharma S, Sangwan PL, Singh S, Singh G & Nandi U, Pharmacokinetic, pharmacodynamic and safety profiling IS01957,a preclinical candidate possessing dual activity against inflammation and nociception, *Regul Toxicol Pharmacol*, 91 (2017) 216-225.

11 Bajad S, Bedi KL, Singla AK & Johri RK, Piperine inhibits gastric emptying and gastrointestinal transit in rats and mice, *Planta Med*, 67 (3) (2001) 176-179.

12 Bhusari SS, Bhat V, Koul M, Sharma SC, Tikoo MK, Tikoo AK, Satti NK, Suri KA & Jhori RK, Development and validation of a RP-HPLC method for the simultaneous determination of rifampicin and a flavonoid glycoside - a novel bioavailability enhancer of rifampicin, *Trop J Pharm Res*, 8 (6) (2009) 531-537.

13 Joshi JM, Tuberculosis chemotherapy in the 21 century: back to the basics, *Lung India*, 28 (3) (2011) 193-200.

14 World Health Organization report on Global tuberculosis, World Health Organization, 2016, Geneva, Switzerland.

15 Benedetti SM & Dostert P, Induction and autoinduction properties of rifamycin derivatives: a review of animal and human studies, *Environ Health Perspect*, 102 (9) (1994) 101-105.

16 Bhusari SS, Monica P, Sharma SC, Satti NK, Tikoo MK, Tikoo AK, Suri KA, Gupta BD & Johri RK, Pharmacokinetic interaction of some antitubercular drugs with caraway: implications in the enhancement of drug bioavailability, *Hum Exp Toxicol*, 28 (4) (2009) 175-184.

17 Kumar N, Vishwas KG, Kumar M, Reddy J, Parab M, Manikanth CL, Pavithra BS & Shandil RK, Pharmacokinetics and dose response of anti-TB drugs in rat infection model of tuberculosis, *Tuberculosis*, 94 (3) (2014) 282-286.

18 Cho H & Yoon I, Pharmacokinetic interactions of herbs with cytochrome P450 and P-glycoprotein, *J Evid Based Comple Altern Med*, 2015 (2015) 1-10. Check

19 Zhou S, Lim LY & Chowbay B, Herbal modulation of P-glycoprotein, *Drug Metab Rev*, 36 (2004) 57–104.

20 Tripathi SM, Sharma RJ, Bansal AK, Bhutani KK & Singh IP, Development of chewable tablet of *Trikatu churna* and standardization by densitometry, *Indian J Tradit Knowle*, 16 (2) (2017) 256-262.

21 DeVane CL, Clinical significance of drug binding, protein binding, and binding displacement drug interactions, *Psychopharmacol Bull*, 36 (3) (2002) 5-21.

22 Kar A, Mukherjee PK, Saha S, Bahadur S, Ahmmed SKM & Pandit S, Possible herb-drug interaction of *Morus alba* L. – a potential anti-diabetic plant from Indian traditional medicine, *Indian J Tradit Knowle*, 14 (4) (2015) 626-631.

23 Wanwimolruk S & Prachayasittikul V, Cytochrome P450 enzyme mediated herbal drug interactions (Part 1), *EXCLI J*, 13 (2014) 347–391.