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Original article

Pharmacokinetic and pharmacodynamic interaction of Rosuvastatin calcium with guggulipid extract in rats

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ABSTRACT

Background & objectives: Rosuvastatin calcium (RC) is a potent and competitive synthetic inhibitor of HMG-CoA reductase used for the treatment of dyslipidemia. Guggulipid obtained from *Commiphora mukul* is used in the treatment of a wide variety of diseases such as atherosclerosis, hypercholesterolemia, rheumatism, and obesity. The present study evaluates the pharmacokinetic and pharmacodynamic interactions between RC and the standardized guggulipid extract in rats.

Materials and methods: The guggulipid extract was standardized for the presence of guggulsterones. The pharmacokinetic interaction was determined after a single dose administration of RC alone or in combination with the guggulipid extract or after multiple-dose administration of RC alone or RC along with the guggulipid extract for 14 days. To determine the pharmacodynamic interaction, RC and guggulipid extract were administered to hyperlipidemic rats for 14 days. The level of significance was determined using unpaired student's *t*-test, one way ANOVA, the post-ANOVA Tukey test.

Results: Standardization of guggulipid extract showed it contains 7.5%w/w of guggulsterones. Guggulipid extract increased the bioavailability of RC in both single-dose and multiple-dose studies. Guggulipid extract reduced the rate of absorption (Ka) of RC but showed an increase in maximum serum concentration (Cmax). An in-vitro study using isolated rat intestine revealed that guggulipid extract decreased the rate of absorption of RC in the intestinal lumen. The hypolipidemic activity of RC was augmented by the guggulipid extract in hyperlipidemic rats.

Interpretation & conclusion: Therefore it is concluded that guggulipid extract increases the bioavailability of RC by delaying its Ka and augments its hypolipidemic action. However, it is recommended that a combination of RC with guggulipid extract should be used only after an adverse effect(s) of this combination are determined.

1. Introduction

The benefits of statins, a class of hypolipidemic agents are well documented. Statins are mostly substrates of the CYP3A4 metabolic enzyme (Hirota and Ieiri, 2015) and their effects are altered when they are administered along with inducers or inhibitors of CYP3A4. Fluvastatin is metabolized by CYP2C9, while pravastatin is not significantly metabolized by CYP (Schachter, 2005). Rosuvastatin calcium (RC) has hepatic selectivity, minimal metabolism,

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and enhanced potency. Less than 10% of RC undergoes metabolism by CYP enzymes and the remainder is eliminated unchanged (Palleria et al., 2020).

Guggul extract is one of the commonly used herbal preparation of folklore as a cholesterol-lowering substance (Satyavati, 1988). It is obtained from resins of Commiphora mukul (mukuk myrrh tree) reported to be effective for the treatment of atherosclerosis and inflammatory disorders (Dev, 1997). The sterols of the plant, especially E- and Z-guggulsterone are the main active constituents responsible for its biological activities. The cholesterol-lowering property of the guggul extract is possibly due to the antagonistic effect of guggulsterone on the farnesoid X (FXR) (Cui et al., 2003) and the bile acid receptors (BAR) (Wu, et al., 2002).

Guggulipid, a standardized guggul extract, obtained regulatory approval in India in 1987 as a lipid-lowering agent, whereas, it is used as a dietary supplement in the USA (Szapary et al., 2003). It is known to stimulate CYP3A4 and p–glycoproteins leading to reduced bioavailability of several drugs (Brobst et al., 2004). Hence, administration of guggulipid with statins that are substrates of CYP3A4 may lead to alteration in their pharmacokinetics. As guggulipid has shown an enzyme-inducing effect on several drugs, there is a possible role of guggulipid on the pharmacokinetics of concurrently administered drugs that undergoes metabolism by the same CYP enzymes. Although the metabolism of RC is mediated through CYP enzymes, most of it gets eliminated unchanged and only 10% gets metabolized by CYP2C9 and CYP2C19. Guggul is known to decrease the expression of CYP2C19 (Chhonker et al., 2018), which may lead to an inhibition of RC metabolism by guggulipid. Moreover, RC has hepatoselectivity and high hydrophilicity. Its pharmacokinetics is considered as unique compared to other statins. An interesting fact about RC is the drug-drug and drug-herb interactions. Co-administration RC with drugs that increase its bioavailability is known to produce deleterious effects on kidney. Furthermore, drugs that prevent organic anion transporter protein 1B1-mediated intake of RC are known to have significant interactions (Kostapanos, et al., 2010). Administration of RC with bezafibrate, gemfibrozil, raltegarvir and red yeast rice is reported to increase the myopathic effect of RC while co-administraation with clopidogrel and different protease inhibitors is reported to be safe (Bajaj and Giwa, 2020). Hence, it is advised that combination of RC with other drugs should be used with caution till the all adverse effects of this combination are determined. Also, if there is a minimal pharmacokinetic effect of guggulipid on RC, there is still possible interaction or counteraction of each other on the hypolipidemic profile through synergism or additive effects. To the best of our knowledge, there is no published data that explains the outcome of the combined use of guggulipid and RC.

Therefore, this study was aimed to evaluate the pharmacokinetic changes of RC when administered with guggulipid, also to determine the effect of this combination on the lipid-lowering potential of each other in standardized animal experimental models.

2. Methods

2.1. Experimental animals

Male albino Wistar rats weighing between 220 and 250 g were used in the study. The animals were maintained under standard laboratory conditions. Before the study, the animals were fed with standard pellet diet and water *ad libitum*. The Institutional ethical committee approved the experimental protocol (VECP/IAEC/2018-19/12).

2.2. Chemicals

All chemicals, reagents, and enzyme kits used for this study were procured from standard companies. HDL-Cholesterol estimating kit (Accurex Bio Medical Pvt. Ltd, Mumbai, India), triglycerides estimating kit (Accurex Bio Medical Pvt. Ltd, Mumbai, India), total cholesterol estimating kit (Crest Biosystems Ltd, Goa, India), cholesterol (Merck Specialities Pvt Ltd, Mumbai, India), acetonitrile HPLC grade (Merck Laboratories Ltd, Mumbai, India), potassium dihydrogen ortho phosphate (Merck Laboratories Ltd, Mumbai, India), ortho phosphoric acid (Merck Laboratories Ltd, Mumbai, India), disodium EDTA (CDH Laboratories Ltd, New Delhi, India), Sodium carboxy methyl cellulose high viscosity (CDH Laboratories Ltd, New Delhi, India), sodium chloride (CDH Laboratories Ltd, New Delhi, India), potassium chloride (CDH Laboratories Ltd, New Delhi, India), potassium phosphate monobasic (anhydrous) (Finar Reagents, Ahmadabad, India), sodium phosphate dibasic (Moly Chem Ltd, Mumbai, India), rosuvastatin calcium (USU Ltd, Mumbai, India), guggulipid extract (Sami Laboratories, Bangalore, India).

2.3. Standardization of guggulipid extract

The guggulipid extract was standardized using methods described by Badmaev *et al* (Badmaev et al., 2003) and in the British pharmacopeia (British Pharmacopoeia, 2005).

2.4. Effect of guggulipid extract on the pharmacokinetics of RC after single-dose administration

The overnight fasted rats with water *ad libitum* were divided into two groups consisting of five each. Animals of the first group received RC at a dose of 100 mg/kg orally (Vaghasiya et al., 2019) in the form of a suspension prepared using 0.5% w/v sodium carboxymethylcellulose (Sodium CMC). Animals in the second group were treated with a combination of RC (100 mg/kg) and guggulipid extract at a dose of 400 mg/kg orally (Mithila and Khanum, 2014), both in the form of a suspension prepared using 0.5% w/v sodium carboxymethylcellulose. The volume administered to both groups was kept as close as possible by adjusting the concentration of stock solutions. Following the administration of drugs, blood samples were collected at 0.5, 1.5, 3, 6, 12, and 24 h into Eppendorf's tubes containing EDTA (Nezasa et al., 2002). After blood withdrawal, an equal volume of normal saline was administered to rats by intraperitoneal route for fluid replacement. The plasma was separated and stored at −20 °C until analysis. Rosuvastatin levels were estimated following the HPLC method described by Kumar et al., 2006. Different pharmacokinetic parameters such as K_a, K_e, C_{max}, T_{max}, V_d, Cl, $t_{1/2}$, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\alpha}$ were calculated using PK solver software (Zhang et al., 2010).

2.5. Effect of guggulipid extract on the pharmacokinetics of RC after multiple-dose administration

Two groups of rats each consisting of five were given either RC (100 mg/kg; p.o) or a combination of RC (100 mg/kg; p.o) and guggulipid extract (400 mg/kg; p.o) once a day for 14 days. The blood sampling time and method of analysis were the same as mentioned above under the pharmacokinetic interaction between guggulipid extract and RC after single-dose administration (Section 2.4).

2.6. In-vitro absorption study

This study was conducted using the ileum part of the isolated rat intestine (Yamamoto et al., 1990). The animal was sacrificed by cervical dislocation and the whole small intestine was isolated

and flushed with ice-cold saline. Pieces of ileum each measuring about five (5) cm were used for the study and a minimum of six (6) pieces were used for studying the absorption pattern of RC alone and another six (6) pieces were used for studying the absorption of RC in the presence of guggulipid.

For studying the absorption pattern of RC, 0.5 ml of RC suspension (5 mg/ml) and 0.5 ml of 0.5% sodium CMC suspension were introduced into the mucosal side of the sac and both ends of the sac were closed tightly and ligated. The sac was immersed into 30 ml of Dulbecco's phosphate buffer containing 25 mmol of glucose. The solution was pre-warmed to 37 °C and oxygenated with 5% CO_2 and 95% O_2 throughout the experiment.

The flow of RC to the serosal surface from the mucosal area of the ileum was measured by collecting samples from the serosal medium periodically at different time intervals viz., 0, 15, 30, 45, 60, 75, 90, 105, and 120 min. The amount of the drug absorbed was estimated spectrophotometrically at 238 nm. The absorption rate constant (K_a) was determined by plotting a graph of percentage unabsorbed drug Vs time. The K_a was calculated using the formula

$$K_a = -\text{slope} \times 2.303$$

For studying the interaction of RC with guggulipid, 0.5 ml of RC suspension (5 mg/ml) and 0.5 ml of guggulipid suspension (20 mg/ml) were used and the same procedure as mentioned above was followed.

2.7. Effect of guggulipid extract on the pharmacodynamics of RC in high-fat diet-fed rats

Hyperlipidemia was induced in all groups of animals by feeding them with a high-fat diet for ten (10) days. The high-fat diet was prepared by mixing cholesterol (2% w/w) with standard rat chow (68% w/w) and saturated vegetable fat (30% w/w) (Asdaq et al., 2009). Three groups of animals were used each consisting of five. The animal of the first group was kept as control and administered the only vehicle in sodium CMC suspension orally for 14 days. The animals in the second group received 100 mg/kg of RC orally, while the third group was treated with a combination of 50 mg/kg of RC (low dose) and 400 mg of guggulipid extract orally. Finally, the last group of animals was given a combination of 100 mg/kg of RC (high dose) with 400 mg/kg of guggulipid extract by the oral route. At the end of the treatment period of 14 days, animals fasted overnight and blood was withdrawn. The serum samples were subjected to biochemical analysis for high-density lipoproteins (HDL), low-density lipoproteins (LDL), triglycerides (TG), and total cholesterol (TC) levels using commercially available kits.

2.8. Statistical analysis

The data in the result section is given in the form of mean ± standard error of the mean (SEM). The level of significance was determined using unpaired student's *t*-test. After one way ANOVA, the post-ANOVA Tukey test was done for comparing pharmacodynamic parameters. Statistical analysis was done using Graphpad Instat (version 3.01).

3. Results

3.1. Standardization of guggulipid extract

The guggulipid extract was found to contain total guggulsterone −7.5% w/w, water-soluble extractives − 48% w/w, and alcohol soluble extractives − 40% w/w. The ash value was 5.5% w/w, acid insoluble ash was 3.5%w/w, and loss on drying was 11.8% w/w.

3.2. Effect of guggulipid extract on the pharmacokinetics of RC after single-dose administration

An increase in the bioavailability of RC was found after acute administration of guggulipid extract (Fig. 1). The absorption rate constant (K_a), elimination rate constant (K_e), the volume of distribution (V_d), and the clearance (Cl) were decreased significantly when RC was administered along with the guggulipid extract ($P < 0.001$). An extremely significant increase ($P < 0.001$) in the C_{max} (maximum plasma concentration), T_{max} (time to reach C_{max}), $t_{1/2}$ (half-life), and $AUC_{0\text{-}t}$ and $AUC_{0\text{-}\infty}$ (area under the curve) of RC was noticed in the group that concurrently received guggulipid extract compared to the group that received RC treatment alone (Table 1).

3.3. Effect of guggulipid extract on the pharmacokinetics of RC after multiple-dose administration

The bioavailability of RC was increased after its chronic administration along with the guggulipid extract for 14 days (Fig. 2). However, the increase in bioavailability of RC was less when compared to the group that received a single dose of RC with the guggulipid extract. Similar to the results observed in the single-dose administration study, an extremely significant decrease ($P < 0.001$) in the K_a, K_e, C_{max}, V_d, and Cl and an extremely significant increase ($P < 0.001$) in the T_{max}, $t_{1/2}$, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ was observed (Table 2).

3.4. In-vitro absorption study

The K_a of RC was found to decrease significantly ($P < 0.001$) when it was introduced into the intestinal lumen along with the guggulipid extract indicating a reduction in the rate of absorption (Table 3).

3.5. Effect of guggulipid extract on the hypolipidemic effect of RC in high-fat diet-fed rats

As expected, 14 days of administration of both RC and a combination of RC with the guggulipid extract produced a significant decrease in the serum level of LDL, TC, and TG with an elevation in HDL levels compared to control. The change in the above-mentioned parameters was significantly more in animals treated with the combination of RC with the guggulipid extract compared to animals treated with RC alone (Table 4).

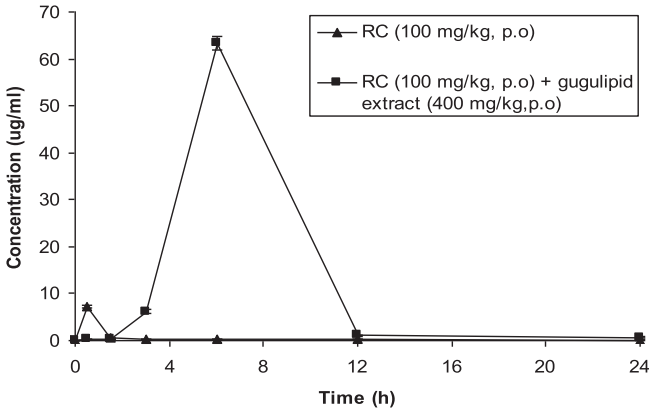

Fig. 1. Serum concentration-time curve after single dose administration of RC (100 mg/kg, p.o) and RC (100 mg/kg, p.o) + guggulipid extract (400 mg/kg, p.o) combination, All values are mean ± SEM, n = 5.

Table 1
Effect of guggulipid extract on the pharmacokinetics of RC after single-dose administration.

Pharmacokinetic parameter	RC[#]	RC[#] + guggulipid extract[##]
$K_a(hr^{-1})$	33.18 ± 0.61	0.19 ± 0.00***
$K_e(hr^{-1})$	2.73 ± 0.06	0.15 ± 0.00***
T_{max}(hr)	0.08 ± 0.00	5.65 ± 0.03***
C_{max}(mcg/ml)	20.64 ± 1.51	26.68 ± 0.67***
V_d(lit)	3.94 ± 0.26	1.55 ± 0.051***
Cl (lit/hr)	10.74 ± 0.50	0.24 ± 0.00***
$t_{1/2}$ (hr)	0.02 ± 0.00	3.49 ± 0.08***
AUC_{0-t} (mcg.hr/ml)	9.38 ± 0.47	381.14 ± 9.17***
$AUC_{0-\alpha}$ (mcg.hr/ml)	9.38 ± 0.47	413.76 ± 9.60***

All values are mean ± SEM, n = 5; ***P < 0.001 when compared with RC alone group; [#](100 mg/kg, p.o); [##](400 mg/kg,po).

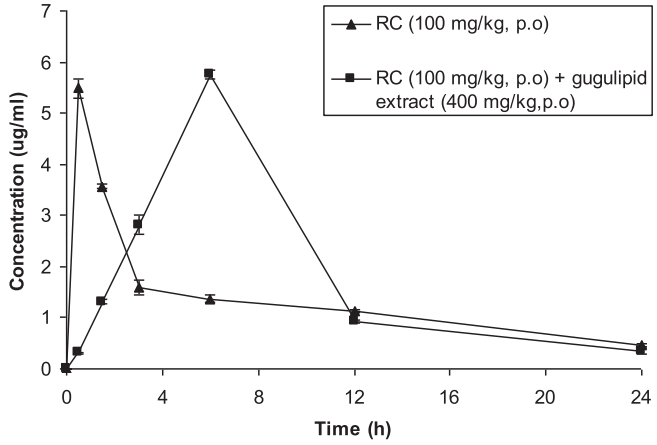

Fig. 2. Serum concentration-time curve after chronic administration of RC and +guggulipid extract combination for 14 days, All values are mean ± SEM, n = 5.

Table 2
Effect of guggulipid extract on the pharmacokinetics of RC after multiple-dose administration.

Pharmacokinetic parameter	RC[#]	RC[#]+ guggulipid extract[##]
$K_a(hr^{-1})$	39.89 ± 1.01	0.20 ± 0.00***
$K_e(hr^{-1})$	0.35 ± 0.041	0.19 ± 0.00***
T_{max}(hr)	0.11 ± 0.00	5.03 ± 0.04***
C_{max}(mcg/ml)	5.96 ± 0.33	3.76 ± 0.05***
V_d(lit)	16.27 ± 0.94	10.09 ± 0.14***
Cl (lit/hr)	5.607 ± 0.40	1.936 ± 0.01***
$t_{1/2}$ (hr)	0.016 ± 0.00	3.372 ± 0.02***
AUC_{0-t} (mcg.hr/ml)	18.18 ± 1.31	49.00 ± 0.43***
$AUC_{0-\alpha}$ (mcg.hr/ml)	18.20 ± 1.32	51.53 ± 0.43***

All values are mean ± SEM, n = 5; ***P < 0.001 when compared with RC alone; [#](100 mg/kg, p.o); [##](400 mg/kg,po).

Table 3
Effect of guggulipid extract on K_a of RC.

Treatment	K_a
RC[#]	0.0080 ± 0.00
RC[#]+ guggulipid extract[##]	0.0064 ± 0.00***

All the values are mean ± SEM, n = 6; ***P < 0.001 when compared to RC alone; [#](100 mg/kg, p.o); [##](400 mg/kg,po).

4. Discussion

This study was done to investigate the pharmacokinetic interaction of RC with guggulipid extract in rats. An attempt was also made to determine the interaction or counteraction of RC and guggulipid as an antihyperlipidemic agent in experimental animal models. The results observed indicate that co-administration of guggulipid extract with RC enhances the antihyperlipidemic activity of the latter at least partly by enhancing its bioavailability.

The concurrent administration of two medicinal substances is known to increase or decrease the pharmacological potential of each other. Combinational therapy may sometimes result in beneficial effects by synergism while antagonisms are also reported in some studies (Asdaq and Inamdar, 2009). Similar to any other medicinal substance, herbal therapies are also equally detrimental if used in combination with a potential conventional regimen without its validated concomitant use. Several studies have reported serious adverse interactions when herbal therapies are combined with conventional drugs (Parvez and Rishi, 2019, Asher et al., 2017, Thompson et al., 2016). Therefore, it is imperative to determine the role of commonly used herbal therapies or their bioactive constituents, in presence of potential modern drugs to confirm the safety index of their combined use. This is particularly important for those herbs and drugs that are known to be mediated through similar metabolic pathways and have identical pharmacological effects.

Lipid-lowering drugs, especially statins that inhibit 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) are widely used in the prevention and treatment of atherosclerotic disease. The risk of myopathy, as well as rhabdomyolysis of statins including RC is known to get enhanced when administered with other drugs or herbs without validation of their combined use (Danielak et al., 2018). Earlier studies with drug-drug and drug-herb interactions of RC have revealed varying effects. Drugs such as bezafibrate, gemfibrozil, raltegarvir and herbs such as red yeast rice are reported to increase the myopathic effect of RC while clopidogrel and different protease inhibitors are reported to increase bioavailability of RC without increasing its adverse effects (Bajaj and Giwa, 2020). Furthermore, co-administration of RC with drugs that increase its bioavailability is known to produce deleterious effects on kidney (Kostapanos, et al., 2010). Hence, the combination of RC and guggulipid should be used with caution till the adverse effects of this combination are determined.

As mentioned earlier, RC is a more effective inhibitor of HMG-CoA compared to several other statins available in the market. As a potent lipid lower agent, it works by both decreasing the production of cholesterol and enhancing the ability of the liver to remove LDL cholesterol present in the blood (Schachter, 2005).

As per the Ayurvedic system of medicine (ancient Indian medical system), guggul has been considered a valuable therapeutic agent for atherosclerosis, hypercholesterolemia, rheumatism, and obesity (Siddiqui and Mazumder, 2012). Guggul is the oleogum resin obtained from the plant *Commiphora mukul* and its extract is prepared by steam distillation followed by lyophilization. In the current study, a commercially available extract was procured and it was standardized in our lab for the presence of active constituents; guggulsterones. Earlier reports suggest that guggulipid acts as antagonists of the farnesoid X receptor (FXR), the bile acid receptor (BAR), and nuclear hormone that is involved in the regulation of cholesterol metabolism (Cui et al., 2003, Wu et al., 2002). Guggulipid is a known hypolipidemic agent that is used clinically for the treatment of hyperlipidemia. In the current study, hypolipidemic activity of guggulipid was not evaluated because the aim was to determine the influence of guggulipid on the pharmacokinetics and pharmacodynamics of RC and not to determine the influence of RC on guggulipid effect. Hence, the well-known hypolipidemic effect of guggulipid was not shown in the study.

The 14-day treatment period was selected based on earlier studies (Ha et al., 2020; Ventura et al., 2018). This time period allows enough drug/herb to accumulate in the body and this time

Table 4
Influence of guggulipid extract on the hypolipidemic effect of RC in high fat-fed rats.

Treatment	Percentage decrease on 14th day compared to 0th-day value			
	LDL level	TC level	TG level	HDL level
Vehicle[#]	46.69 + 0.757	62.48 + 0.63	23.30 + 0.41	0.16 + 0.06
RC[##]	35.71 + 0.678[**]	58.45 + 0.31[**]	12.63 + 0.16[**]	2.20 + 0.05[***]
RC[##+] guggulipid extract[###]	20.98 + 0.261[**++]	49.99 + 0.46[**++]	10.796 + 0.59[**+]	4.30 + 0.01[***+++]

All values are mean ± SEM, n = 5; [**]P < 0.01, [***]P < 0.001 when compared with vehicle treated control, [+]P < 005, [++]P < 0.01, [+++]P < 0.001 when compared with RC treated group;[#](1 ml/kg, po); [##] (100 mg/kg, p.o); [###](400 mg/kg,po).

period is also required for induction/inhibition of proteins in the body that may be responsible for drug-herb interaction (Rombolà et al., 2020).

The AUC of RC after single dose administration was exponentially high compared to multiple dose administration. There are very few studies on the pharmacokinetics on RC in rats and there is no study that determined the difference in pharmacokinetics of RC after single and multiple dose administration of RC in rats. However, an earlier report on liver clearance of RC in rat liver shows that RC undergoes very high hepatic uptake and biliary clearance compared to other statins that is modulated through OATP1B1/Mrp2 in rats (Kitamura et al., 2008). Though there is no direct report on the effect of multiple dose administration of RC on hepatic uptake and biliary clearance in rats, a study conducted with ciproflaxicin has showed that hepatic uptake and bilairy clearance of unchanged ciprofloxacin increases by up to 10 fold in rats after multiple dose administration compared to single dose administration (Parry et al., 1988). It is difficult to predict from the results of the present study if such an effect occurred after single and multiple dose administration of RC. Further investigation about hepatic intake and biliary clearance of RC after single and multiple dose administration in rats may provide at least one explanation for the difference in AUC. There may also be other unknown factors that might be responsible for this difference in AUC of RC between single and multiple dose.

The result of the current investigation showed that the bioavailability of RC was increased by the guggulipid extract. The effect of guggulipid extract on the bioavailability of RC was more after single-dose administration compared to that observed after multiple-dose administration. The exact reason for the difference in the bioavailability of RC after acute administration and chronic administration is difficult to explain. However, guggulipid mediated increase in the Tmax of RC in both single-dose and multiple-dose studies indicate that there is a delay in the absorption of RC. Furthermore, the increase in Cmax is an indication of the enhanced absorption of RC when given along with the guggulipid extract. Moreover, an increase in Ka pointed towards a delay in the rate of absorption and a decrease in Ke is an indication of decreased elimination of RC when given along with the guggulipid extract. RC is mainly excreted unchanged in the urine and undergoes metabolism to a lesser extent compared to other statins. CYP2C9 and CYP2C19 enzymes (Schachter, 2005) mediate the metabolism.

The decrease in the rate of absorption of RC by the guggulipid extract was confirmed by an in-vitro absorption study using isolated ileum. Since the in-vitro absorption study was carried out in small intestinal segments; the decrease in Ka is most probably due to physicochemical interaction between the RC and the guggulipid extract rather than the effect of guggulipid on gastrointestinal motility. The effect of p-glycoproteins in this interaction can be ruled out because RC is not a substrate for p-glycoproteins (Werba et al., 2018) and guggulipid extract is reported to stimulate p-glycoproteins rather than inhibiting it, which may lead to a decrease in bioavailability than an increase as observed in the present study. Furthermore, RC is a substrate of organic anion transporting polypeptide 1B1 (OATP1B1) and breast cancer resistance protein (BCRP) mediated transport (Fan et al., 2008, Lehtisalo et al., 2020). Guggulipid extract may increase the expression of both these transporters due to its effect on PXR receptors (Deng, 2007) that may further lead to a decrease in bioavailability of RC rather than an increase that is observed in the present study. Hence, the exact mechanism involved in the delayed absorption of RC by guggulipid is not known. However, further studies about physicochemical interaction between guggulipid and RC and involvement of intestinal enzymes in this interaction should be studied to elucidate the mechanism(s) responsible for this interaction.

The increase in bioavailability of RC was more in the single-dose study compared to the multiple-dose study. Earlier studies with other drugs involving single-dose and multiple-dose interaction studies suggest that this type of difference occurs due to induction and/or inhibition of proteins involved in drug transport and/or drug metabolism. As discussed above, the role of protein transporters namely OATP1B1 and BCRP in this interaction can be ruled out. Only 10% of RC is metabolized in the body and almost 90% is excreted unchanged in urine (Schachter, 2005). CYP2C9 and CYP2C19 enzymes mediate the metabolism. As mentioned earlier, guggul is known to decrease the expression of CYP2C19 (Chhonker et al., 2018), which suggests an inhibition of metabolism of RC by guggulipid. Hence, the increase in bioavailability of RC when administered along guggulipid could also be due to metabolic interaction between RC and guggulipid. However, guggulipid extract is believed to interact with many drugs due to its interaction with PXR receptors, which may lead to increased expression of many genes including those that encode many CYP enzymes (Zhou, 2008; Hedrich et al., 2016). There are no reports on the effect of guggulipid extract on the renal excretion of drugs.

The co-administration of guggulipid extract with RC in both single-dose and multiple-dose studies has caused a reduction in elimination rate constant and clearance of the RC from plasma, which has eventually increased the half-life. The Vd was significantly reduced after both acute and chronic administration of RC with guggulipid extract. The plasma protein binding of RC is around 88% and there are no reports on the effect of guggulipid on plasma protein binding.

Apart from pharmacokinetic interaction, there is also a pharmacodynamic interaction between guggulipid extract and RC. The co-administration of guggulipid extract with RC enhanced the antihyperlipidemic activity of the latter. The current study was done using a crude extract of guggul that was standardized for the presence of guggulsterones; constituents known for hypolipidemic effect. However, from the results, it is difficult to predict the constituent(s) that are responsible for pharmacokinetic and pharmacodynamic interactions.

Increased lipid level is a well-known risk for the development of lesions of atherosclerotic plaques in the coronary arteries that eventually may lead to cardiovascular derangements and metabolic dysfunctions. While studying the effect of drugs in experi-

mental animals, hyperlipidemia has to be induced and there are several established techniques available to do so. In this study, a standard high fat diet was prepared and used to induce hyperlipidemia in rats (Asdaq and Inamdar, 2010).

Pharmacodynamic interaction study of guggulipid extract with RC demonstrated that guggulipid extract increased the hypolipidemic effect of RC. This may be due to enhanced bioavailability of RC when administered along with guggulipid. There may also be interaction at the site of action that needs further investigation. Finally, combined administration of herb and drug, both with known antihyperlipidemic potentials, has significantly elevated the antihyperlipidemic effect. This eventually may have a beneficial effect if the addition of guggul extracts helps in reducing the adverse effect of RC.

5. Conclusion

To conclude, guggulipid extract augmented the antihyperlipidemic activity of RC and also increased its bioavailability. The combined therapy showed a reduction in serum TC, TG, and LDL levels. However, the results of the present study did not determine the effect of the combination on the adverse effects of RC. Hence, it is recommended that a combination of RC with guggulipid extract should be used only after an adverse effect(s) of this combination are determined.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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