Research Article

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Synthetic Characterization of Complexes of Rosuvastatin and Some ACE Inhibitors: Pharmacological Evaluation

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Abstract

Rosuvastatin is an anti-hypercholesterolemic agent with good anti-inflammatory and antinociceptive responses. The objective of present study was to assess the pharmacological effects of interaction of rosuvastatin with co-prescribed ACE inhibitors. For this purpose complexes of Rosuvastatin with enalapril, captopril and lisinopril were synthesized and characterized. Their spectroscopic analyses suggest that hydrogen bonded complexation occurs between rosuvastatin and selected ACE inhibitors at their carboxylic (COOH) and hydroxyl OH sites.

The anti-nociceptive effect of complexes was assessed by formalin induced nociception in mice, anti-inflammatory effect was evaluated by carrageenan induced paw edema in rats. Neuropharmacological behaviors were also studied on mice.

All the complexes of Rosuvastatin showed analgesic behavior in rats and mice. Anti-inflammatory activity of complexes is found insignificant. Enalapril complex keeps sedative activity while complexes with captopril and lisinopril contain anti-depressant behaviors.

Results suggest that the interaction of rosuvastatin with ACE inhibitors have consumed the active reacting sites of Rosuvastatin for which its anti-inflammatory, analgesic and gross locomotor behaviors have been affected. So it is suggested that Rosuvastatin should not be co-administered with any of these ACE inhibitors.

Key words

Hypercholesterolemia; Rosuvastatin; ACE inhibitors; Anti-inflammatory; Analgesic; Neuropharmacology

Abbreviations

ROS: Rosuvastatin; RCC: Rosuvastatin-captopril-complex; REC: Rosuvastatin-enalapril-complex; RLC: Rosuvastatin-lisinopril-complex

Introduction

Rosuvastatin being a potent HMG CoA reductase inhibitor in controlling biosynthesis of hepatic cholesterol is used for the treatment of coronary artery disease [1], heart attacks, hyperlipidemia, hypercholesterolemia and hypertriglyceridemia. At contrary, continuous dosage of statins also causes myositis, rhabdomyolysis [2-4], atherosclerosis and liver transaminase elevation in patients with common symptoms of headache, gastrointestinal disorders, rash, diabetes, hypertension and nephrotic disease. Enalapril, Captopril and lisinopril belong to angiotensin-converting enzyme (ACE) inhibitors (Figure 1). They block the conversion of angiotensin I to angiotensin II in hypertension, collagen vascular and cardiovascular disease [5,6], cardiac arrhythmias, myocardial infarction [7] and reduce the activity of blood pressure regulating rennin-angiotensin-converting enzyme in Barter's syndrome.
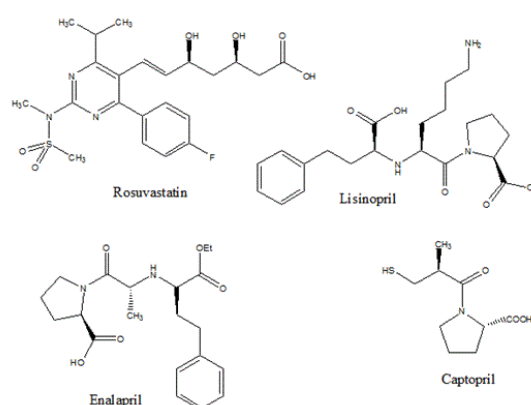

Figure 1: Chemical structures of drugs.

As clinical complexity of patients at high cardiovascular risk and with multiple comorbid conditions increases so does potential for drug-drug interactions (DDIs). Large surveying studies in various clinical fields have shown that large proportion of patients are co-prescribed a statin with potentially interacting therapies, suggesting

that the impact of polypharmacy on the safety profile of statins should be considered deeply.

Literature survey reveals a number of studies carried on derivatization of ROS [8-15], captopril [16-19], enalapril [20] and lisinopril [21,22] but there is no study yet present on analogues of ROS and ACE inhibitors. Owing to the tendency of DDI of ROS due to the presence of number of electron donating sites, in vitro interactions were carried out it was reacted with anti-hypertensives and synthesis of complexes of ROS with enalapril, captopril and lisinopril are reported in this paper. Complexes were characterized with physical and spectroscopic techniques and potential in vivo pharmacological studies.

Experimental

Materials and reagents

Reference standards of ROS, enalapril, captopril and lisinopril were kind gifts from Pharm Evo Pvt. Ltd., Merck Sharp and Dome Ltd., BMS Pvt. Ltd. and Atco Laboratories Ltd. respectively. Analytical grade hydrochloric acid (11 N), HPLC grade methanol and acetonitrile were purchased from Merck, Darmstadt, Germany. Deionized doubly distilled water was used throughout. Pyrex A grade glasswares were used after washing with diluted chromic acid, water and deionized waters.

Synthesis of compounds

Rosuvastatin-enalapril-complex (REC), rosuvastatin-captopril-complex (RCC) and rosuvastatin-lisinopril-complex (RLC) were synthesized. ROS (1 mmole) and enalapril (2 mmole) were separately dissolved in 40 mL methanol. Solutions were mixed in 100 mL round bottomed flask with constant stirring and refluxed for 3-4 hours at 70°C till the consumption of drugs. The progress of reaction was monitored by TLC. The volume of reaction mixture was reduced by rotary-evaporator. The precipitates were filtered and washed with chloroform at room temperature. The content was purified by re-crystallization with methanol:chloroform (2:8) system. Crystals obtained were again checked by TLC and constant melting points (Table 1).

Compounds	Color	State	Melting point	Solubility
REC	Transparent	Crystalline	110	DMSO
RCC	Brownish	Crystalline	85	DMF
RLC	White	Crystalline	70	MeOH

Table 1: Physical characteristics of compounds.

Instrumentation

IR studies were carried out on Shimadzu Model FTIR Prestige-21 spectrophotometer using Shimadzu IR solution 1.2 software. 1H-NMR spectra were recorded on a Bruker AMX 300 MHz spectrometer in CD3OD and deutrated water with TMS as an internal standard.

Pharmacological activities

Animals: Male Swiss albino mice of 20-25 g and Wistar rats 200-225 g were used as test animals. They were housed in standard cages at room temperature under controlled light with free access to food and water. Animals submitted to oral administration of test samples were fasted for 12 hours.

Analgesic activity studies (Formalin test): Formalin solution (20 μL; 2%) was injected in sub-plantar region of right hind paw of the mice to induce pain in the form of licking and biting responses. This test is done for the evaluation of antinociceptive effect. Aspirin as standard drug was administered orally 30 minutes before formalin injection to act as positive control while distilled water was given to serve as negative control. The test samples were administered orally (400 mg/kg) prior to the reaction. Licking and biting responses of the injected paw was considered as indicator of pain response [23,24]. The responses were counted in early phase for 5 min and for 30 min in late phase of formalin injection. Inhibition of pain or reduction in licking and biting responses was calculated by the following formula:

% inhibition = (mean of control group) − (mean of treated group) / (mean of control group) ×100

Anti-inflammatory activity studies: Studies were carried out after inducing inflammation in animals through carageenan injection [25]. Five groups of 6 rats each were taken. Test (or zero) group was treated intraperitoneal 30 minutes before carageenan injection. Group 1 was assigned positive control after receiving 300 mg/kg of aspirin; group 2 was made negative control after giving 0.1 mL distilled water/kg. Group 3 and 4 were given 400 mg and 800 mg/kg of REC. Plethysmometer (Ugo Basile) was used to measure increase in paw diameter immediately and till four hours following carageenan injection. Difference in sizes of right and left hind paw indicates the extent of inflammation. The procedure was repeated for RCC and RLC and ROS with other groups of animals.

Neuropharmacological activity studies Gross behavioral activity: Method described by Irwin [26,27]was followed for gross behavioral activities. Shivering, licking/ biting, rashes, fits, irritation and urination were observed.

Locomotor and muscle relaxant activity: Methods described by Ahmad et al. [28] and Verma et al. [29]in their prior studies were followed for CNS activities of compounds. Cage cross, head dip, rearing, open field, traction test, light/dark and forced swim tests were performed by placing animals in specified chambers and observed their movements for 30 minutes.

Open field activity was carried out in a square box of 76x76 cm and 42 cm high walls. The floor was divided into 25 equal squares. Mice were individually placed on the central square and number of squares crossed by each mouse with all four paws was counted for 15 minutes.

1000 mL beaker lined with white paper at the bottom was taken for rearing test on control and treated mice. Mice were placed in the beaker individually and allowed to move upward with erect position of the body. Each erect position was counted for 30 minutes [30-32].

Square-shaped head-dip box having three holes on each side was designed. Mice were placed individually. Number of head dips from the holes was counted for 30 minutes [33-35].

Traction was performed in such a way that mice were allowed to move on an iron rod of 1 m in length. Mice were first trained to walk on the rod. Time taken to travel on the rod was noted [36-38].

In cage cross test mice were allowed to cross the cages individually to a specifically designed rectangular box. Their crossing movement was observed for 30 minutes [39-41].

In forced swim test (FST) mice were individually forced to swim in a glass tank of 56 cm height and 20 cm width with water contained to a height of 22 cm at 25°C for 6 minutes. The level of water in the tank was set so that mice neither touch the bottom of the tank nor jump out of it. Swimming behavior was noted and movement was counted.

Statistical analysis: Statistical data were expressed as mean ± SEM [42]. Student's t-test values of $p<0.05$ was applied for significance of difference between means.

Results

Spectroscopic studies of synthesized compounds

IR studies: In the IR spectrum of Rosuvastatin, a broad region of band was seen between 3625-3200 cm^{-1} that was indication of OH group. The presence of characteristic carbonyl stretched peak at 1654 cm^{-1}confirmed the OH peak was mainly of carboxylic group with overlapping hydroxyl group in the region of 3625-3200 cm^{-1}. Adjacent to this band a sharp peak at 2926 cm^{-1} was seen which is specific for olefinic C-H of heptenoic side chain. Peak for bending vibrations of methyl C-H was present at 1453 cm^{-1} respectively. Peaks for distinguished sulfoxide group of the drug were confirmed by the presence of asymmetric and symmetric strong stretching vibrations at 1336 and 1155 cm^{-1} respectively. The C-F stretch of aromatic ring present in the compound was followed by the peak at 1230 cm^{-1} [43,44].

The spectrum of lisinopril was observed with broad band of carboxylic OH between 3570-3510 cm^{-1}. sp^2–CH stretch was found at 3099 cm-1. Carboxylic carbonyl stretching peak of at 1642 $cm^{-1}$and amide carbonyl stretch was present at 1575 cm^{-1}. Primary amine asymmetric stretch was present at 1610 cm^{-1} and symmetric stretch of primary amine was found at 1506 cm^{-1}. C=C aromatic ring stretch was found at 1450 cm^{-1}. 918-702 cm^{-1} was observed for =C-H absorption [45].

In captopril, broad band of carboxylic OH was seen at 3543-3320 cm^{-1}. Stretching peak of carboxylic carbonyl was present at 1747 $cm^{-1}$and amide carbonyl was observed at 1591 cm^{-1}. SH stretch of Captopril was seen at 2565 cm^{-1} [46].

In the IR spectrum of enalapril it was found that NH stretch was present at 3200 cm^{-1}. Peak for carboxylic OH was present at 3600-3300 cm^{-1} and carbonyl at 1725 cm^{-1}. A peak at 1381 cm^{-1} was present for C-N bond. Aromatic ring C=C was observed at 1649 cm^{-1} [47].

In complex RLC, there is a band at 3525-3150$cm^{-1}$for carboxylic OH of heptenoic acid site of ROS. In RCC is the band at 3460-3260 cm^{-1} that shows carboxylic OH and 1544 cm^{-1} for carbonyl peak. In REC, OH band is present at 3480-3100cm^{-1} and carbonyl peak is present at 1562 cm^{-1}.

^{1}H-NMR studies: Characteristic ^{1}H-NMR signals of ROS in $CDCl_3$ at 300 MHz showed a doublet at 1.23 ppm for methyl hydrogen. Singlet proton at 1.40 ppm for CH of heptenoic acid chain and another singlet was seen at 4.32-4.81 ppm for hydroxyl OH. Singlet for protons of ethylene was observed at 6.25 and 6.65 ppm. Quartet of benzyl protons is present at 7.30-8.15 ppm. Proton of carboxylic acid was seen at 10.25 ppm as singlet [48].

The ^{1}H-NMR signals of lisinopril in $CDCl_3$ at 300 MHz produced a singlet was present at 2.00 ppm for primary and secondary amine. Singlet at 1.92 and 2.02 ppm for CH_2 protons of pyrollidone are observed. Quartet of benzyl protons was observed at 7.29-7.40 ppm. Singlet proton of carboxylic OH was present at 10.20 ppm.

The ^{1}H-NMR spectrum for captopril in $CDCl_3$ taken at 300MHz produced a singlet at 1.5 ppm for mercapto proton. Methyl protons are seen in singlet at 1.19 ppm. Quartet for the protons of pyrrolidine was observed at 2.02-2.33 ppm. Doublet for protons of methylene bonded with sulfur was present at 2.68-2.93 ppm. Another doublet for β-CH of pyrrolidine was found at 3.41-3.51 ppm. Singlet proton at 10.20 ppm was present for the presence of carboxylic hydroxyl proton.

In the ^{1}H-NMR spectrum of enalapril, chemical shifts of amine proton as singlet at 2.00 ppm, pyrollidine protons as quartet at 4.33 ppm were observed. Phenyl protons were another quartet at 7.27-7.40 ppm. A singlet for the proton of carboxylic OH wa present at 9.50 ppm.

In RLC notable peak was present at 11.90 ppm for carboxylic acid. In RCC peaks for carboxylic acid were present at 11.00 ppm, while in REC the same signal was present at 9.61 ppm.

Pharmacological studies of synthesized complexes

Analgesic activities: The licking and biting response of rats due to REC and RCC was 24 ± 2.43 and 24 ± 1.76 (with 56.204 % inhibition each), while RLC showed 31 ± 2.46 response with 43.431 % inhibition against control (54.8 ± 4.45) and aspirin standard (1.498 ± 0.029, 70.887% inhibition) in early phase of formalin injection.

In late phase of injection REC showed (17.6 ± 1.44, 57.281%), RCC (24 ± 1.79, 41.747%) and RLC (20.6 ± 0.75, 50 %) number of responses against control (41.2 ± 4.23) and Aspirin standard (1.21 ± 0.04, 86.854%) in licking and biting. % inhibition to pain is calculated in Table 2 and Figure 2.

Compounds	phase 1		phase 2	
	Licking/biting	% inhibition	Licking/biting	% inhibition
Control	54.8 ± 4.45	--	41.2 ± 4.23	--
REC(400mg/kg)	24 ± 2.43	56.204	17.6 ± 1.44	57.281
REC(800mg/kg)	18.2 ± 0.665	66.788	15.954 ± 0.546	61.276
RCC	24 ± 1.76	56.204	24 ± 1.79	41.747
RLC	31 ± 2.46	43.431	20.6 ± 0.75	50
Aspirin	1.498 ± 0.029	70.887	1.21 ± 0.04	86.854
ROS	1.34 ± 1.34	97.554	1.564 ± 0.43	96.203

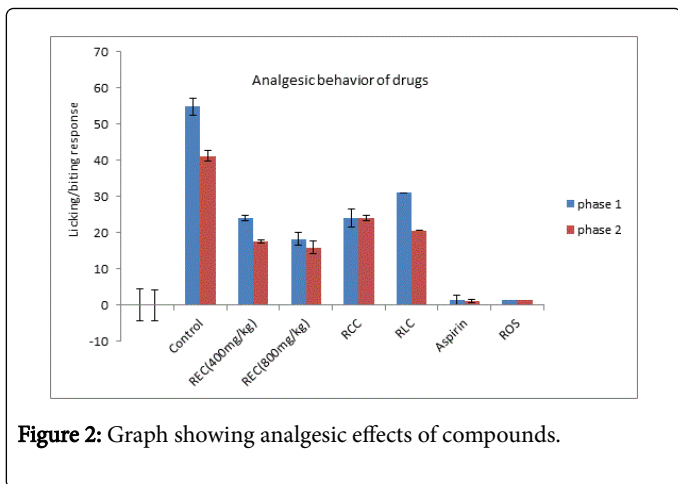**Table 2:** Formalin induced licking and biting response of compounds.

Figure 2: Graph showing analgesic effects of compounds.

Anti-inflammatory studies: Carageenan induced edema in animals showed negligible change in paw size in all cases of REC, RCC and RLC. The graph (Figure 3) shows very little anti-inflammatory response to edema when examined for four hours of drug injection. Difference of change in paw size is shown in Table 3.

CNS depressant activities Gross behavioral activities: After 30 minutes of drug administration (400mg/kg), shivering in animals was found considerable with REC and RCC while it was lesser with RLC. Hyper sensitivity produced licking and biting in animals to high degree in all test compounds, RCC showed higher response.

Rashes were produced to a little extent with RCC and RLC and was very low in the presence of REC. Irritation was increased in all compounds. REC increased urination while RCC and RLC did not show such response. Fits were not produced by any compound.

Compounds	0 hr (mm increase)	1 hr (mm increase)	2 hrs (mm increase)	3 hrs (mm increase)	4 hrs (mm increase)
Control	1.418 ± 0.0116	1.302 ± 0.0159	1.27 ± 0.0176	1.176 ± 0.02069	1.186 ± 0.01604
REC(400mg/kg)	1.478 ± 0.025	1.21 ± 0.0406	1.226 ± 0.0232	1.34 ± 0.1203	1.212 ± 0.0097
% inhibition	0.9383	13.5714	12.8023	5.0991	14.1643
REC(800mg/kg)	1.444 ± 0.0202	1.266 ± 0.0108	1.158 ± 0.0185	1.14 ± 0.0131	1.212 ± 0.009
% inhibition	6.8387	4.0909	17.8723	17.9856	12.8057
RCC	1.502 ± 0.017	1.404 ± 0.011	1.438 ± 0.022	1.442 ± 0.015	1.47 ± 0.018
% inhibition	5.9238	7.8341	13.2283	22.619	23.946
RLC	1.48 ± 0.015	1.43 ± 0.016	1.394 ± 0.011	1.386 ± 0.007	1.388 ± 0.0037
% inhibition	4.3723	9.831	9.7638	17.8571	17.032
Aspirin	1.98 ± 0.0293	1.21 ± 0.0406	1.226 ± 0.0232	1.34 ± 0.1203	1.212 ± 0.0097
% inhibition	8.333	13.05	3.597	12.806	32.708
ROS	1.1 ± 0.01	1.00 ± 0.02	0.94 ± 0.03	0.56 ± 0.03	0.47 ± 0.03
% inhibition	22.42	23.19	25.98	52.38	60.37

Table 3: Anti-inflammatory activity of compounds.

800 mg/kg dose of REC given to animals showed elevated response. Shivering, licking and biting and urination were very high while irritation was highest, however fits and rashes were not observed (Table 4).

Locomotor and muscle relaxant activities: Locomotor responses in the presence of these three test compounds were observed and compared with those of diazepam and ROS. Animals were allowed to cross the cage after 30 minutes of administration of compounds. REC took 6.6 ± 1.367 minutes, RCC took 18.4 ± 0.814 minutes and RLC took 20.8 ± 1.74 minutes to come out of the cage while diazepam standard and control took 7 ± 0.07 and 28 ± 1.143 minutes respectively.

Head dip response of animals during 30 minutes after administration of compounds: as compared to diazepam (6 ± 0.06 min) was 5.1 ± 1.725 minutes with REC, 15.2 ± 3.82 minutes with RCC, 22.8 ± 1.72 minutes with RLC and 27.6 ± 0.929 minutes with control (27.6 ± 0.929 min).

Rearing response as compared to diazepam (4 ± 0.03 minutes) was 4 ± 0.03 minutes for control, 9.2 ± 1.659 minutes for REC, 19.4 ± 0.93 minutes for RCC and 21.6 ± 1.69 minutes for RLC.

Open field response of mice to assess the fearfulness and emotional reactivity of mice was observed for 30 minutes during which diazepam showed 26 ± 0.09 counts, control showed 232.2 ± 6.321, REC (131.8 ± 14.877), RCC (142.6 ± 3.49) and RLC (158 ± 2.99) counts.
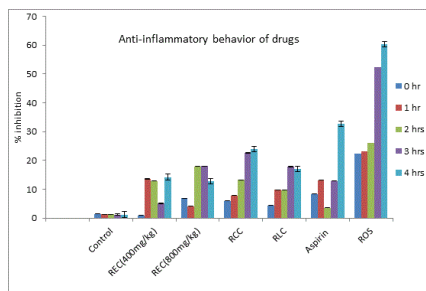
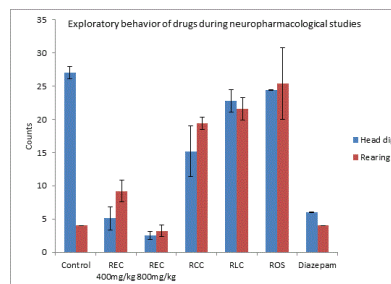**Figure 3:** Graph showing anti-inflammatory effects of compounds.

Compounds	Shivering	Licking/biting	Rashes	Fits	Urine	Irritation
REC (400mg/kg)	2+	2+	2-	3-	2+	2+
REC (800mg/kg)	3+	6+	4-	4-	3+	9+
RCC	1+	3+	1+	1-	1-	2+
RLC	2-	2+	1+	1-	1-	2+

Table 4: Gross behavioral activities of compounds.

In traction test carried out for muscle relaxant activity of mice, diazepam administered animals fell within 0.6 ± 0.02 seconds while control took 7.4 ± 1.252 sec, REC (31.4 ± 2.16), RCC (33.426 ± 1.74) and RLC (1.37 ± 0.08) seconds. After subjecting them for light dark activity test, mice were in dark for 6.44 ± 0.15 minutes with REC, 6.818 ± 0.14 minutes with RCC and 7.102 ± 0.145 minutes with RLC while diazepam dosed mice took 8.01 ± 1.03 minutes. The control had 6.91 ± 0.26 minutes in dark.

Mobility time of animals in FST was noted by placing them in a tank filled with water explained as above and observing their movement from one end to other. Animals with REC took 1.78 ± 0.12 minutes, RCC took 1.25 ± 0.025 minutes, RLC took 1.338 ± 0.05 minutes against control (4.124 ± 0.037 minutes) and diazepam (1.044 ± 0.02) minutes. (Figures 4a-d)
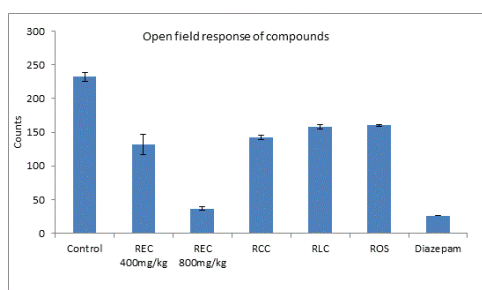

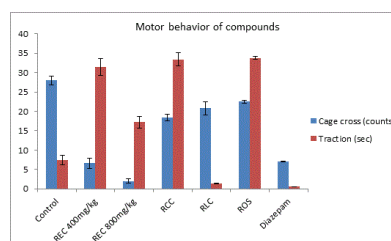**Figure 4a :** Graph showing open field response of compounds during neuropharmalogical studies.

Figure 4b: Graph showing neuropharmalogical effects of compounds.

Figure 4c : Graph showing motor behavior of compounds during neuropharmalogical studies.
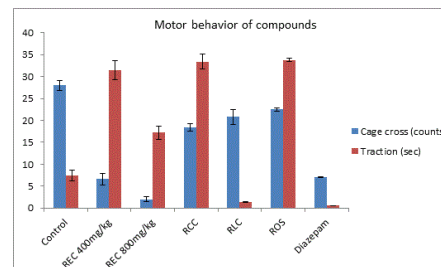

Figure 4d : Graph showing motor behavior of compounds during neuropharmalogical studies.

Discussion

Complexes of rosuvastatin with ACE inhibitors were subjected to characterization by IR and 1H-NMR spectroscopic studies so that nature of bond can be elucidated.

Spectroscopic studies of synthesized complexes

IR studies: The IR spectral analysis of RLC provided that the major changes as the absorption frequencies of carbonyl of both ROS and lisinopril were shifted towards right. The band for OH of heptenoic acid site also shifted to lower frequencies 3525-3150 cm^{-1}. This indicates the participation of OH and carbonyl bonds of both molecules (Figure 5a).

Similar trend in shifting of carbonyl and OH bands towards lower frequency was seen in complexes RCC and REC. In RCC, the OH was

observed at 3460-3260 cm^{-1} and carbonyl stretch was shifted to 1544 cm^{-1}. In REC, OH shift was found at 3480-3100 cm^{-1} with carbonyl peak at 1562 cm^{-1}. (Figure 5b and 5c)
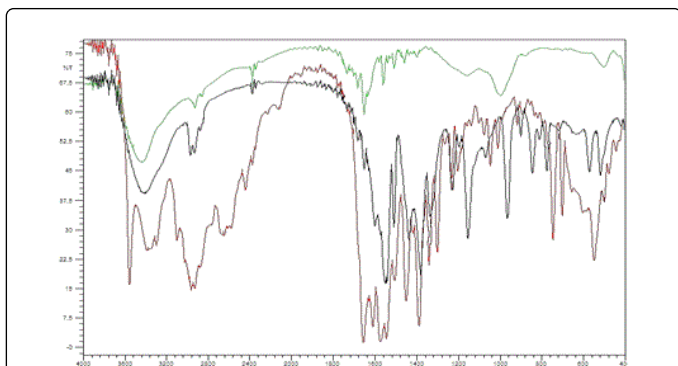

Figure 5a: Representative IR spectra of rosuvastatin (black), lisinopril (red) and their complex (green).
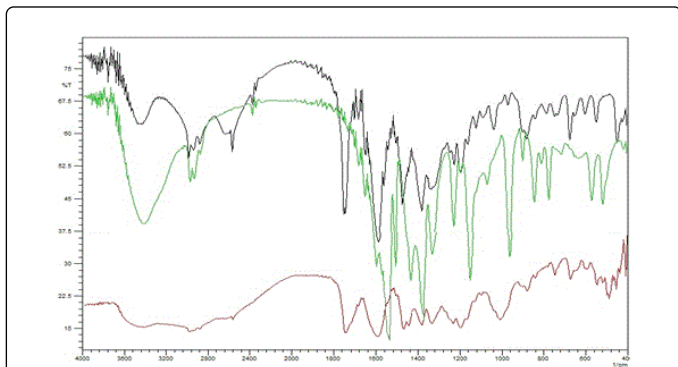

Figure 5b: Representative IR spectra of rosuvastatin (green), captopril (black) and their complex (red).
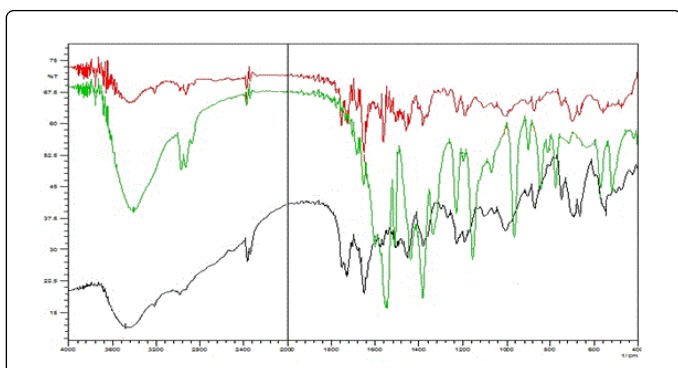

Figure 5c: Representative IR spectra of rosuvastatin (green), enalapril (red) and their complex (black).

The comparison of IR spectra of ROS with its complexes showed that the changes were occurred at the OH site of carboxyl of each interacting compounds while rest of the peaks had undisturbed shifts (Table 2). The changes show the involvement of these sites in complexation.

1H-NMR studies: The results of proton NMR analyses of these compounds are in accordance with the results of IR studies.

On comparing the spectrum of RLC with ROS and lisinopril the signals of carboxylic acid protons there was deshielding effect due to presence of hydrogen bonding between carboxylic acid site of lisinopril and heptenoic acid site of ROS.

Downfield shift in heptenoic acid OH of ROS was from 10.25 to 11.00 ppm and 10.20 from 10.50 ppm in captopril. In the complex with enalapril, there was downfield shift seen at 10.58 ppm for ROS and 9.61 ppm for the carboxylic of enalapril [48].

There is downfield shift in carboxylic and hydroxyl OH of ROS with indifferent changes in other signals. In lisinopril, captopril and enalapril the signals of carboxylic OH have been shifted down field. Characteristic IR and NMR signals have been demonstrated in Table 2.

The spectral studies show that the new compounds have been formed by multiple hydrogen bonding between OH (carboxylic and hydroxyl) of ROS and OH (carboxylic) of interacting compounds lisinopril, captopril and enalapril because no bond breaking seen. The complexes possess distinguished physical characteristics also (Table 1 and 5).

Compounds	phase 1		phase 2	
	Licking/biting	% inhibition	Licking/biting	% inhibition
Control	54.8 ± 4.45	--	41.2 ± 4.23	--
REC(400mg/kg)	24 ± 2.43	56.204	17.6 ± 1.44	57.281
REC(800mg/kg)	18.2 ± 0.665	66.788	15.954 ± 0.546	61.276
RCC	24 ± 1.76	56.204	24 ± 1.79	41.747
RLC	31 ± 2.46	43.431	20.6 ± 0.75	50
Aspirin	1.498 ± 0.029	70.887	1.21 ± 0.04	86.854
ROS	1.34 ± 1.34	97.554	1.564 ± 0.43	96.203

Table 5: Formalin induced licking and biting response of compounds.

Pharmacological studies of complexes

Analgesic activity studies: Results of formalin test indicated that all compounds (REC, RCC, RLC) showed significant response to licking and biting produced by formalin injection in both phases than standard drug (aspirin used). They exhibited high rate of inhibition (57.281, 41.747 and 50 % respectively) but it is less than that of standard (70.887 and 86.854%). Compared from studies carried out by Ghaisas and coworkers [49] rate of inhibition of ROS is high and is greater than that of standard. This shows that the compounds are less effective than standard and their parent drug; however they have potent analgesic effect to pain. (Table 3 and Figure 3)

Anti-inflammatory activities: The anti-inflammatory effects of compounds were studied using carageenan induced edema model. From statistical correlation of our studies, REC, RCC and RLC showed less inhibition to inflammation than aspirin standard (Table 4 and Figure 4).

Further REC was examined in two dosages (400 mg/kg and 800 mg/kg). Both doses did not show distinguished inhibition to pain. Rate of

inhibition of ROS (60%) was in correspondence with the work done by Ghaisas and coworkers [49]. The graph shows that all three test compounds show lesser anti-inflammatory response to edema than their parent drug i.e, ROS having potent anti-inflammatory response. Percent inhibition observed for four hours of drug injection is shown in Table 4. Figure 4 shows significance of inhibition of compounds.

CNS depressant activity studies

Gross behavioral activities: Table 4 shows that all three compounds with 400 mg/kg dose exhibited hypersensitivity in mice and less shivering with RLC. Fits were absent in all. 800 mg/kg dose of REC had all the parameters with prominent high response making it toxic. Diuresis was found with REC in both doses. (Table 5)

Effect on locomotor activities: Locomotor and muscle relaxant activities of mice were observed by cage cross, head dip, rearing, open field, traction test and mobility by FST.

Open field activity of mice is done for their general locomotor behavior and anxiety level [50,51]. In experiments at 400mg/kg dose of all test drugs, open field response of mice is shown from lowest to highest degree: Diazepam > REC > RCC > RLC > Control (Figure 5a). Similar increasing trend was observed in head dip activity i.e., REC > diazepam > RCC > RLC and control. Rearing response of RCC and RLC-treated mice were very high while REC showed low activity. Results with REC were like those of standard and control. These results showed high exploratory behavior with RCC and RLC like ROS while low with REC (Figure 5b).

Locomotor activity of mice shows sedative or stimulating effect of drug. In cage cross activity REC took less time to come out of cages unlike diazepam while RCC and RLC showed ease and took longer time to cross the cages and their results were like controlled and parent-drug treated mice. Traction test showed that all three test-drugs had muscle relaxing response as they all withstood longer however they all fell down at last. Results were similar to those of ROS. Diazepam-dosed mice could not move more than 0.6 ± 0.2 sec (Figure 5c).

During light/dark activity mice showed moderate movement in light. They all behaved like controlled and standard mice. Their response was similar to ROS also. In forced swim test, test-drug dosed mice behaved like those of diazepam and ROS. Increased immobility shows less struggle and all these compounds have response similar to their parent ROS (Figure 5d).

When 400 mg/kg REC with dose was compared with new dose of 800 mg/kg, the effect on all these activities was quite near the results of diazepam with increased irritation and diuresis.

Overall REC at both doses (400 and 800 mg/kg) showed it sedative like diazepam however high dose induced irritation also. RCC and RLC showed them as anti-depressant and results were like rosuvastatin.

Conclusion

Interacting products i.e., REC, RCC and RLC were synthesized. Interaction between drugs had suffered the drugs from formation of hydrogen-bonded complexes that have consumed the active sites of drugs. Newly formed complexes also have analgesic response but less than that of rosuvastatin. They all show less anti-inflammatory character unlike ROS. RCC and RLC can act as anti-depressant while REC as sedative. Results also conclude that the complexation of parent drugs when co-administered can also reduce their efficacy reducing their availabilities inside body. Hence it is suggested that administration of rosuvastatin with any of enalapril, captopril or lisinopril should be avoided.

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