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PHARMACOKINETIC AND PHARMACODYNAMIC INTERACTIONS OF ATORVASTATIN WITH GUGGULSTERONE IN HYPERLIPIDEMIC RATS

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Keywords:

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ABSTRACT: Atorvastatin is an HMG-CoA reductase inhibitor used in the treatment of hyperlipidemia. Guggulsterone is a herbal product used in hyperlipidemia. The present work was aimed to study the pharmacokinetic and pharmacodynamic interactions of atorvastatin with guggulsterone in hyperlipidemic rats. Wistar albino rats with induced hyperlipidemia were divided into six groups (n=6). Blood samples were collected at predetermined time intervals from the groups treated with atorvastatin alone and in combination with guggulsterone for kinetic analysis and lipid profiles. Plasma samples were quantified for atorvastatin concentration by HPLC, and then the concentration-time data were analyzed. C_{max}, $t_{1/2}$, AUC, and MRT were significantly ($p<0.05$) decreased during kinetic study, and clearance was significantly ($p<0.05$) increased in comparison with atorvastatin alone. Further, there was no significant difference in T_{max} in all groups. The study revealed lower values [C_{max} AUC, $T_{1/2,}$ and MRT] of atorvastatin in guggulsterone treated groups. The combination of atorvastatin and guggulsterone in hyperlipidemic rats produced a significant change in lipid profiles when compared with hyperlipidemic control, but less than atorvastatin alone.

INTRODUCTION: Atorvastatin (AT) is a synthetic lipid-lowering agent. It is a selective competitive inhibitor of 3-hydroxy- 3-methyl glutaryl coenzyme A reductase (HMG-CoA), which catalyzes the conversion of HMG-CoA to mevalonate, an important rate-limiting step in cholesterol biosynthesis [1]. It is used in the treatment of hyperlipidemia [2]. It is a substrate for the CYP3A4 isoenzyme. It has shown susceptibility to inhibitors and inducers of CYP3A4 to produce increased or decreased plasma concentrations, respectively [3].

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The usage of herbal therapies, along with prescription and Over The Counter (OTC) medications is increasing day by day. Guggulsterone from guggul resin (*Commiphora mukul*) is a well-known herbal product that has been targeted for its hypolipidemic activity [4]. The farnesoid X receptor (FXR) antagonism by guggulsterone has been proposed as a mechanism for its hypolipidemic effect. It inhibits lipogenic enzymes and HMG-CoA reductase in the liver [5]. It also increases biliary and fecal excretion of cholesterol [6]. Guggulsterone is reported to be more beneficial in reducing blood cholesterol, triglycerides levels, and to reduce the risk of cardiovascular disease without any apparent risk of liver damage [7, 8].

The present work was aimed at studying the pharmacokinetic and pharmacodynamic herb-drug interactions of guggulsterone with atorvastatin in

hyperlipidemic rats. The standard cholesterol diet [9] has been successfully used to induce hyper-lipidemia in rats, due to its convenience, reproducibility, and availability.

MATERIALS AND METHODS:

Drugs and Chemicals: Atorvastatin pure drug was a kind gift from Stallion Laboratories Pvt. Ltd., Bavla, Ahmedabad, Gujarath. *Commiphora mukul* standardized extract (2.6% w/w total guggulsterones) was procured from Natural Remedies Pvt. Ltd, Bangalore, India. HPLC grade acetonitrile, methanol and water were purchased from Finar Chem. Ltd., Hyderabad, India. Diclofenac sodium was a kind gift from Farmson Pharmaceutical Pvt. Ltd. Gujarat. Cholesterol kit (Enzymatic Method), Triglycerides kit and HDL-C kit were procured from Excel Diagnostics Pvt. Ltd., Hyderabad, India.

Experimental Animals: Wistar albino adult male rats weighing 170-200g were purchased from Mahaveer Enterprises, Hyderabad and housed in polypropylene cages in a room where the congenial temperature was 27 ± 1 ℃ and 12 h light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet and water ad *libitum.* The Institution Animals Ethics Committee (IAEC), Vaagdevi College of Pharmacy, India had approved the experimental protocol (VCOP/2012/ 11/1/S-II/6), and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare, and Government of India (1047/ac/07/ CPCSEA). Before induction of hyperlipidemia, the weight of the individual animal and plasma cholesterol levels were estimated. The standard cholesterol diet, along with butter (0.5 ml twice a day) was administered for 30 days to induce hyperlipidemia.

At the end of one month, the blood was withdrawn from retro-orbital sinuses to analyze for lipid profiles (TC, TG, LDL-C and HDLC levels) to confirm the induction of hyperlipidemia [10].

Anti-Hyperlipidemic Studies: The hyperlipidemic rats were divided into six groups of six rats each and dosed as below.

Group I: (HL) 1% Tween 80 (hyperlipidemic control).

Group II: (AT) atorvastatin (10 mg/kg p.o).

Group III: (GE) guggulsterone standardized extract (25 mg/kg p.o.).

Group IV: (GC) guggulsterone marketed preparation (capsule) (25 mg/kg p.o.).

Group V: (AT-GE) pretreated with guggulsterone standardized extract (25 mg/kg p.o.) for seven days and administered atorvastatin on the 8th day (10 mg/kg p.o.).

Group VI: (AT-GC) pretreated with guggulsterone marketed preparation (25 mg/kg p.o.) for seven days and administered atorvastatin (10 mg/kg) on 8th day [11].

Collection of Blood Samples: On 8th day, blood samples of 0.5 mL were withdrawn at different time intervals through retro-orbital sinus into heparinized Eppendorf tubes at 0, 1, 2, 4, 8, 12 and 24 h and equal amount of saline was administered intraperitoneally to replace blood volume at every blood withdrawal time [12]. Plasma was obtained by immediate centrifugation of blood samples using REMIULTRA cooling centrifuge at 3000 rpm for 10 min at room temperature. All samples were stored at -20 ℃ until analysis.

Preparation of Standard Graph: Stock solution of atorvastatin was prepared by dissolving it in methanol at a concentration of 1 mg/ml. This solution was diluted with methanol to obtain the working standard solutions in the range of 0.05-5 µg/ml. Chromatogram of Blank rat plasma is shown in **Fig. 1**. Plasma standards were prepared in the range of 0.05-5 µg/ml by taking 100 µl of working standard solutions in a disposable tube, evaporating to residue by heating at 45 ºC. To the residue, 1 ml of pooled untreated plasma was added and mixed in a vortex mixer for 30 sec and used for standard curve preparation. A standard curve was prepared from working plasma standard in the range of 0.005-5 µg/ml.

After extraction of the drug, standard samples were injected into the HPLC system, and peak areas were recorded and plotted against respective known concentrations of plasma atorvastatin to obtain a linear regression line (r^2=0.991).

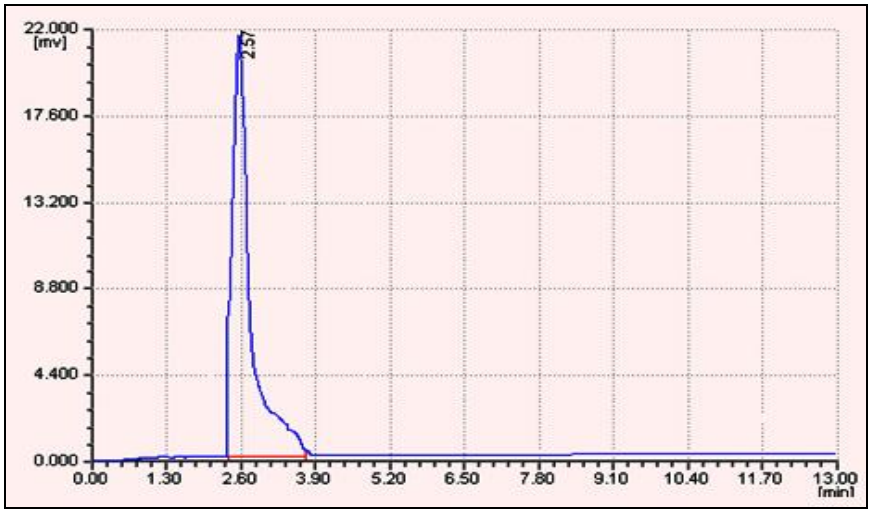

FIG. 1: BLANK RAT PLASMA CHROMATOGRAM

Measurement of Plasma Atorvastatin Concentration: To the plasma sample (100 µl), internal standard (Diclofenac sodium) (20 µl) and methanol (400 µl) was added and vortexed for 1 min. After centrifugation (4000 rpm for 10 min), the supernatant was collected into a fresh eppendroff's tube and evaporated to dryness and then reconstituted in 0.2 ml of methanol, 20 µl of this was injected into the High-Performance Liquid Chromatography (HPLC) system (Shimadzu LC-10AD) containing SPD-10A VP solvent delivery UV-Visible spectrophotometer detector.

The same extraction procedure was applied for the standard samples as well as those to be assayed for pharmacokinetic analysis [13]. The chromatography column was C-18 (Phenomenox, USA; 100A size 250 × 4.60 mm) coated with 0.5 µ silica gel. The mobile phase used was an isocratic solution of acetonitrile: water of 60:40 v/v [14], which was filtered through 0.2-µm nylon filter paper. The flow rate was adjusted to 1 ml/min. The peak was detected at 247 nm. The retentions times of atorvastatin and diclofenac sodium (internal standard) were 6.09, 10.19 min, respectively **Fig. 2**.

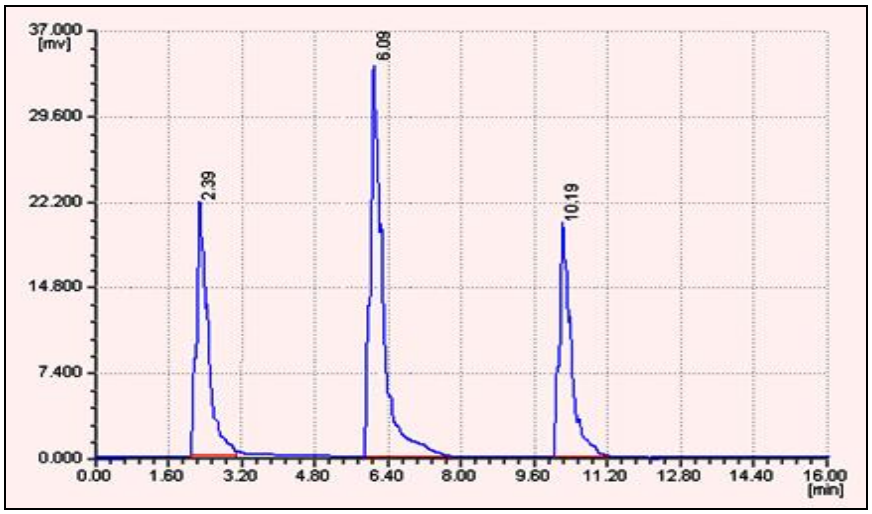

FIG. 2: CHROMATOGRAM OF STANDARD ATORVASTATIN WITH 5µg/ml IN RAT PLASMA

Biochemical Analysis: Plasma lipid levels (TC, TG, and HDL-C) were determined using respective diagnostic commercial kits [15, 16] from Excel Diagnostics Pvt. Ltd., Hyderabad, India and LDL-C in plasma was calculated as per Friedewald estimation[17, 18], LDL-C (mg/dl)= TC (TG/5+HDL)-C.

Statistical Analysis: The pharmacokinetic data were statistically analyzed by Student *t*-test; KINETICA (version 5.0). Other data were analyzed statistically by one-way Analysis of Variance (ANOVA) with Dunnett's test with significance level at 0.05 using Graph pad prism version 6.0.

RESULTS AND DISCUSSION:

Pharmacokinetic Analysis: Pharmacokinetic parameters like area under the curve [AUC], elimination half-life [$t_{1/2}$], volume of distribution [V_d], total clearance [CL], peak plasma concentrations [C_{max}], mean residence time [MRT] and time to reach peak plasma concentrations [T_{max}] were calculated for each subject using non compartmental pharmacokinetic model "KINETICA" (5.0 version). The plasma levels (mg/ml) of atorvastatin in groups treated with atorvastatin, atorvastatin-guggulsterone extract, and atorvastatin-guggulsterone capsules at different time points, on day 8th were shown in **Table 1, Fig. 3** and respective pharmacokinetic parameters were shown in **Table 2, 3**.

TABLE 1: COMPARISON OF MEAN ± SD PLASMA CONCENTRATION-PROFILE OF AT ALONE, AT+GE, AT+GC IN HYPERLIPIDEMIC RATS

Time (hrs)	AT alone	AT+GT	AT+GC
0	0.00±0.00	0.00±0.00	0.00±0.00
1	0.04±0.00	0.04±0.00[a]	0.01±0.00[b]
2	2.43±0.84	0.88±0.17[c]	0.68±0.11[b]
4	1.46±0.73	0.63±0.09	0.34±0.12[b]
8	1.03±0.25	0.45±0.08[b]	0.22±0.02[b]
12	0.90±0.02	0.34±0.01[b]	0.15±0.01[a]
24	0.50±0.00	0.13±0.00[a]	0.05±0.00[a]

All values expressed as Mean ± SD, n=6 [a] p<0.05 significant, [b] p<0.01 highly significant, [c] p<0.001 very highly significant
AT= Atorvastatin, GE= Guggulsterone standardized extract, GC= Guggulsterone capsule

TABLE 2: PHARMACOKINETIC PARAMETERS OF PLASMA ATORVASTATIN ALONE AND IN COMBINATION WITH GUGGULSTERONE STANDARDIZED EXTRACT, GUGGULSTERONE CAPSULE ON DAY 8 (n=6)

Parameters	AT alone	AT+GE	AT+GC
C_{max} (µg/ml)	2.43±0.97	0.88±0.17	0.68±0.13[c]
T_{max} (hr)	2±0.00	2±0.00	2±0.00
AUC (µg/ml*hr)	28.21±4.98	10.31±0.38[b]	5±0.47[c]
$t_{1/2}$ (hr)	10.95±0.16	8.8±0.62	7.5±2.69[b]
MRT(hr)	16.14±0.23	13.38±0.40[a]	10.73±0.43
V_d/F(ml)	65983.58±512.18	82010.28±427.25[a]	49851.2±102.26[a]
CL/F(ml/min)	2689.18±135.21	16483.31±105.23[c]	59065.8±124.2[c]

All values expressed as Mean ± SD, n=6 [a] p<0.05 significant, [b] p<0.01 highly significant, [c] p<0.001 very highly significant (Compared by one way ANOVA followed by Dunnett's test).

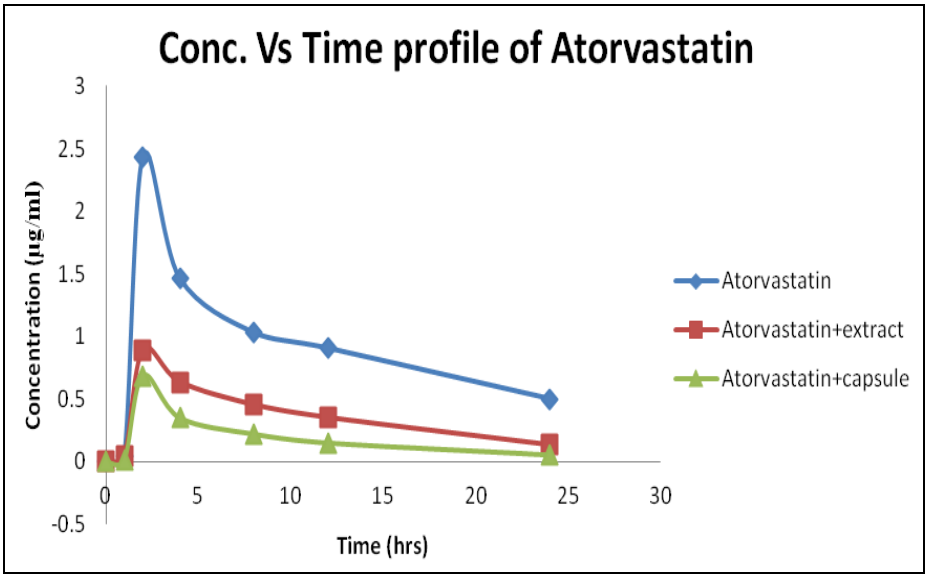

FIG. 3: MEAN PLASMA CONCENTRATION (µg) OF ATORVASTATIN ALONE AND COMBINATION WITH GUGGULSTERONE STANDARDIZED EXTRACT AND GUGGULSTERONE MARKETED PREPARATION ON DAY 8TH IN HYPERLIPIDEMIC RATS (n=6)

TABLE 3: MEAN PLASMA LIPID PROFILE CONCENTRATION (mg/dl) IN HYPERLIPIDEMIC RATS

Groups	TC	TG	LDL-C	HDL-C	VLDL-C
Disease control-I	241±6.05	140±8.48	194.7±3.67	18.2±3.86	28±1.69
AT (10mg/kg)- II	151.5±4.43^{c}	93.2±4.92^{c}	98.6±3.89^{c}	33±3.39^{b}	18.6±0.98^{c}
GE (25 mg/kg)-III	171.25±6.39^{c}	109±7.74^{c}	120.7±4.78^{c}	28.7±5.31^{a}	21.8±1.54^{c}
GC (25 mg/kg)-IV	192±5.16^{c}	113.7±5.56^{c}	143.5±5.69^{c}	25±3.55	22.7±1.11^{c}
AT+ GE(10 mg/kg + 25 mg/kg-V	204.5±6.19^{c}	119±5.83^{b}	157.2±8.24^{c}	23.5±2.88	23.8±1.16^{b}
AT+GC(10 mg/kg + 25 mg/kg)-VI	212±6.32^{c}	125±4.32^{a}	164.9±4.37^{c}	20.7±5.56	24.8±0.69^{a}

All values expressed as Mean ± SD, n=6 ^{a}p<0.05 significant, ^{b}p<0.01 highly significant, ^{c} p<0.001 very highly significant
AT= Atorvastatin, GE= Guggulstearone standardized extract, GC= Guggulstearone capsule.
(TC=Total Cholesterol, TG=Triglycerides, LDL-C=low density lipoprotein cholesterol, HDL-C=high density lipoprotein cholesterol, VLDL-C=very low-density lipoprotein cholesterol) (Compared by one way ANOVA followed by Dunnett's test)

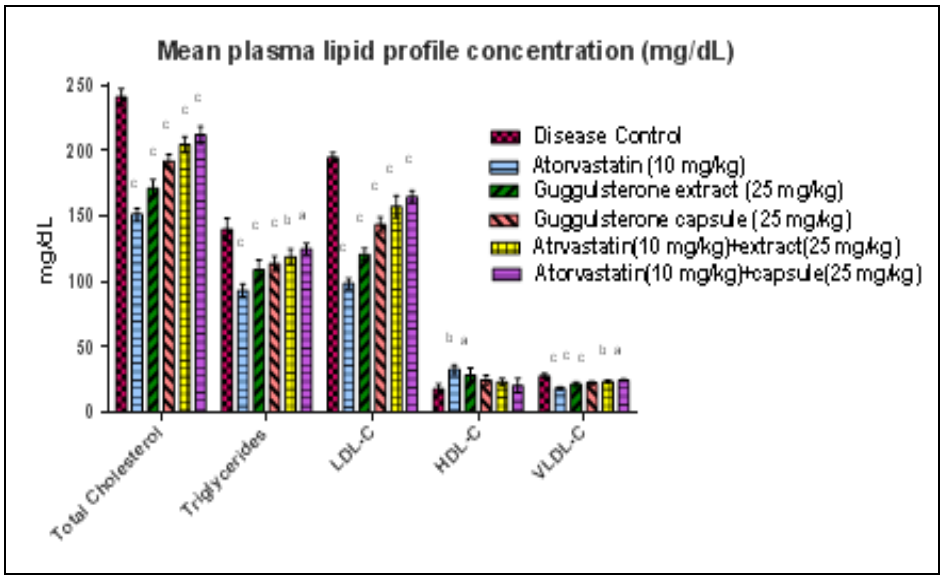

FIG. 4: MEAN PLASMA LIPID PROFILE CONCENTRATION (mg/dl) IN HYPERLIPIDEMIC RATS. ^{a}p<0.05 significant, ^{b}p<0.01 highly significant, ^{c}p<0.001 very highly significant

Herb-Drug interactions have both a pharmacokinetic and pharmacodynamic basis, most of which is attributed to the induction or inhibition of hepatic and intestinal microsomal enzymes (primarily cytochrome P450), and drug transporters [19]. Although, popular belief about the herbal products is that many of these preparations are considered natural and safe, they require attention for potential risk as they are pharmacologically active. Most of these herbal remedies can interact with allopathic drugs, resulting in altered activity and toxicity.

Some of the herbs that interact with Atorvastatin are red yeast Rice (*Monascus purpureus*), grapefruit, grape fruit juice, Garlic (*Allium sativum*) [11].

Atorvastatin lowers plasma cholesterol and lipoprotein levels by inhibiting HMG CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic low-density lipoprotein (LDL) receptors on the cell surface for enhanced uptake and catabolism of low-density lipoprotein (LDL), it also decreases triglycerides (TG) levels but, increases high-density lipoprotein (HDL) levels [20]. It was reported that the atorvastatin therapy produced statistically significant changes in total cholesterol, LDL-C, TG, and HDL-C within 24 hours [21].

The present study investigated the pharmacokinetic and pharmacodynamic interactions of atorvastatin with guggulsterone standardized extract and guggulsterone marketed preparation in hyper-lipidemic rats.

The observed peak plasma concen-trations (C_{max}) of atorvastatin was 2.43 ± 0.97 µg/ml at 2 h after oral administration on day 8, but in combination with guggulsterone, standardized extract or guggulsterone marketed preparation it was significantly changed to 0.88 ± 0.17, 0.68 ± 0.13 µg/ml (p<0.001) respectively.

The AUC of atorvastatin was significantly decreased to 63.45%, 82.27% when it was combined with guggulsterone standardized extract or guggulsterone marketed preparation.

While there was an increase in the clearance (CL) of atorvastatin in combination with guggulsterone standardized extract or guggulsterone marketed preparation. These results suggest that there is a significant pharmacokinetic interaction between atorvastatin and guggulsterone. In particular, guggulsterone (a CYP inducer) probably enhanced the metabolism of atorvastatin. Further, there was also a significant decrease in lipid levels of AT+GE, AT+GC with hyperlipidemic control, but the results were found to be less significant than atorvastatin alone.

CONCLUSION: In conclusion, the therapy of atorvastatin with guggulsterone alters the pharmacokinetics of atorvastatin in hyperlipidemic rats producing a significant change in lipid profiles when compared with hyperlipidemic control.

However, the hypolipidemic effect resulted from the combination therapy was found to be less significant than atorvastatin alone. Thus, it can be suggested that the combination of atorvastatin with guggulsterone has no potential benefits in safety, efficacy, and tolerability than individual drugs.

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CONFLICT OF INTEREST: Nil

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