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In Vitro In Vivo Pharmacokinetic Interaction Study of Escitalopram Oxalate when Co Administered with Caffeine/caffeinated Beverages

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Abstract: The effect of caffeine and caffeinated beverage (green tea) on the pharmacokinetics of escitalopram oxalate was evaluated by *in vitro* and *in vivo* studies.

In the *in vitro* study, the release of escitalopram oxalate in the presence and absence of caffeine/green tea (Caffeinated beverage) was studied using dissolution procedure. Water was used as the dissolution media. For the *in vivo* study, rabbits were used to predict the pharmacokinetic data. The escitalopram oxalate concentrations in the dissolution and plasma samples were analyzed by a Reverse phase High performance liquid chromatographic method (RP HPLC method). This method was carried out on a Hibar C_{18} (250 mm × 4.6 mm id., 5µ) using mobile phase containing 25mM potassium dihydrogen ortho phosphate (pH adjusted to 3.0 with ortho phosphoric acid) and acetonitrile with the ratio of 60:40 % v/v with UV detection at 234 nm. The flow rate was maintained at 1.2 ml/min. The developed method was validated according to the ICH guidelines using various validation parameters. The dissolution study was carried out for one hour in the presence of synthetic caffeine and green tea decoction. The presence of synthetic caffeine and green tea decreased the release of escitalopram oxalate by 33.75 and 13.12% respectively. In the *in vivo* study carried out with new zealand rabbits a difference was observed in the C_{max} and $AUC_{(0-t)}$ values when the drug was co administered with caffeine and green tea.

The absorption of escitalopram oxalate was decreased to a maximum of 28% and 18% when co administered with caffeine and green tea respectively.

Keywords: RP HPLC, *in vitro* study, *in vivo* study, Escitalopram oxalate, Caffeine.

INTRODUCTION

Drug interactions may make our drug less effective, cause unexpected side effects or increase the action of a particular drug. Some drug interactions can even be harmful. Drug interactions are pharmacodynamic, pharmacokinetic or clinical responses to the administration of a drug combination that differ from the known effects of the individual drugs administered alone. Pharmacokinetic drug interactions are a consequence of altered levels of exposure to the drug or its metabolites through one or more of the following mechanisms viz altered absorption, altered distribution, altered transport, induction, inhibition and altered excretion. Drug interaction studies fall into three broad categories viz drug – drug interactions, drug – food/beverage interactions and drug – condition interactions.

Drug – food/beverage interactions result from drugs reacting with foods or beverages. Most drug food interactions occur through three mechanisms: reduced rate or extent of absorption, increased rate or extent of absorption or through chemical/pharmacological effects.

Escitalopram oxalate is an orally administered selective serotonin reuptake inhibitor. It is the pure S enantiomer of the racemic bicyclic phthalane derivative citalopram. It is primarily used for the treatment of major depressive disorder and general anxiety disorder in adults [1].

A detailed literature review carried out revealed the interaction of anti depressant drugs with caffeine/caffeinated beverages. Laswell WL Jr and co-workers [2] have reported the *in vitro* interaction of neuroleptics and tricyclic antidepressants with coffee, tea and gallotannic acid. Fukasawa T and coworkers [3] have reported the effect of caffeine on the kinetics of fluvoxamine and its major metabolite in plasma after a single oral dose of the drug. A study on the effect of caffeine on clozapine pharmacokinetics in human volunteers was reported [4]. Helen J Cheeseman *et al.* [5] have reported the interaction of

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chlorpromazine with tea and coffee. Further spectrop-hotometric and High Performance Liquid Chromatographic [HPLC] methods [6-10] were reported for the estimation of escitalopram oxalate alone or in combination with other drugs in the formulations and plasma.

The present study was carried out to evaluate the influence of the presence of caffeine/caffeinated beverage on the *in vitro* dissolution study and *in vivo* pharmacokinetics of escitalopram oxalate in rabbits. An RP HPLC method was developed for the simultaneous estimation of escitalopram in the presence of caffeine and validated in accordance with ICH guidelines.

MATERIALS AND METHODS

Drugs and Reagents Used

The Escitalopram solid dosage formulation assayed was citopam 10 mg (Sun Pharmaceuticals, India). Working standards used were: escitalopram oxalate (Crescent Pharmaceuticals, India) and synthetic caffeine RS (Sigma Aldrich, India). Sample of green tea was purchased from the market. Acetonitrile HPLC grade, ortho phosphoric acid AR grade and potassium dihydrogen ortho phosphate AR grade were supplied by Qualigens fine chemicals. Water HPLC grade was obtained from Milli Q RO water system.

Instruments Used

Shimadzu gradient HPLC system equipped with LC 2010 AT VP solvent delivery system (pump), Rheodyne 7725i auto injector with 20 µl loop volume and 2010 A HT UV detector. Class VP data station was used for data collection and processing.

In Vitro Dissolution Study

Dissolution profiles were determined at 37±0.5°C in 900 ml of water [11]. Commercially available dissolution equipment employing the paddle apparatus as described in the USP XXII was used. Rotation speed was maintained at 50 rpm. The paddle was positioned to extend to exactly 2.5 cm above the flask bottom. Samples (5 ml) were taken with a graduated pipette at the following times.

- Escitalopram tablet: 0, 5, 10, 15, 20, 30, 45 and 60 min.

Each sample was replaced by an equal volume of dissolution media to keep the total volume constant. A correction was made to take into account the total cumulative removed volumes when determining the total amount dissolved as a function of time. All samples were suitably filtered, placing a filter paper at the end of the sample probe, and kept at 5°C until immediate analytical determination.

One tablet of the corresponding escitalopram was placed in each filled flask (6 tablets per run) when establishing the dissolution profiles of the drug in the absence of caffeine/caffeinated beverages. These profiles were considered as reference curves.

To evaluate the influence of caffeine / caffeinated beverages on escitalopram dissolution kinetics, the corresponding synthetic caffeine (10mg) and decoction of green tea were prepared [2.5 gm powder in 100 ml (29.8 mg of caffeine)] and added to each flask at the same time as escitalopram formulation. The volume of caffeinated beverages was selected based on the normal daily intake of the beverage.

In Vivo Study

Subject

New zealand rabbits (6–8 months old) were used for the study.

Study Design

This was a single dose, randomized study. Animals were separated in four equal groups (six in one group). First group received the product Citopam, second group received product escitalopram tablet along with synthetic caffeine, third group received product escitalopram tablet along with decoction of green tea and fourth group was kept as control receiving water. The dosage administered was calculated based on the animal weight and they were administered via oral route.

Blood Sampling and Assay

Blood samples were drawn from the jugular vein of the rabbit just before the administration of drug and after 1, 2, 4, 6, 8, 12, 24, 36, 48, 60 and 72 hrs of drug administration in pre citrated tubes. The withdrawn samples were centrifuged at 4000 rpm for 10 minutes to separate plasma immediately after withdrawal. They were transferred into airtight containers and stored at - 20°C until further analysis.

To the plasma samples, 0.05 ml of internal standard (cetrizine HCl) and 0.40 ml of acetonitrile were added and centrifuged twice at 4000 rpm for 20 min. Then the supernatant solutions were used for further analysis. The plasma concentration levels were measured by the below mentioned high performance liquid chromatographic method.

Pharmacokinetic and Statistical Analysis

Pharmacokinetic parameters like AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, T_{max}, K_{eli} and $t_{\frac{1}{2}}$ were calculated for different groups by using Pk_1 and Pk_2 solutions software.

Chromatographic Conditions

Samples from all assays were analyzed by an RP HPLC method.

An isocratic HPLC separation was carried out using a Shimadzu LC 2010AT VP system (Shimadzu Technologies, Japan). A Hibar C_{18} column (250 X 4.6 mm id., 5µ), was used as stationary phase. A mobile phase consisting of a mixture of 25 mM potassium dihydrogen orthophosphate [Adjusted to pH 3.0 using ortho phosphoric acid]: acetonitrile in the ratio of 60: 40 % v/v was delivered at a flow rate of 1.2 ml/min with detection at 234 nm. The mobile phase was filtered through a 0.2 µ filter and degassed prior to analysis. Cetrizine hydrochloride was used as internal standard in plasma samples. The developed method was validated as per ICH guidelines.

RESULTS AND DISCUSSION

RP HPLC Method

Optimization of the method was carried out using various concentrations of acetonitrile while keeping the pH of the
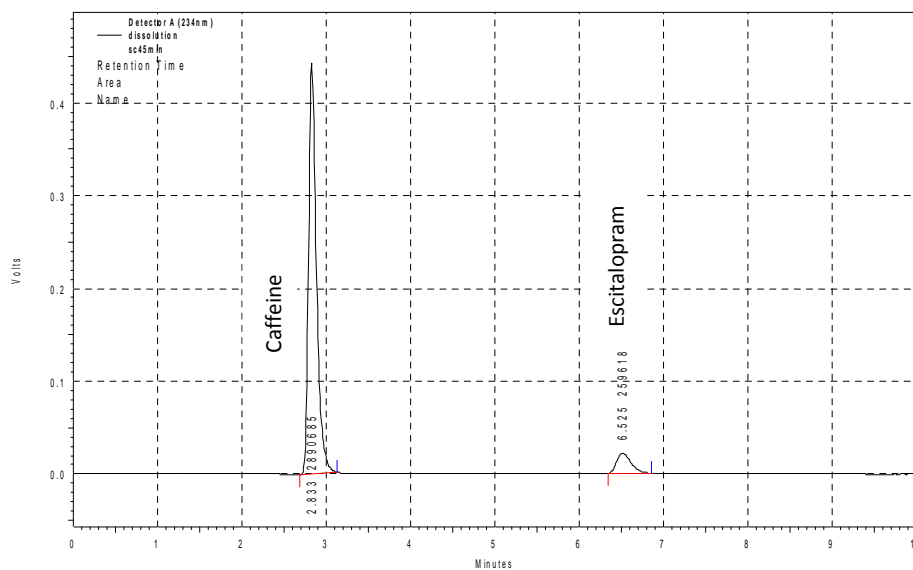
Fig. (1). Typical Chromatogram of Escitalopram oxalate in presence of caffeine (Dissolution sample).
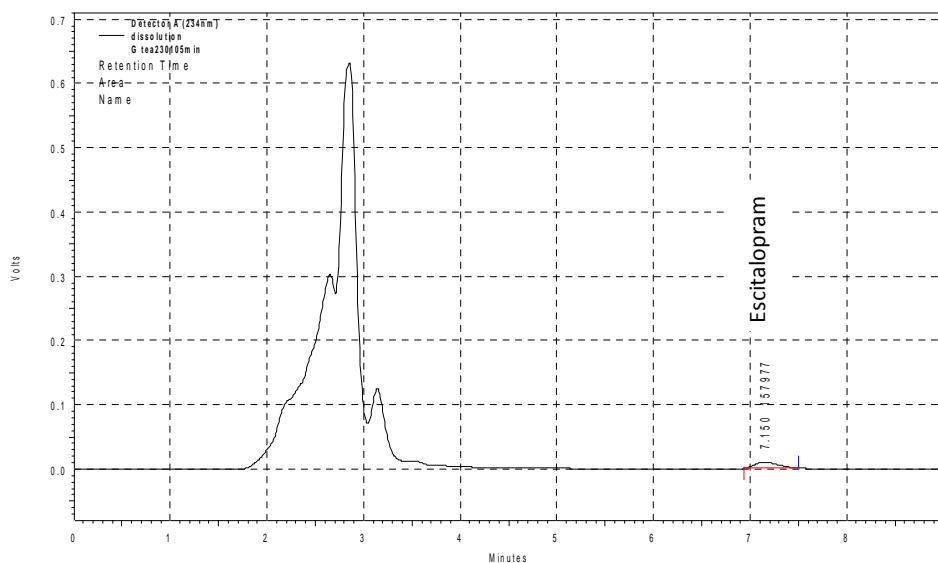

Fig. (2). Typical Chromatogram of Escitalopram oxalate in presence of green tea (Dissolution sample).

aqueous phase constant. A solvent combination of 25 mM potassium dihydrogen ortho phosphate [Adjusted to pH 3.0 using ortho phosphoric acid]: Acetonitrile (60:40 % v/v) gave a satisfactory separation of the compounds of interest. This optimized mobile phase separated caffeine at 2.8 min, internal standard (Cetrizine HCl) at 11.3 min and escitalopram oxalate at 6.5 min respectively. Increasing the concentration of organic phase in the solvent system resulted in faster elution but loss of resolution. On the other hand increasing the aqueous phase concentration caused peak broadening and increase in retention time.

In Vitro **Dissolution Study**

In vitro interaction of escitalopram and caffeine / green tea was determined by the effect of caffeine / green tea on dissolution profile of escitalopram tablets. Dissolution profiles were determined at 37±0.5°C in 900 ml of water. The dissolution study was carried out for 60 minutes and

samples were withdrawn at the intervals of 0, 5, 10, 15, 20, 30, 45 and 60 mins. The samples withdrawn at different time intervals were analyzed using the developed HPLC method and the chromatograms are shown in Figs. (**1** and **2**). The mean percentage release of escitalopram in presence and absence of caffeine/green tea decoction are tabulated in Table **1**. The effect of caffeine on the dissolution profile of escitalopram tablets can be interpreted from Fig. (**3**).

The results revealed a significant modification of the dissolution profiles of escitalopram oxalate as a consequence of the presence of synthetic caffeine ($P<0.01$) and green tea ($P<0.05$). Synthetic caffeine decreased the release of escitalopram oxalate in the dissolution samples by 33.15%. The presence of green tea also produced a decrease of 13.15% in the total amount of escitalopram oxalate dissolved and was more relevant to the effect of synthetic caffeine on the drug.

Table 1. Release Profile of Escitalopram Oxalate in Presence and Absence of Caffeine / Caffeinated Beverages

Time (min)	% Release of Escitalopram (n = 6)		
	Sample A	Sample B	Sample C
0	0.00 ±0.00	0.00±0.00	0.00±0.00
05	37.8 ±0.82	22.17±1.37	41.4±1.06
10	63.21±0.65	36.88±0.94	68.63±0.97
15	76.16±0.43	37.97±0.83	78.91±1.78
20	76.58±0.98	49.70±0.97	79.64±1.38
30	95.00±1.21	58.97±1.56	82.85±0.81
45	95.52±1.47	63.98±0.87	83.3±0.49
60	103.24±0.99	66.85±1.16	86.28±0.96

A = Escitalopram tablet
B = Escitalopram tablet in presence of synthetic caffeine (raw chemical, 10 mg)
C = Escitalopram tablet in presence of decoction of green tea (29.8 mg of caffeine)
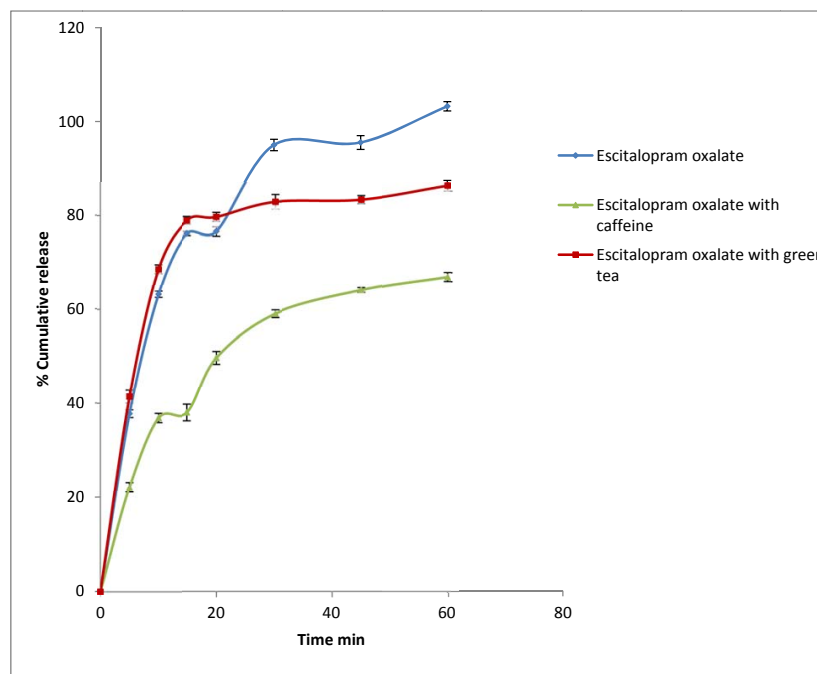

Fig. (3). Dissolution profiles of escitalopram oxalate corresponding to *in vitro* tests carried out in absence and in presence of caffeine/green tea.

In Vivo **Pharmacokinetic Study**

In vivo pharmacokinetic interaction study of escitalopram oxalate with caffeine/green tea was carried out in new Zealand rabbits. Fig. (**4**) shows the mean observed time course of escitalopram oxalate plasma concentration for the three groups treated with escitalopram alone, with caffeine and with green tea. The comparision of mean plasma concentrations of escitalopram oxalate alone with that of caffeine/green tea are summarized in Table **2**. Summaries of the pharmacokinetic parameters are provided in Table **3**.

The results revealed a significative difference in the AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} values between the groups(P<0.05). The absorption of escitalopram oxalate was decreased to a maximum of 28% and 18% when co administered with caffeine and green tea respectively.

The Prediction from *In Vitro* to *In Vivo*

In the *in vitro* study, the release of escitalopram oxalate in the dissolution study carried out for one hour in the presence of caffeine and green tea was slower than that in the absence of the same. The *in vivo* study carried out also revealed that the absorption of orally administered escitalopram oxalate was impaired in the presence of caffeine/green tea. Approximately 28 % and 18 % decrease in the bioavailability of escitalopram oxalate was observed with the co administration of caffeine/green tea.

Thus the results suggest that the *in vitro* data of this study corresponded to the data of the *in vivo* study. Accordingly, it may be possible to predict the *in vivo* drug food beverage interaction caused by the formation of complexes from the *in vitro* release tests in the study.
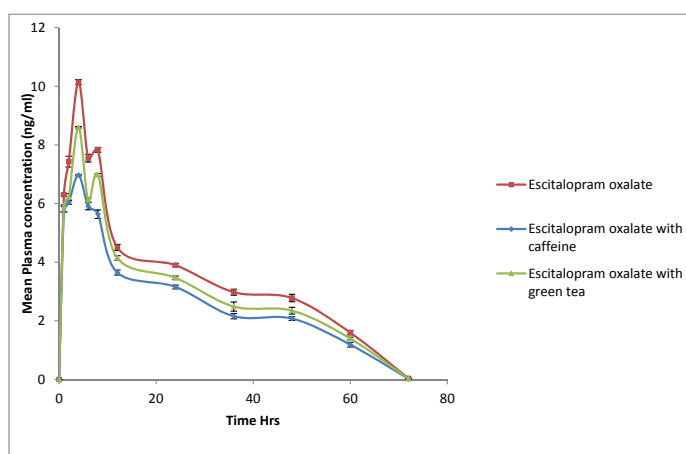
Fig. (4). Comparison of plasma concentration data of escitalopram oxalate in presence and absence of caffeine/ green tea.

Table 2. Comparison of Plasma Concentration data of Escitalopram Oxalate in Presence and Absence of Synthetic Caffeine/Green Tea

Time	Mean Plasma Concentration (ng/ml)			% Decrease in mean Plasma Concentration of Escitalopram Oxalate	
	Group 1	Group 2	Group 3	Group 2	Group 3
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00	0
1 hr	6.298±0.0378	5.721±0.0221	5.932±0.0062	9.16	5.81
2 hr	7.432±0.1850	6.052±0.1205	6.22±0.0709	18.56	16.28
4 hr	10.144±0.0871	6.971±0.0252	8.605±0.0254	31.27	15.17
6 hr	7.548±0.1320	5.91±0.0754	6.125±0.1216	21.70	18.85
8 hr	7.831±0.0869	5.64±0.0503	6.98±0.1450	27.97	10.86
12 hr	4.504±0.1050	3.65±0.0757	4.148±0.0850	18.96	7.90
24 hr	3.9±0.0556	3.158±0.0608	3.47±0.0655	19.02	11.02
36 hr	2.98±0.1014	2.16±0.1562	2.49±0.0907	27.51	16.44
48 hr	2.78±0.1266	2.08±0.1153	2.35±0.0635	25.17	15.46
60 hr	1.6±0.0750	1.19±0.0472	1.4±0.080	25.62	12.5
72 hr	0.04±0.0005	0.03±0.0095	0.029±0.0041	25	27.5

Group 1: Escitalopram oxalate
Group 2: Escitalopram oxalate with synthetic caffeine
Group 3: Escitalopram oxalate with green tea decoction

Table 3. Pharmacokinetic Parameters

Parameter	Group 1	Group 2	Group 3
T_{max} (Hrs)	4	4	4
C_{max} (ng/ml)	10.144	6.971	8.605
$t_{1/2}$ (Hrs)	12.434	12.424	12.111
k_{eli}	0.0557	0.05578	0.0572
$AUC_{0-\infty}$	248.588	190.417	216.123
AUC_{0-t}	247.8701	189.879	215.617

Group 1: Escitalopram oxalate
Group 2: Escitalopram oxalate with synthetic caffeine
Group 3: Escitalopram oxalate with green tea decoction

From the *in vitro* and *in vivo* study it was also evident that the presence of synthetic caffeine showed significative decrease in the bioavailability of escitalopram when compared with that of the group administered with green tea (containing 29.8 mg of caffeine).

CONCLUSION

Caffeine and caffeine containing products like green tea, are found to interfere with the pharmacokinetic profile of escitalopram oxalate. Synthetic caffeine decreased the

bioavailability of escitalopram oxalate tablets by 33.15% (*in vitro*) and 28% (*In vivo*). Green tea decoction containing 29.8 mg of caffeine decreased the dissolution of escitalopram tablet by 13.72% (*in vitro*) and 18 % (*in vivo*). More over the *in vitro* study on the release of escitalopram oxalate is one of the methods for predicting the absorption of a drug caused by the formation of complexes between the drug and caffeine.

ABBREVATIONS

AUC_{0-t} =   Area under the curve (0 to 72 hrs)

$AUC_{0-\infty}$ =   Area under the curve (0 to infinity time)

C_{max} =   Maximum plasma concentration

T_{max} =   Time in hours at maximum plasma concentration,

K_{eli} =   Elimination rate constant

$t_{1/2}$ =   Half life

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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