A study of pharmacokinetic interaction of Carbamazepine with Atorvastatin in dyslipidemic rats

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ABSTRACT

Introduction: Every time a drug is administered with any other prescription medicine, OTC products, herbs or even food we expose ourselves to the risk of a potentially dangerous interaction.A drug interaction occurs when the pharmacological effects of the object drug alters the intensity of the precipitant drug. Whenever two or more drugs are taken concurrently there is a chance of an interaction among the drugs that could manifest as an increase or decrease in their effectiveness or an adverse reaction or a totally new side effect that is not seen with either drug alone.

Materials & Methods: The study was done on male Sprague dawley rats, divided into 5 groups each having at least six animals.The groups were initially treated with carbamazepine at different doses for two weeks. Then all the groups were administered orally with a dose of 10 mg/kg of atorvastatin.Blood was collected from retro orbital plexus at 0, 1, 2, 4, 6,8,10 and 12 h after oral administration of atorvastatin and then subjected for analysis.

Results: The influence of carbamazepine on the pharmacokinetics of atorvastatin is decrease in Cmax, bioavailability and T1/2 indyslipidemic rats.

Conclusion: From the present investigation it is shown that the carbamazepine having considerable inducing effect on atorvastatin. The decreased bioavailability of atorvastatin in the study might be attributed to the induction of CYP-3A4 by carbamazepine. It might be true that, concurrent administration of the atorvastatin and carbamazepine could lead to alternations in the concentrations of either drug as both of them are substrates for CYP-3A4.

Keywords: Carbamazepine, Atorvastatin, Male Sprague dawley rats, dyslipidemia

INTRODUCTION

A drug interaction occurs when the pharmacological effects of the object drug alters the intensity of the precipitant drug. Whenever two or more drugs are taken concurrently there is a chance of an interaction among the drugs that could manifest as an increase or decrease in their effectiveness or an adverse reaction or a totally new side effect that is not seen with either drug alone. Some drugs that are most likely to precipitate interactions include those that are highly protein bound drugs that stimulate the metabolism of other drugs carbamazepine, rifampicin etc., or those that inhibit the metabolism of drugs which include ketoconazole, quinolones and MAO inhibitors and drugs that alter renal elimination like diuretics. In general certain groups of drugs anti epileptics, oral contraceptives, antibiotics, statins, antipsychotics, drugs enhancing G.I. motility, and drugs having a low therapeutic index, pose a daily challenge for practicing physicians[1].

The exact incidence of drug interactions in real life situations is largely unknown because a fair number do not get reported, do not result in any substantial harm to patients or may not end in hospitalization and even when it does it gets recorded as an adverse reaction rather than a drug interaction. Drug- drug interactions reflect the modulation of the pharmacological activity of the object drug by concomitantly administering the precipitant drug resulting in decrease or increase in the pharmacological properties of either drug.The mechanisms usually responsible for adverse effects associated with drug interactions are those in which one drug affects the pharmacokinetic profile of absorption, distribution, metabolic biotransformation, excretion or elimination of another; or Pharmacodynamic, such as interactions between agonists and antagonists at drug receptors, leading to vastly altered clinical response and implications.

The CYP450 complex is essential for metabolism of drugs and interactions mediated by it and what is significant is that out of 50 enzymes in this class, each encoded by a different gene, just 6 of them (CYP1A2, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5) together account for 90- 95% of bio transformations; with the 3A4 and 2D6 sub families being responsible for a large number of clinically important interactions. However, there is a considerable variability in

enzyme activity between patients due to medical, environmental, nutritional and genetic polymorphisms reasons with polymorphism being especially significant for CYP2D6, CYP2C9, and CYP2C19 and CYP3A4[2].

Atorvastatin, a lipid lowering drug primarily acting by inhibiting the enzyme HMG CoA reductase indicated as an adjunct to lifestyle changes, including diet, for the reduction of elevated total cholesterol (total-C), LDL-C, triglycerides (TG), apolipoprotein B (apo B), the Total-C/HDL-C ratio and for increasing HDL-C in hyperlipidemia and dyslipidemic conditions, including: Primary hypercholesterolemia (Type IIa); Combined (mixed) hyperlipidemia (Type IIb).Atorvastatin is metabolized by the Cytochrome P-450 isoenzyme, CYP 3A4. Concomitant use of Atorvastatin with any of the inhibitors or inducers of cytochrome P-450 enzymes can have an effect on its lipid lowering action.

Carbamazepine a synthetic compound of the benzodiazepine class, used as an anticonvulsant and analgesic drug. Carbamazepine is a potent inducer of CYP3A4 and other phase I and phase II enzyme systems in the liver[3]. Itis also known to exhibit auto induction, a phenomenon that can occur anytime[4].Due to induction of the hepatic mono-oxygenase enzyme system, carbamazepine may lower the plasma concentration and or diminish or even abolish the activity of certain drugs that are metabolised by this system[3].

MATERIALS AND METHODS

Male Sprague dawley rats weighing about 200 to 230 gm were procured and acclimatized. All the rats were maintained on standard diet throughout the study period. They were kept in special cages at room temperature of 25 ± 2^0 C , relative humidity of 75 ± 5% & 12hr light dark cycle and standard pellet diet and water ad libitum. The experimental protocol was approved by the institutional animal ethics committee and experiments were conducted in accordance with principles prescribed for laboratory animal use.Maximal induction by carbamazepine occurred after 10 days of treatment at a dose of 100 mg/kg intraperitoneally[4].

The rats were divided into 5 groups each having at least six animals and fed with high cholesterol diet for two weeks to induce hyperlipidemia. The atherogenic diet consisting of 2gm cholesterol and 8gm of saturated fat and 100mg calcium were added to the standard commercial pellet diet and thoroughly mixed[5].

The groups were initially treated with carbamazepine at different doses for two weeks. On the next day all the groups were administered orally with a dose of 10 mg/kg of atorvastatin. Before this the rats were kept fasting overnight, making water freely available. The blood samples were withdrawn at different time intervals [Table 1].

Table: 1 Treatment of different group's with Carbamazepine and Atorvastatin

Group	Carbamazepine (i.p)	Atorvastatin (Oral)
1	Control	10mg/kg
2	30 mg/kg	10 mg/kg
3	60 mg/kg	10 mg/kg
4	100 mg/kg	10 mg/kg
5	200 mg/kg	10 mg/kg

Blood samples collection: Blood (approximately 500 µl) was collected from retro orbital plexus at 0, 1, 2, 4, 6,8,10 and 12 h after oral administration of atorvastatin into heparinised containers and plasma was separated by centrifugation at 5,000 rpm for 10 min and stored at -20°C till analysis[6].

PHARMACODYNAMIC STUDY:

- Serum total cholesterol
- HDL cholesterol
- Serum total triglycerides
- LDL cholesterol&VLDL cholesterol

Estimation of Serum Cholesterol by CHOD/POD method:

After collection of blood specimen, it was allowed to clot, and then serum was separated by centrifugation at 3000rpm, for 10 minutes. Serum Cholesterol was estimated by making following dilutions [Table 2].

Table 2: Estimation of serum cholesterol.

Reagents	Blank	Standard	Sample
Cholesterol enzyme reagent	1000 µl(1ml)	1000 µl(1ml)	1000 µl(1ml)
Standard	--	10µl(0.01ml)	--
Sample	--	--	10µl(0.01ml)

Mix well and incubate at37^{0}C for 5 or 10 minutes at room temperature (25^0 – 30^{0}C).

Read the absorbance against reagent blank at 510 nm.

Calculations:

Cholesterol concentration in sample (mg/dl) =

(Absorbance of sample ÷ Absorbance of standard) x Concentration of standard

Serum HDL Cholesterol:

Principle : Phosphotungstate/Mg2+ precipitates chylomicrons, LDL and VLDL fractions of lipids present in serum. High density lipoprotein (HDL) fraction remains unaffected in supernatant.

Cholesterol content of HDL fraction is assayed using reagent kit [Table 3].

Serum/plasma → Phosphotungstate/Mg^{2+} →

↓

HDL Fraction+

↓

(Supernatant)

(LDL+VLDL+ Chylomicrons.)

↓

(Precipitate)

Table 3: Estimation of HDL cholesterol.

Components	Blank	Standard	Sample
Working reagent	1000µl(1ml)	1000µl(1ml)	1000µl(1ml)
Standard Cholesterol	--	50µl(0.05ml)	--
Supernatant	--	--	50µl(0.05ml)

Mixed well; incubated for 10 min. at 37°C, then read the OD at 510 nm against Blank.

Calculations:

HDL concentration (mg/dl) = (Absorbance of sample ÷ Absorbance of Standard) x 100.

*Factor of 100 (not 50) is used for calculation due to serum dilution during precipitation step.

Normal range: for men-30-60 mg/dl, for women33-70 mg/dl.

Friedwald's equation for LDL = Total Cholesterol-[HDL+ (TGL÷5)]

Serum triglycerides estimation:

After collection of blood specimen, it was allowed to clot, and then serum was separated by centrifugation at 3000rpm, for 10 minutes. Serum Triglycerides were estimated by making following dilutions in table [Table 4]. Mixed well; incubated at 37°C for 5 min. or at 20-25°C for 30 min and then read optical density (OD) against blank at 546nm or green filter.

Table 4: Estimation of Serum Triglycerides.

Components	Blank	Standard	Test
Working reagent	1000µl (1ml)	1000µl (1ml)	1000µl (1ml)
Standard	--	10µl	--
Serum Sample	--	--	10µl

Calculations:

Triglycerides concentration (mg/dl) = (OD of Test ÷OD of

PHARMACOKINETIC EVALUTION:

Measurement of plasma atorvastatin concentration:

Preparation of standard graph: Standard curve was prepared from working plasma standards in the range of 0.1- 20 µg/ml. 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 (Standard curve).

Preparation of test plasma samples: After taking the blood samples from the rat they were centrifuged and stored at -20^0 c until use. The proteins are removed by precipitation. To the plasma sample (200 µl), internal standard (rosuvastatin) (50 µl) and 1ml of the phosphate buffer (0.1 M, pH 7) were added, mixed well and subjected to liquid–liquid extraction using 5ml ethyl acetate as extracting solvent. After vortex mixing for 30s and centrifugation (10 min at 400 rpm), the organic phase was removed and evaporated to dryness. The residue was reconstituted in 0.2 ml of methanol, 20 µl of this was injected into the High-Performance Liquid Chromatography (HPLC) system (Shimadzu LC-20AD). Same extraction procedure was applied for the standard samples as well as those to be assayed for pharmacokinetic analysis[7].

The concentration of atorvastatin in the plasma samples was determined by using linear regression formula obtained from a standard curve.

$$Y = a+bx$$

Concentration (y) = a+b×peak area of the chromatogram

Where, a= Y-intercept of the regression line (standard curve)

b= Slope of the regression line (standard curve)

By substituting the respective peak area of the chromatogram(x value in the above equation), the concentration of atorvastatin in the test plasma sample could be calculated.

CALCULATION OF PHARMACOKINETIC PARAMETERS:

Pharmacokinetic parameters such as C_{max},T_{max}, AUC AUMC, clearance etc were calculated using "KINETICA" software. All the data were expressed as Mean ± Standard deviations and samples comparisons were performed using One Way ANOVA (Dunnett test).The whole statistical analysis was performed by using graph pad prism version 6.0.

RESULTS

PHARMACOKINETIC DATA:

Standard graph or Calibration curve of atorvastatin in rat serum: A calibration curve from different concentrations (0.5, 1, 2, 3, 5 & 10µg/ml) was plotted. The equation of the calibration curve obtained was Y= 0.339x + 0.832 [Figure 1].

Standard graph of atorvastatin:

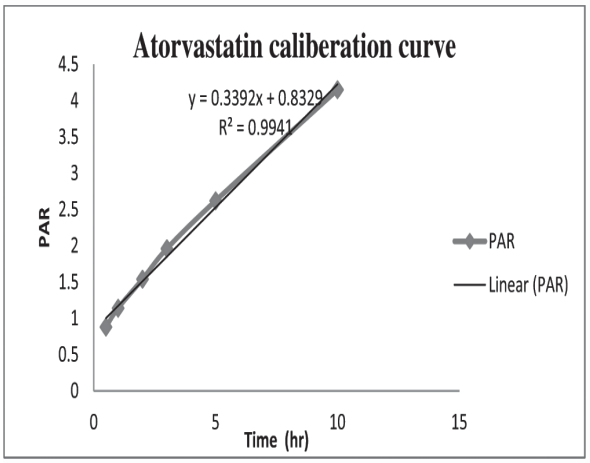


Figure 1: Calibration curve of atorvastatin in rat serum

HPLC chromatograms of blank serum [Figure 2].

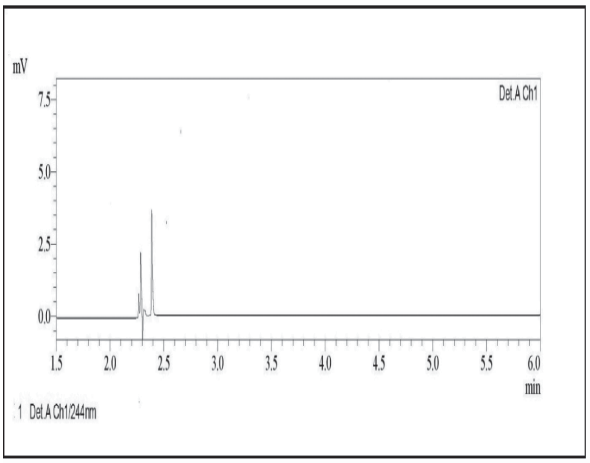


Figure 2: HPLC chromatograms of blank serum.

HPLC chromatograms of atorvastatin in rat serum samples

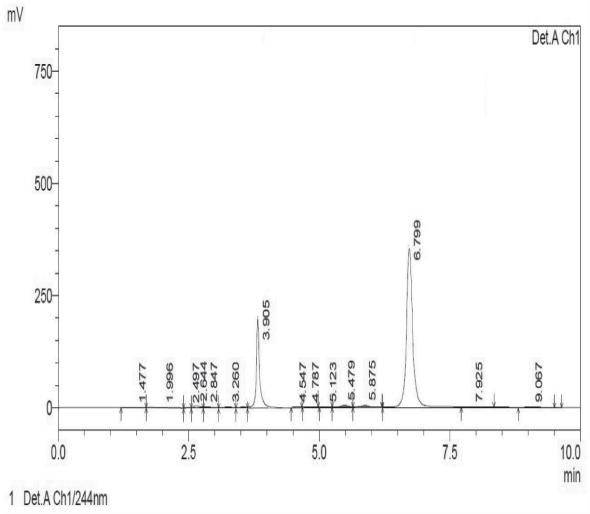


Figure 3: HPLC chromatograms of atorvastatin in rat serum samples

[Drug name: Atorvastatin (RT: 6.7), Internal standard: Rosuvastatin (RT: 3.9).]

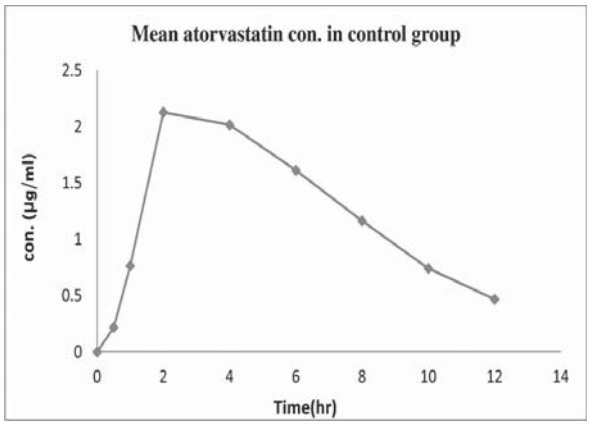


Figure 4: Mean serum concentrations of atorvastatin in rats control group

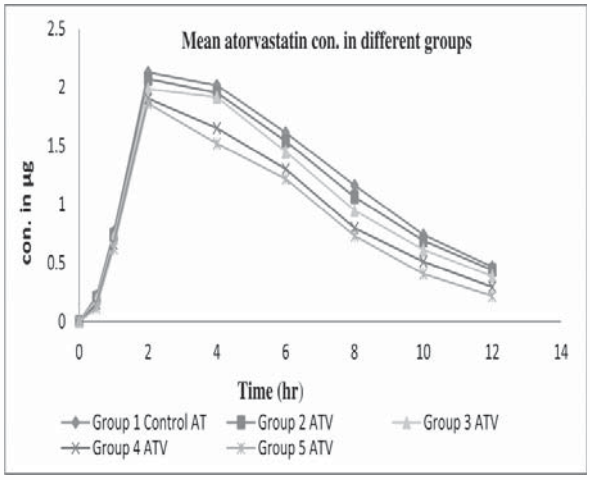


Figure 5: Mean Serum concentrations of atorvastatin different groups (SD).

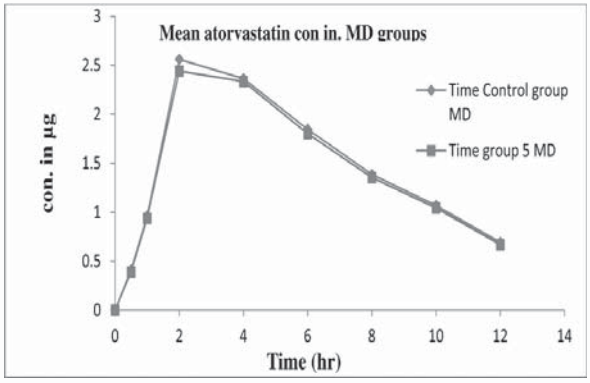


Figure 6: Mean serum concentrations of atorvastatin in MD treated Groups

Table 5: Mean pharmacokinetic parameters of atorvastatin in SD groups.

All values are expressed as Mean±SD, when compared to control value(s); * p< 0.05; **p<0.01; ***p<0.001; Statistical analysis was performed using One way ANOVA (Dunnett test).

P.K. Parameters	Group 1 At	Group 2 At	Group 3 At	Group 4 At	Group 5 At
C_{max}(µg/ml)	2.128±0.07	2.073±0.04	1.986±0.11*	1.903±0.12**	1.861±0.1***
T_{max}(hr)	2±0	2±0	2±0	2±0	2±0
AUC_{tot}(µg.hr/ml)	17.36±0.61	16.59±0.68	15.5±0.75***	13.34±0.68***	11.89±0.71***
$AUMC_{tot}$(µg.hr2/ml)	115.42±8.18	110.18±7.68	100.77±8.17***	81.16±7.03***	66.56±6.07***
T ½ (hr)	3.03±0.09	3.13±0.08	3.15±0.11	2.82±0.09**	2.28±0.10***
MRT (hr)	6.64±0.07	6.62±0.06	6.49±0.06***	6.06±0.05***	5.59±0.05***
CL(ml/hr)	576±30	602±35	644.81±41*	749.28±45***	840.58±53***
V_d (ml)	2524±86.	2725±91**	2931±96***	3053±110***	2775±84**
V_{ss} (ml)	3828±125	4002±132	4189±138***	4557±146***	4703±150***

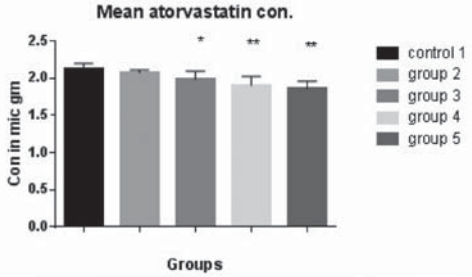
Table 6: Mean pharmacokinetic parameters of atorvastatin in MD groups.

P.K. Parameters	Control (MD)	Group 5 (MD)
C_{max}(µg/ml)	2.561±0.07	2.441±0.06**
T_{max}(hr)	2±0	2±0
AUC_{tot}(µg.hr/ml)	23.30±0.35	22.6±0.5*
$AUMC_{tot}$(µg.hr2/ml)	187.90±3.98	180.98±3.61*
$T_{½}$ (hr)	4.61±0.042	4.54±0.045*
MRT (hr)	8.06±0.03	8±0.03**
Cl(ml/hr)	429±8	442±8*
V_d(ml)	2856±32	2903±33*
V_{ss} (ml)	3459±43	3542±43**

All values are expressed as Mean±SD, when compared to control value(s); * p< 0.05;

**p<0.01 ***p<0.001; Statistical analysis was performed using unpaired Student t test.

Figure 7: Comparison of C_{max} of atorvastatin in different groups (SD) groups.



PHARMACODYNAMIC DATA:

Table 7: Serum Lipid Levels, in different groups on day 0.

Groups	TC	TG	HDL	LDL	VLDL
Group 1	179±5.20	157±4.69	36±3.08	114±4.13	31±2.92
Group 2	186±5.86	192±6.13	38±3.68	110±3.92	38±3.63
Group 3	157±4.67	168±4.26	41±2.97	83±3.61	33±2.93
Group 4	163±5.43	175±5.60	43±4.05	84±3.14	35±2.34
Group 5	168±4.92	181±5.89	39±3.42	93±4.06	36±3.07

TC=total cholesterol; TG=total triglycerides; HDL=high density lipoproteins

LDL=low density lipoproteins; VLDL=very low density lipoproteins

Table 8:Serum lipid levels in different groups on day 7.

Groups	TC	TG	HDL	LDL	VLDL
Group 1	164±5.69	141±3.86	42±3.12	95±3.8	28±1.94
Group 2	173±6.84	178±5.67	44±2.76	94±4.35	35±2.64
Group 3	146±5.31	157±4.81	46±3.28	71±2.91	31±3.12
Group 4	155±4.87	166±5.34	47±2.51	75±3.75	33±3.07
Group 5	161±5.27	174±5.61	42±2.94	83±4.12	35±2.86

TC=total cholesterol; TG=total triglycerides; HDL=high density lipoproteins

LDL=low density lipoproteins; VLDL=very low density lipoproteins.

Table 9: Percentage reduction (-) or rise (+) in serum lipid levels on day 7.

Groups	TC	TG	HDL	LDL	VLDL
Group 1	-9%	-11%	+16%	-17%	-10%
Group 2	-7%	-8%	+15%	-15%	-8%
Group 3	-7%	-7%	+12%	-15%	-7%
Group 4	-5%	-6%	+9%	-11%	-5%
Group 5	-4%	-4%	+7%	-10%	-3%

DISCUSSION

Atorvastatin is a statin class of drug used in hyperlipidemia. The bioavailability is only of 14%. The mechanism of action of the drug is mainly confined to liver where it inhibits the Cholesterol synthesis & is metabolized by CYP 3A4 enzymes.

The carbamazepine is having antiepileptic properties, also used in trigeminal neuralgia. The drug was having potent inducing effects on the CYP P450 isoenzymes particularly CYP 3A4.

When atorvastatin is used with the CYP3A4 inducers such as carbamazepine the metabolism of the drug is increased resulting in decreased concentrations.

In the previous studies the effects of CYP 3A4 inducers such as rifampicin were studied in which considerable decrease in the atorvastatin concentrations were noted (up to 80%)[9]. Similar interactions were also observed in the co-administration of the St.-John's-wort and atorvastatin in which increased cholesterol levels has been observed[9].

When, the concentrations of atorvastatin administered with phenytoin, compared with atorvastatin alone administered, reductions in AUC and Cmax were observed by 54% and 24% respectively[10].

In the present investigation we tested the influence of carbamazepine on the Pharmacokinetics and Pharmaco dynamics of atorvastatin and found that, T_{max} was unaltered but the C_{max}, bio availability and $T_{1/2}$ were decreased by 13.55%, 32%, and 24.75% respectively. Clearance and $V_d$were also altered significantly.The decreased serum concentrations of atorvastatin in the present study might be attributed to the CYP3A4 induction by the carbamazepine.

CONCLUSION

From the present investigation it is shown that the carbamazepine having considerable inducing effect on atorvastatin. The decreased bioavailability of atorvastatin in the study might be attributed to the induction of CYP-3A4 by carbamazepine. It might be true that, concurrent administration of the atorvastatin and carbamazepine could lead to alternations in the concentrations of either drug as both of them are substrates for CYP-3A4.

Further long term studies over a large number of animals and patients with different doses of drugs are required to correlate the results of the present study and to outweigh the risk-benefit ratio.

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Please cite this article as: Suresh D, Devde R, Reddy N Y, Rao V D, Khan I.A. Study of pharmacokinetic interaction of Carbamazepine with Atorvastatin in dyslipidemic rats. Perspectives in medical research 2016;4 (3):32-38.

Sources of Support: Nil,Conflict of interest:None declared.