National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

A study on the effect of atorvastatin on the pharmacokinetic and antidepressant activity of fluoxetine

Bimbishar Bhattarai[1], Shivakumar Swamy[2], Sanjay Raj Baral[3], Chandra Prakash Acharya[4]

[1]Save the Children, Nepal Country Office, Kathmandu, Nepal, [2]Department of Pharmacology, Mallige College of Pharmacy, Silvepura, Bengaluru, Karnataka, India, [3]Department of Pharmacology, Chitwan Medical College, Chitwan, Nepal, [4]Department of Quality Assurance, Time Pharmaceuticals Pvt., Ltd., Nawalparasi, Nepal

Correspondence to: Bimbishar Bhattarai, E-mail: bhattaraibimbishar@gmail.com

Received: June 26, 2017; **Accepted:** July 19, 2017

ABSTRACT

Background: Polypharmacy is of wide concern in drug interaction. It is the leading cause of adverse drug reaction, and consequently, increasing the possibility of hospitalization and escalating the cost of care. The incidence of coexisting depression and hyperlipidemia in patients is generally managed by providing numerous drugs for a longer period of time. **Aims and Objectives:** This study was conducted to observe the effect of atorvastatin on pharmacokinetic parameters of fluoxetine in healthy albino rabbits and to find possible interactions of atorvastatin on the antidepressant activity of fluoxetine using animal screening test. **Materials and Methods:** Two drugs: Fluoxetine and atorvastatin used concurrently were selected. Healthy male albino rabbits were used to determine the effect of atorvastatin on fluoxetine addressing its pharmacokinetic parameters, whereas rats and mice were used to assess the antidepressant activity of fluoxetine. Two animal models were used to determine its antidepressant activity. Forced swim test (FST) and tail suspension test (TST) were employed in exploring antidepressant activity. High-performance liquid chromatography was used to estimate fluoxetine concentration. **Results:** The concentration of serum fluoxetine showed slight increment after the atorvastatin treatment for 7 days at 2nd, 4th, 8th, 16th, and 24th h. The pharmacokinetic parameters: Area under the curve, area under first-order moment curve, $t_{1/2}$, and C_{max} of fluoxetine varied after atorvastatin treatment for 1 week in healthy albino rabbits. ‘’Furthermore, atorvastatin treatment for a week revealed a reduction in immobility time in rats and mice as shown by the FST and TST respectively. **Conclusion:** The results revealed the possibility of drug-drug interaction between fluoxetine and atorvastatin.

KEY WORDS: Depression; Fluoxetine; Atorvastatin; Drug-drug Interaction; Forced Swim Test; Tail Suspension Test

INTRODUCTION

Drug interactions are prevalent and generally result from intersecting pathways of drug action or shared pathways of metabolism. Some interactions can be determined from the analysis of these pathways, whereas others require pragmatic approaches and become evident usually after the drugs are on the market. Interactions can lead to precautions in prescribing, absolute contraindications for combination use, or even withdrawal of drugs. Understanding interactions between commonly prescribed drugs are a matter of paramount clinical significance.[1]

Depression is a global public health issue, not only due to its lifetime prevalence but also its link with substantial disability. It is rated as the fourth major cause of disease burden in 2000 and accounts for 4.4% of total disability-adjusted life years.[2] It is a neuropsychiatric illness from the eyes of conventional

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2017.7.0622519072017	

public and media.[3] Various terms are employed to describe the state of the disease such as "disorder", "episodes," "remission," "recovery," "relapse," and "recurrence."[4] This kind of disorder not only makes one prone to visible and expected charges but also causes unseen expenditure such as rising cost of health care and time one spends away from work. Broadly, over forty billion dollars are estimated annually as the cost of these disorders, considering the cost of treatment, absenteeism in work, and other costs. Suicide is the ultimate cost.[5]

The decreased level of high-density lipoprotein and elevated level of low-density, serum total cholesterol, and very low-density lipoprotein are characteristic of hyperlipidemia.[6] These kinds of lipid disorders cause atherosclerotic cardiovascular disease. Between this hypertriglyceridemia and hypercholesterolemia are narrowly linked with ischemic heart disease.[7] According to the American Heart Association, hyperlipidemia "is a high level of fats in the blood. These fats called lipids include cholesterol and triglycerides. There are various types of hyperlipidemia depending on which lipid levels are high in the blood."[8]

Depression and hyperlipidemia are common conditions that often coexist and may clinically interact with each other. Depression has a negative impact on medication adherence.[9] In addition, depressed hyperlipidemic diabetes patients tend to refill their statin prescriptions less often than those without depression.[10] Both the disorders are dealt clinically providing drugs for a long time. In such a scenario, the effect of one drug could be altered by another drug. Moreover, research has shown the neuroprotective effect by atorvastatin in traumatic brain injury,[11] and other data also show that there is a decrease in the incidence of anxiety and depression with statins therapy,[12] though the mechanism of action is not established.

MATERIALS AND METHODS

Animals

Healthy adult male albino rabbits, healthy albino rats, and healthy albino mice weighing 2.0-2.5 kg, 160-180 g, and 20-25 mg, respectively, were selected. All the animals were housed in their individual case for 7 days before testing and acclimatized to standard laboratory conditions of temperature (25 ± 2°C). A standard condition of a normal light cycle (12 h light/dark) was maintained for keeping animals. Animals were provided with free access to water and food. Rabbits were housed in stainless steel cages, whereas plastic cages were used for rats and mice. The study protocol was approved by the Institutional Animal Ethical Committee, Reg. No.1432/PO/a/11/CPCSEA, and was conducted in Mallige College of Pharmacy, Bengaluru.

Drugs and Chemicals Used

Fluoxetine and atorvastatin were provided as a gift samples by Time Pharmaceuticals Pvt., Ltd., Nepal. Normal saline was used from Claris Life Sciences Ltd. and tween 80 from Merck India Ltd.

Preparation of Fluoxetine and Atorvastatin Standard Solution

Fluoxetine pure sample was dissolved in saline (0.9% w/v). The final volume was made up in a volumetric flask using saline. Atorvastatin pure sample was dissolved in saline (0.9% w/v) after triturating with 10% tween-80. The final volume was made up in a volumetric flask using saline.

Procedure

Experiment 1: To observe pharmacokinetic parameters of fluoxetine after atorvastatin treatment in healthy albino rabbits

Four male albino rabbits weighing around 2-2.5 kg were taken and marked suitably. All the rabbits received fluoxetine (5 mg/kg) solution orally and time of administration was noted. After that, blood samples were collected at 0.5, 2, 4, 8, 16 and 24 h in blood collection tube and kept aside for 30-40 min. Serum samples were obtained after centrifugation (Laboratory Centrifuge-Remi R8C) at 3000 rpm for 15-20 min. The transparent supernatant liquid (serum) obtained was transferred into a clean dry Eppendorf tube. Serum samples were stored at 2-8°C for analysis.

After blood collection, animals were left for a washout period of 15 days with a normal diet. The next part of this experiment was conducted on the same group of animals. All the rabbits received atorvastatin (2.5 mg/kg) orally once a day for 1 week. On the 8th day, atorvastatin (2.5 mg/kg) was administered orally to all the animals; time of administration was noted. After 60 min of atorvastatin administration, fluoxetine (5 mg/kg) was administered orally. Blood samples were collected in a blood collection tube at prefixed time intervals that are 0.5, 2, 4, 8, 16, and 24 h after fluoxetine dosing, serum was separated from blood and stored at (2-8)°C for analysis. High-performance liquid chromatography (Agilent 1200 series) was used to estimate serum concentration of fluoxetine. The serum concentration of fluoxetine before and after treatment atorvastatin was applied to software Ramkin to calculate pharmacokinetic parameters.

Experiment 2: Effect of atorvastatin treatment on antidepressant activity of fluoxetine in healthy albino rats using forced swim test (FST)

Six male albino rats weighing 160-180 g were taken and marked suitably. A glass tank of 45 cm height and 17 cm width with water to a height of 15 cm was used to place rats individually,

and 25°C temperature was maintained. Twenty-four h before the experiment, pretest sessions of 15 min in swimming tank were provided to animals. After that, animals were removed from swimming tank. Then, they were dried and returned to their respective cages. Any animal showing wound or nasal bleeding or sinking during the pretest session was discarded.

In this experiment, animals were administered with fluoxetine (10 mg/kg, p.o.). Actual time of the drug administration was noted for all the animals. The animals were forced to swim and immobility duration was measured for the duration of 5 min at 0, 2, 4, 8, 16, and 24 h after drug administration. Individual animals were considered to be immobile only if it remained floating motionless in water to make desired movement which helps to maintain its head above water and stopped struggling.[13] All the rats were left for a washout period of 15 days.

After that, same group of animals with a gap of 15 days were administered with atorvastatin (10 mg/kg, p.o.) once a day for 1 week. Atorvastatin (10 mg/kg, p.o.) was administered to all the animals on the 8th day, and the time of administration was noted. After 60 min of atorvastatin administration, fluoxetine (10 mg/kg, p.o.) was administered, the test was repeated, and the total duration of immobility for 5 min was measured at 0, 2, 4, 8, 16, and 24 h after fluoxetine administration.

Experiment 3: Effect of atorvastatin treatment on antidepressant activity of fluoxetine in healthy albino mice using tail suspension test (TST)

Six male albino mice weighing 20-25 g were taken and marked suitably. Test mice were hanged on the edge of a shelf, 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The total time of immobility was noted for 5 min of duration. When the mice were hung passively with total immobility, then it was considered as immobile.[14]

In this experiment, animals were administered with fluoxetine (15 mg/kg, p.o.). The time of drug administration was noted for all the animals. Animals were subjected to the TST. The duration of immobility was calculated for 5 min at 0, 2, 4, 8, 16, and 24 h after drug administration.

After that, the same group of animals with a gap of 15 days were administered with atorvastatin (10 mg/kg, p.o.) for 1 week, once a day. On the 8th day, atorvastatin (10 mg/kg) was administered, and after 1 h, fluoxetine (15 mg/kg, p.o.) was administered, the test was repeated, and total time of immobility for 5 min was measured.

Statistical Evaluation

For each treatment group, data are expressed as mean ± standard error of the mean. Students *t*-test using parametric statistics and Graph Pad Prism version 6.02 were used to evaluate data from each response measures. A value of $P < 0.05$ was considered as statistically significant, in all tests.

RESULTS

As seen from Figure 1 and Table 1 that treatment with fluoxetine alone and the combination of fluoxetine with atorvastatin showed changes in the pharmacokinetic data in healthy albino rabbits. The effect of fluoxetine alone showed peak concentration at 4th h, i.e., 0.204 µg/ml, then the concentration decreased till 24th h. After that, atorvastatin treatment for 7 days, and on the 8th day, its combination with fluoxetine significantly increased the serum concentration of fluoxetine. Similarly, pharmacokinetic parameters such as C_{max}, T_{max}, area under curve (AUC_{0-t}), and Area under first-order moment curve ($AUMC_{0-t}$) have been increased, but $t_{1/2}$ did not change after the combination of both drugs rather than fluoxetine alone.

The result shown in Figure 2 indicates that fluoxetine exhibited immobility time of 55 s at the initial state and least

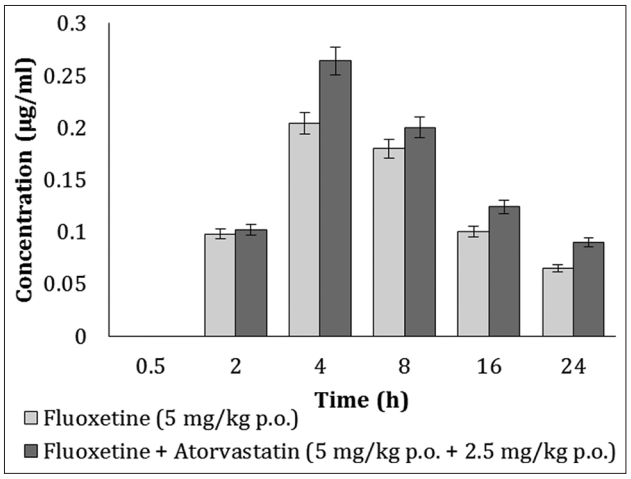

Figure 1: Graphical representation of serum fluoxetine concentration before and after atorvastatin treatment in healthy albino rabbits. $n = 4$ animals. Values are expressed as mean ± standard error of the mean

Table 1: Data showing the pharmacokinetic parameters of fluoxetine before and after atorvastatin treatment in healthy albino rabbits

Pharmacokinetic parameter	Drug treatment	
	Fluoxetine	Fluoxetine+atorvastatin
AUC_{0-t} (µg/ml/h)	2.948	3.548
$AUMC_{0-t}$ (µg/ml/h)	40.29	52.69
$t_{1/2}$ (h)	10.88	13.88
C_{max} (µg/ml)	0.204	0.264
T_{max} (h)	4	4
MRT (h)	20.366	24.68

AUC: Area under curve, AUMC: Area under first-order moment curve, $t_{1/2}$: Terminal half-life, C_{max}: Concentration maximum, T_{max}: Time of maximum concentration, MRT: Mean residential time

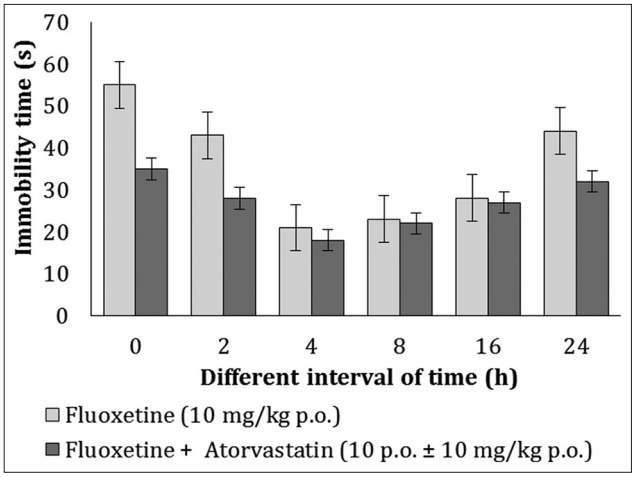

Figure 2: Graphical representation of atorvastatin treatment on immobility time of fluoxetine in rat by forced swim test. $n = 6$ animals. Values are expressed as mean ± standard error of the mean

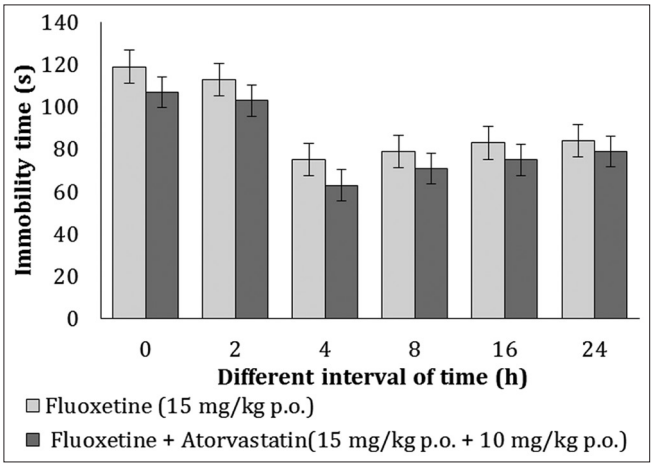

Figure 3: Graphical representation of atorvastatin treatment on immobility time of fluoxetine in mice by tail suspension test. $n = 6$ animals. Values were stated as mean ± standard error of the mean

of 21 s at 4th h. Atorvastatin treatment for 1 week decreased the immobility time in healthy albino rats significantly at 0, 2nd, 4th, 8th, 16th, and the 24th h.

The result shown in Figure 3 indicates that mice treated with fluoxetine showed greater immobility time at 0 h, i.e., 119.16 s. The lowest immobility time was observed at 4th h, i.e., 75 s, and immobility time was increased till 24th h. Atorvastatin treatment for 1 week showed the difference in immobility time. The immobility time at 0, 2nd, 4th, 8th, 16th, and 24th h was recorded as 107, 103, 63, 71, 75, and 79 s, respectively.

DISCUSSION

With the growing literature about drug interaction, our study explores if there may be any pharmacokinetic or pharmacodynamics drug interaction of fluoxetine and atorvastatin on pharmacokinetic parameter and animal screening model. As these, pharmacokinetic parameter test in rabbits and animal screening models in rats and mice served as quick review for observing possibility of drug interaction. Interestingly, results revealed that there was interaction between atorvastatin and fluoxetine. If we see the research conducted on human about knowing the status of depression and its relation with hyperlipidemia, then about 10-15% of the population is affected by depression at some time in their lives.[15] Depression and hyperlipidemia are common conditions that often coexist and may clinically interact if drugs used to treat these conditions are given with each other. Depression has a negative impact on medication adherence.[9]

Any alteration in drug metabolism often causes pharmacokinetic interactions. A wide range of drugs is oxidized by cytochrome P450 (CYP) by different metabolic processes. Both the systemic and pre-systemic drug dispositions are affected because of the location of CYP3A4 in the liver and small bowel. Rhabdomyolysis occurs when hydroxy-methyl-glutaryl-CoA reductase inhibitors (statins), i.e., 3-hydroxy-3-methylglutaryl-coenzyme is coadministered with CYP3A4 inhibitors.[16]

In this study, we observed the influence of atorvastatin on the antidepressant activity of fluoxetine in healthy rats and mice and the pharmacokinetic parameters in healthy rabbits. The healthy animal screening test served quickly to identify the interactions. It was found that both the drugs are metabolized by common enzymes CYP3A4. Hence, atorvastatin may also alter the effects of fluoxetine by altering the pharmacokinetic parameters similar to other drugs metabolized by the common way.[16,17]

Our experiment results revealed that atorvastatin increased the concentration of fluoxetine at absorption site; hence, the AUC and AUMC were also increased. This confirms the drug interaction at absorption site and may be due to the displacement of protein-bound fluoxetine by atorvastatin, as it is largely protein-bound, >98%.[18] We have also observed that mean residential time (MRT) and $t_{1/2}$ of fluoxetine are increased by atorvastatin, the literature survey revealed that both the drugs are metabolized by the same enzyme CYP3A4.[19] The possible reason for the increase in MRT may be due to the reduction of metabolism rate of fluoxetine by the enzyme CYP3A4, as the same enzyme is associated with the metabolism of atorvastatin.

Both TST and FST are accepted, sensitive and selective screening test to evaluate depression. When rats are exposed to stress, they initially struggle, however, after this struggle period, they become immobile. This sort of immobility signalizes behavior despair, which resembles the state of mental depression.[20] The exposure of animals to such stress causes depletion of biogenic amines such as norepinephrine and serotonin. This is one of the reasons for the prevalence of depression.

In FST, immobility time was reduced due to combination of fluoxetine and atorvastatin, confirming its antidepressant activity in an experimental condition. Similar study conducted by Sonawane et al.[21] proposed that, combination of atorvastatin and fluoxetine also showed decrease in the immobility time in rats rather than single drug, confirming its antidepressant activity. This could be due to selective serotonin reuptake inhibitors (SSRIs) like drugs stimulating the serotonergic system and also stimulate active swimming in the water tank. The climbing behavior could be preferentially accelerated by drugs primarily blocking noradrenaline uptake. The swimming behavior was increased by the combination of fluoxetine with statins. It is disputable to guarantee the effect of statins along with fluoxetine on animal models of depression. However, one of the findings, combination of lovastatin with low dose of fluoxetine augments antidepressant-like effect. As indicated by decreased immobility and accelerated swimming among rats, lovastatin increases the antidepressant efficacy of fluoxetine in laboratory animals,[22] and the action of lovastatin may involve the serotonergic instead of noradrenergic pathways, confirming augmentation of serotonergic function by statins. The reduction in immobility time by atorvastatin in our experiment may be due to increased activity in blocking the noradrenaline uptake. On the other hand, study conducted by Santos et al.[23] found that fluoxetine potentiated effect of simvastatin (statin).

In TST, the immobility time was decreased with combination therapy rather than fluoxetine alone. Likewise, a study conducted by Ludka et al.[13] showed that atorvastatin mediates antidepressant-like effect with fluoxetine. This decrease in immobility or presence of antidepressant-like effect was reported due to the N-methyl-D-aspartate (NMDA) receptor activation and/or nitric oxide-cyclic GMP (cGMP) synthesis.[13] Brain-derived neurotrophic factor (BDNF) is one of the neurotrophic factors which has numerous properties on inducing and sustaining elements of brain plasticity.[24] It was found that serum BDNF is higher in healthy participants than those of drug-free depressive patients, and after treatment of chronic antidepressant, there is an increase in serum BDNF levels in depressed patients.[25] According to the literature, pro-BDNF cleavage to BDNF was enhanced by statins which result in an antidepressant-like effect.[26] Moreover, atorvastatin increased the mice hippocampal BDNF protein level.[13]

It is of utmost importance to comprehend underlying principles behind the interaction of drugs. This assists in discovering drugs possibly having metabolic interactions. It is essential to consider any alteration in clinical effect as well as which was noticed in our research. Although our experiment revealed interaction between two drugs, results obtained from our experiments were conducted on a small number of animals. Thus, it should be confirmed by conducting experiments on a large number of animals and further by clinical trials before considering therapeutic use. Moreover, when evaluating parameters and results, receptor level was not included which could provide precise results.

CONCLUSION

Our experimental results conclude the interaction of fluoxetine and atorvastatin. The atorvastatin may enhance the effect of fluoxetine due to protein displacement or due to the diminished metabolism of fluoxetine or NMDA receptor activation and/or Nitric oxide-cGMP synthesis or due to the increase in BDNF protein level.

ACKNOWLEDGMENT

The authors would like to thank all the faculty members of Mallige College of Pharmacy for their continuous support and encouragement. The authors would like to thank all the Post Graduate students in our department for creating a positive environment to conduct this research.

REFERENCES

1. Tatonetti NP, Denny JC, Murphy SN, Fernald GH, Krishnan G, Castro V, et al. Detecting drug interactions from adverse-event reports: Interaction between paroxetine and pravastatin increases blood glucose levels. Clin Pharmacol Ther. 2011;90(1):133-42.
2. Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: Results from the World Health Surveys. Lancet. 2007;370(9590):851-8.
3. Wingert P, Kantrowitz B. Young and depressed. Newsweek. 2002;7:53-61.
4. Frank E, Prien RF, Jarrett RB, Keller MB, Kupfer DJ, Lavori PW, et al. Conceptualization and rationale for consensus definitions of terms in major depressive disorder. Remission, recovery, relapse, and recurrence. Arch Gen Psychiatry. 1991;48(9):851-5.
5. Antonouccio DO, Thomas M, Danton WG. A cost-effectiveness analysis of cognitive behavior therapy and fluoxetine (prozac) in the treatment of depression. Behav Ther. 1997;28:187-210.
6. Ghule BV, Ghante MH, Saoji AN, Yeole PG. Antihyperlipidemic effect of the methanolic extract from *Lagenaria siceraria* Stand. Fruit in hyperlipidemic rats. J Ethnopharmacol. 2005;124(2):333-7.
7. Jeyabalan S, Palayan M. Antihyperlipidemic activity of *Sapindus emarginarus* in triton WR-1339 induced albino rats. Res J Pharm Technol. 2009;2(2):319-23.
8. Jain KS, Kathiravan MK, Somani RS, Shishoo CJ. The biology and chemistry of hyperlipidemia. Bioorg Med Chem. 2007;15(14):4674-99.
9. Sokol MC, McGuigan KA, Verbrugge RR, Epstein RS. Impact of medication adherence on hospitalization risk and healthcare cost. Med Care. 2005;43(6):521-30.
10. Lin EH, Katon W, Von Korff M, Rutter C, Simon GE, Oliver M, et al. Relationship of depression and diabetes self-care, medication adherence, and preventive care. Diabetes

Care. 2004;27(9):2154-60.
11. Lu D, Mahmood A, Qu C, Goussev A, Lu M, Chopp M. Atorvastatin reduction of intracranial hematoma volume in rats subjected to controlled cortical impact. J Neurosurg. 2004;101(5):822-5.
12. Young-Xu Y, Chan KA, Liao JK, Ravid S, Blatt CM. Long-term statin use and psychological well-being. J Am Coll Cardiol. 2003;42(4):690-7.
13. Ludka FK, Zomkowski AD, Cunha MP, Dal-Cim T, Zeni AL, Rodrigues AL, et al. Acute atorvastatin treatment exerts antidepressant-like effect in mice via the L-arginine-nitric oxide-cyclic guanosine monophosphate pathway and increases BDNF levels. Eur Neuropsychopharmacol. 2013;23(5):400-12.
14. Vogel HG, editor. Drug Discovery and Evaluation, Pharmacological Assays. 2nd ed. Heidelberg: Springer-Verlag; 2002.
15. Gilmans G, editor. The Pharmacology Basis of Therapeutics. 12th ed. New York: McGraw Hill; 2001.
16. Dresser GK, Spence JD, Bailey DG. Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition. Clin Pharmacokinet. 2000;38(1):41-57.
17. Ouellet D, Hsu A, Qian J, Lamm JE, Cavanaugh JH, Leonard JM, et al. Effect of fluoxetine on pharmacokinetics of ritonavir. Antimicrob Agents Chemother. 1998;42(12):3107-12.
18. Lennernäs H. Clinical pharmacokinetics of atorvastatin. Clin Pharmacokinet. 2003;42(13):1141-60.
19. Andrade C. Selective serotonin reuptake inhibitor drug interactions in patients receiving statins. J Clin Psychiatry. 2014;75(2):e95-9.
20. Prostal RD, Bertin A, Jalfre M. Behavioural despair in mice: A primary screening test for antidepressants. Arch Int Pharmacodyn Ther. 1997;229(2):327-36.
21. Sonawane DV, Petare AU, Tripathi RK. Evaluation of antidepressant activity of statins alone and in combination with fluoxetine in acute and chronic behavioural models of depression. Indian J Physiol Pharmacol. 2015;59(3):313-21.
22. Renshaw PF, Parsegian A, Yang CK, Novero A, Yoon SJ, Lyoo IK, et al. Lovastatin potentiates the antidepressant efficacy of fluoxetine in rats. Pharmacol Biochem Behav. 2009;92(1):88-92.
23. Santos T, Baungratz MM, Haskel SP, de Lima DD, da Cruz JN, Magro DD, et al. Behavioral interactions of simvastatin and fluoxetine in tests of anxiety and depression. Neuropsychiatr Dis Treat. 2012;8:413-22.
24. Jin Y, Fischer I, Tessler A, Houle JD. Transplants of fibroblasts genetically modified to express BDNF promote axonal regeneration from supraspinal neurons following chronic spinal cord injury. Exp Neurol. 2002;177(1):265-75.
25. Gonul AS, Akdeniz F, Taneli F, Donat O, Eker C, Vahip S. Effect of treatment on serum brain-derived neurotrophic factor levels in depressed patients. Eur Arch Psychiatry Clin Neurosci. 2005;255(6):381-6.
26. Tsai SJ. Statins may enhance the proteolytic cleavage of proBDNF: Implications for the treatment of depression. Med Hypotheses. 2007;68(6):1296-9.

How to cite this article: Bhattarai B, Swamy S, Baral SR, Acharya CP. A study on the effect of atorvastatin on the pharmacokinetic and antidepressant activity of fluoxetine. Natl J Physiol Pharm Pharmacol 2017;7(11):1224-1229.

Source of Support: Nil, **Conflict of Interest:** None declared.