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Research Article

Association between HMG CoA reductase inhibitors and anxiety: an experimental study using elevated plus maze and light dark arena behavioural models

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ABSTRACT

Background: Aims: To investigate the effect of HMG CoA reductase inhibitors on behavioural models of anxiety in male swiss mice using Elevated plus maze test and light-dark arena test.
Methods: It was an experimental study, carried out in department of Pharmacology, J. N. Medical College, KLE University, Belgaum. The *in vivo* behavioural activity of simvastatin, lovastatin, atorvastatin was studied using EPM and light-dark arena test. The mice received the drugs as per their weight and subjected for experimentation. Group mean time spent and number of entries into open arm in EPM test and group mean number of entries and time spent in the light area was calculated in treated and control groups for comparison. Statistical analysis was done using ANOVA followed by Bonferroni's multiple comparison test ($P<0.05$).
Results: Going through the results of this study an evidence for the association between lowered serum cholesterol and symptoms of anxiety can be seen as statins used in the present study failed to show significant anxiolytic effect when compared to standard but in turn showed even more less activity when compared to control, indicating anxiogenic behavior.
Conclusions: Our findings support the evidence of the negative effects of statins on psychological outcomes. It's generally understood that having low cholesterol is a good health sign, combined with other factors; it could actually put a person at risk by causing anxiety and stress. Further research comprising a greater number of studies is required to confirm the effects of this agent on psychological outcomes.

Keywords: Simvastatin, Atorvastatin, Lovastatin, Anxiety, Cholesterol, Serotonin

INTRODUCTION

Anxiety is a normal phenomenon, It is said to be a universal human emotion. If becomes disproportionate and excessive to the situation, it interferes with performance and constitute a psychiatric disorder. May present either as a primary disorder or as a comorbid condition.[1]

HMG CoA (3-hydroxy-3-methyl glutaryl coenzymeA) reductase inhibitors commonly categorized as statins have long been used in patients with atherosclerotic disease and hyperlipidemia. Besides their established cholesterol-lowering property, statins exert a number of pleiotropic effects including anti-inflammatory actions, neuroprotective effects.[2]

Few studies have suggested that cholesterol lowering may have adverse effect on mood as cholesterol plays an important role in the development, function, and stability of synapses. Evidence shows that low serum cholesterol influences the brain biogenic amines especially 5-HT function and reduced number of serotonin receptors in brain, since cholesterol affect the membrane fluidity leading to altered 5HT metabolism which may play a fundamental role in anxiety related behaviours.[3]

In several human studies contradictory results on the association between statins and anxiety was acquired. The reasons for the conflicting outcomes may result from their different methodology. It is highly probable that inhibition on NMDA receptor upregulation in the hippocampus mediate the anxiolytic effects of statins.[4]

Some other studies state that anxiety is usually associated with depression (approximately 60%).[5]

Our previous studies on statins showed significant antidepressant activity on animals.[6]

However there is scarcity of information regarding direct effect of statins on anxiety paradigms in animal models. Therefore the present study was planned to explore the effect of clinically used lipophilic statins viz lovastatin, atorvastatin and simvastatin in their clinically equivalent doses using paradigms of anxiety in male swiss mice.

Objectives

- To investigate the effect of simvastatin, lovastatin, atorvastatin on behavior models of anxiety using elevated plus maze and light dark arena test.
- To compare their activity with that of standard anxiolytic drug alprazolam and control.

METHODS

Animals

Healthy adult male healthy mice weighing 20-30 g were obtained from the central animal house, J. N. Medical College, Belgaum and were acclimatized to 12:12 h light - dark cycle for 10 days prior to the day of experimentation. They were maintained on standard chow pellet (Amrut Brand) and water ad libitum. The study was approved by Institutional Animal Ethical Committee formed as per the guidelines of CPSCEA, New Delhi.

Drugs and doses (Table 1)

Considering the maximum therapeutic dose mice equivalent dose were calculated with the help of converting table devised by Paget and Barnes.[7]

Table 1: Doses of the drugs used in the study.

Treatment	Human dose (mg/day)	Mice dose (mg/kg)
Control	(1 % gum acacia)	0.5 ml
Simvastatin	80	10.4
Lovastatin	80	10.4
Atorvastatin	80	10.4
Alprazolam	0.5	0.065

Mice were divided into several groups of six each (n=6). Control group received 0.5ml of 1% gum acacia suspension, orally, while the other groups received calculated clinical equivalent doses of simvastatin, lovastatin, atorvastatin, alprazolam in 1% gum acacia suspension, orally. Alprazolam is taken as standard anxiolytic drug. The tests were conducted one hour after administration of drugs. Mouse activity was assessed with the help of following paradigms.

Elevated plus maze[8-12]

The elevated plus maze apparatus consists of two open arms (16 cm x 5 cm) and two closed arms (16 cm x 5 cm x 12 cm) and an open roof, with the entire maze elevated 25cms from the floor.

Principle: Exposure of mice to such novel stimuli can evoke both exploratory drive and fear. Elevation of the maze causes greater fear. Open arms are more fear provoking and animals tend to spend time in the closed arm. Anxiolytics would be expected to increase the entries and time spent in the open arms.

Procedure: The pretreated animals were placed individually for 5 minutes at the centre of the elevated plus maze with the head facing towards an open arm. The number of entries into open or closed arm and the time spent in each arm were recorded. The percentage of number of entries (against the total number of entries both in open and closed arms) and percentage time spent in open arm (against total time spent both in open and closed arms) were calculated for each group.

Light-dark arena[8,10]

It consists of a wooden test box (50 x 30 x 35 cm) placed on a table, 1 m above floor level. The box is open at the top. A partition with a gap of 7.5 x 7.5 cm at the centre of its lower border was fixed to separate $2/5^{th}$ of the base from the remaining $3/5^{th}$. The $2/5^{th}$ of the base was painted black and illuminated with dull red light. The other $3/5^{th}$ was painted white and brightly illuminated with a 100 W light source located 17 cm above the box.

Principle: Normally mice spend 60-70% of their time in the dark area. Anxiolytics increase the number of entries and time spent in the bright area.

Procedure: Pretreated mice were placed in the centre of the bright area. The number of entries into and time spent in light area were recorded over a period of 5 min. The mean number of entries, and percentage of time spent were calculated for each group.

Statistical analysis

The results presented here are the means ± SD of 6 mice in each group. The results were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test and Kruskal-Wallis test was used to assess the discrete values. P ≤0.05 was considered statistically significant. All data was analyzed using statistical software SPSS (version 17.0).

RESULTS

This was carried out by employing two models viz. elevated plus maze and light dark arena. Alprazolam was used as a standard anti-anxiety drug for the sake of comparison.

Elevated plus maze

In the elevated plus maze, number of entries and time spent in the open arm were observed over a period of 5 minutes.

The mean percentage of time spent in the open arm (% time spent in open arms of total 300 seconds) in the control group was 8.58 ± 2.92 while it was 21.72 ± 3.72, 13.33 ± 2.71, 9.27 ± 1.69 and 7.66 ± 0.75 in alprazolam, simvastatin, lovastatin, atorvastatin respectively (Table 2a, Figure 1). Statistically significant difference (P<0.05) was observed only in alprazolam group in comparison to control group. Simvastatin, lovastatin and atorvastatin groups in comparison to alprazolam group, indicating that anti-anxiety activity of these statins was less compared to alprazolam (Table 2b, 2c).

Table 2a: Effect of various treatments on the time spent in open arms. a) Mean of % time spent in open arms of the total 300 seconds.

Groups	Control	Alprazolam	Simvastatin	Lovastatin	Atorvastatin
Mean ± SD	8.587 ± 2.92	21.72 ± 3.72	13.33 ± 2.71	9.277 ± 1.69	7.667 ± 0.75

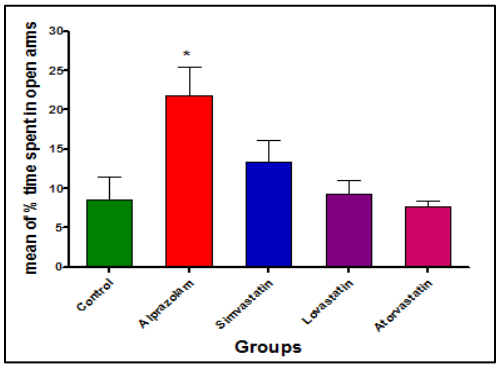

Figure 1: Effect of various treatments on % time spent in open arm of elevated plus maze.

Note: *P<0.05 (in comparison to control group)

Table 2b: Effect of various treatments on the time spent in open arms. b) One way ANOVA.

Source of variation	Sum of squares	df	Mean square	F	P value
Between groups	804.368	4	201.092	5.054	0.003998
Within groups	994.682	25	39.787		
Total	1799.050	29			

The P value indicates that there is non-homogeneity among the five groups compared

Table 2c: Effect of various treatments on the time spent in open arms. c) Bonferrani's multiple comparison test (*P<0.05).

Group	Group	Mean difference	Std. error	P value
1	**2**	-13.13500*	3.64176	0.013*
1	**3**	-4.74500	3.64176	1.000
1	**4**	-.69000	3.64176	1.000
1	**5**	0.92000	3.64176	1.000
2	**3**	8.39000	3.64176	0.049*
2	**4**	12.44500*	3.64176	0.022*
2	**5**	14.05500*	3.64176	0.007*

1 Control
2 Alprazolam
3 Simvastatin
4 Lovastatin
5 Atorvastatin

The effect of various drugs on the number of entries into the open arm as well as closed arm (total entries) were noted to calculate percentage of entry into open arm for each animal and then mean was calculated. The mean percentage of entry into the open arms in the control group was 24.93 ± 4.28 while it was 49.03 ± 6.89, 34.82 ± 4.22, 35.06 ± 2.99 and 37.41 ± 4.49 in the alprazolam, simvastatin, lovastatin, and atorvastatin groups respectively (Table 3, Figure 2).

Table 3a: Effect of various treatments on entries into the open arm. a) Mean of % of entries into open arm to total entries in both open and closed arm.

Groups	Control	Alprazolam	Simvastatin	Lovastatin	Atorvastatin
Mean ± SD	24.93 ± 4.28	49.03 ± 6.89	34.82 ± 4.22	35.06 ± 2.99	37.41 ± 4.49

Table 3b: Effect of various treatments on entries into the open arm. b) One way ANOVA.

Source of variation	Sum of squares	df	Mean square	F	P value
Between groups	1777.051	4	444.263	3.279	0.0272027
Within groups	3386.804	25	135.472		
Total	5163.854	29			

The P value indicates that there is non-homogeneity among the five groups compared

Table 3c: Effect of various treatments on entries into the open arm. c) Bonferrani's multiple comparison test (*P<0.05).

Group	Group	Mean difference	Std. error	P value
1	**2**	-24.09500*	6.71992	0 .014*
1	**3**	-9.88667	6.71992	1.000
1	**4**	-10.12167	6.71992	1.000
1	**5**	-12.47833	6.71992	0.751
2	**3**	14.20833	6.71992	0.446
2	**4**	13.97333	6.71992	0.480
2	**5**	11.61667	6.71992	0.962

1 Control 2 Alprazolam 3 Simvastatin
4 Lovastatin 5 Atorvastatin

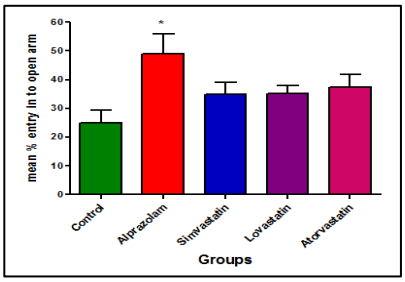

Figure 2: Effect of various treatments on number of entries into the open arm (% of entries into open arm to total entries in both open and closed arm).

Note: *P<0.05 (in comparison to control group)

Light dark arena

The time spent in light arena over a period of 5minutes was noted for each animal and the percentage time spent in the light arena to that of total time spent in light and dark arena was calculated and mean was tabulated. The mean percentage time spent in light arena in the alprazolam treated group is 15.61 ± 1.80 which was statistically significant (p<0.05) more than the control group which was 9.27 ± 2.07. The mean percentage time spent in the light arena in the simvastatin, lovastatin, atorvastatin are 6.38 ± 0.86, 5.38 ± 0.60 and 6.05 ± 0.48 respectively and showed significant change with alprazolam group in comparison to control group (Table 4, Figure 3).

Table 4a: Effect of various treatments on the time spent in light arena. a) Mean of % of time spent in light arena.

Groups	Control	Alprazolam	Simvastatin	Lovastatin	Atorvastatin
Mean ±SD	9.27 ± 2.07	15.61 ± 1.80	6.38 ± 0.86	5.38 ± 0.60	6.05 ± 0.48

Table 4b: Effect of various treatments on the time spent in light arena. b) One way ANOVA.

Source of variation	Sum of squares	df	Mean square	F	P value
Between groups	427.734	4	106.933	9.993	0.0000568
Within groups	267.524	25	10.701		
Total	695.258	29			

The P value indicates that there is non-homogeneity among the five groups compared

The mean number of entries into the light arena in the control group was 1.66 ± 0.33, while it was 2.66 ± 0.33, 1.50 ± 0.22, 1.33 ± 0.21 and 1.67 ± 0.16 in alprazolam, simvastatin, lovastatin, and atorvastatin treated groups respectively. The mean values showed significant decrease in the number of entries as compared to that of control in the test drug treated group of animals (Table 5, Figure 4).

Table 4c: Effect of various treatments on the time spent in light arena. c) Bonferrani's multiple comparison test (*P<0.05).

Group	Group	Mean difference	Std. error	P value
1	2	-6.33500*	1.88865	0.025*
1	3	2.88833	1.88865	1.000
1	4	3.88833	1.88865	0.501
1	5	3.22167	1.88865	1.000
2	3	9.22333*	1.88865	0.0005*
2	4	10.22333*	1.88865	0.0001*
2	5	9.55667*	1.88865	0.0003*

1 Control
2 Alprazolam
3 Simvastatin
4 Lovastatin
5 Atorvastatin

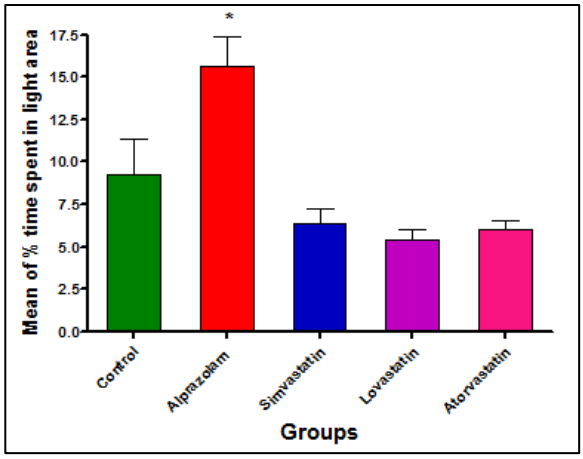

Figure 3: Effect of various treatments on % time spent in light arena.

Note: *P<0.05 (in comparison to control group)

Table 5a: Effect of various treatments on number of entries into the light arena. a) Mean of number of entries into light arena.

Groups	Control	Alprazolam	Simvastatin	Lovastatin	Atorvastatin
Mean ±SD	1.667 ± 0.33	2.667 ± 0.33*	1.500 ± 0.22	1.333 ± 0.21	1.167 ± 0.167

Table 5b: Effect of various treatments on number of entries into the light arena. b) Post-HOC analysis by Kruskal-Wallis test.

Group	Different from group	P value
1 (Control)	2	P<0.05
2 (Alprazolam)	1, 3, 4, 5	P<0.05

P = 0.0183

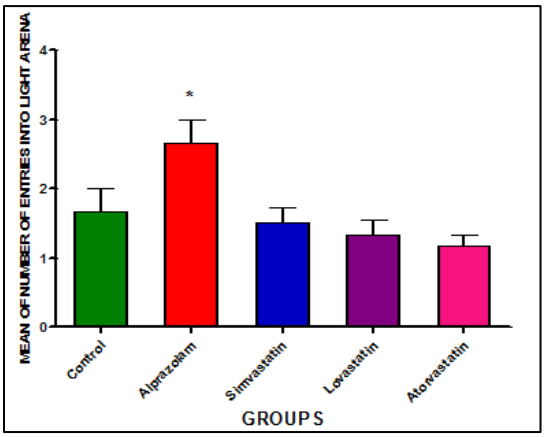

Figure 4: effect of various treatments on number of entries into the light arena.

Note: *P<0.05 (in comparison to control group)

DISCUSSION

In the present study, hydroxymethylglutaryl coenzyme A (HMG CoA) reductase inhibitors viz simvastatin, lovastatin and atorvastatin were investigated for their effects on anxiety using different behavioral models of anxiety in male swiss mice.

Elevated plus maze test and light dark arena test are based on the assumption that unfamiliar, nonprotective, and brightly lit environmental stress provokes inhibition of normal behavior. This normal behavioral inhibition is further augmented in the presence of fear or anxiety‐like state. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places.[13] All the test drugs showed decreased anxiolytic effect compared to standard drug and even less compared to control, which may suggest anxiogenic effects of statins.

Possible link between lower serum cholesterol and psychological well-being remains an issue of debate. How statins affect anxiety and the underlying mechanisms remain unclear. Elevated as well as low cholesterol level may be associated with serotonergic dysfunction. A primary decrease in cholesterol level may directly lead to decreased brain 5-HT activity through a variety of mechanisms, ranging from an alteration in 5HT levels, to 5HT receptor concentration or 5HT transporter activity. In contrast, elevated cholesterol levels may lead

to lower 5HT receptor sensitivity or 5HT transporter activity in depressed patients compared to normal controls, either directly by binding to the various membrane-bound 5HT receptors or transporter or indirectly by altering the fluidity of the neuronal membrane and thereby the conformation of these structures.[14,15]

Various neurotransmitter or neuromodulator systems, such as GABAergic, glutamatergic, serotonergic, noradrenergic, and corticotrophin releasing hormone systems, as well as intracellular signalling proteins, were shown to be involved in anxiety-like behaviors. Genetic mutation or pharmacological manipulations of one or combination of them could lead to abnormal levels of anxiety.[16] Recent study has also demonstrated that statins may stimulate mitochondrial biogenesis. Thus, there is every possibility that prolonged administration of statins (atorvastatin and simvastatin) may alter the mitochondrial density/biogenesis and this may contribute to the effects of statins on anxiety.[3]

However, in several human studies contradictory results on the association between statins and anxiety were acquired. The reasons for the conflicting outcomes may result from their different methodology. It is highly probable that inhibition on NMDA receptor upregulation in the hippocampus mediates the anxiolytic effects of simvastatin. In another study, it was also observed that a similar effect was obtained with inhibition of NMDA receptors in the basolateral complex of the amygdala.[4]

The present study was not planned to probe into the anxiolytic mechanism of statins. Using predetermined criteria, our aim was to conduct the study on animals to confirm the impact of statins on psychological wellbeing with and without hypercholesterolemia.

Further laboratory and clinical research studies are needed to clarify the association between serum cholesterol and anxiety.

CONCLUSION

The present study results may contribute to the hypothesis for the relation between serum cholesterol and symptoms of anxiety and also confirm that the lipophilic statins can lead to anxiety and stress if could be extrapolated to clinical situation as in cardiovascular disease with co-morbidity like generalized anxiety disorder and may put the person at risk. Further research is recommended in particular with the effects of statins on mood.

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Conflict of interest: None declared
Ethical approval: The study was approved by the institutional animal ethics committee formed as per the guidelines of CPSCEA, New Delhi

REFERENCES

1. Stahl SM. Essential psychopharmacology. In: Stahl SM, eds. A Book. 2nd ed. New Delhi: Foundation Books, Cambridge University Press; 2000.
2. Tandon V, Bano G, Khajuria V, Parihar A, Gupta S. Pleiotropic effects of statins. Indian J Pharmacol. 2005;37(12):75-85.
3. Anil Kumar, Aditi Vashist, Puneet Kumar, Harikesh Kalonia, Jitendriya Mishra. Protective effect of HMG CoA reductase inhibitors against running wheel activity induced fatigue, anxiety like behavior, oxidative stress and mitochondrial dysfunction in mice. Pharmacol Rep. 2012;64:1326-36.
4. Fatma S. Kilic, Yasemin Ozatik, Bilgin Kaygisiz, Canan Baydemir, Kevser Erol. Acute antidepressant and anxiolytic effects of simvastatin and its mechanisms in rats. Neurosciences. 2012;17(1):39-43.
5. Brunton LL, Laso JS, Parker KL, Goodman Gilman A. The pharmacological basis of therapeutics. In: Brunton LL, Laso JS, Parker KL, Goodman Gilman A, eds. A Book. 11th ed. New York: McGraw Hill; 2001.
6. Gudadappanavar Anupama M, Hiremath Shrishail V, Torgal Shashikant. Evaluation of antidepressant activity of simvastatin lovastatin and atorvastatin in male swiss mice - an experimental study. Int J Drug Dev Res. 2013 Apr-Jun;5(2):99-102.
7. Gosh MN. Fundamentals of experimental pharmacology. In: Gosh MN, eds. A Book. 4th ed. Kolkota: Hilton And Company; 2005.
8. Vogel GH, Vogel WH. Drug discovery and evaluation, pharmacological assays. In: Vogel GH, Vogel WH, eds. A Book. 2nd ed. New York: Springer-Verlag Berlin Heidelberg; 2002.
9. Kulkarni SK. Handbook of experimental pharmacology. In: Kulkarni SK, eds. A Book. 2nd ed. Delhi: Vallabh Prakashan; 1993. New York: Springer Verlag Berlin Heidelberg; 2002.
10. Veena N, Patil PA. Antidepressant activity of fosinopril, ramipril, and losartan, but not of lisinopril in depressive paradigms of albino rats and mice. Indian J Experiment Biol. 2008;46:180-4.
11. Schneider P, Ho YJ, Spanagel R, Pawlak CR. A novel elevated plus-maze procedure to avoid the one-trial tolerance problem. Front Behav Neurosci. 2011;5:43.
12. Andreatini R, Bacellar LFS. The relationship between anxiety and depression in animal models: a study using the forced swimming test and elevated plus-maze. Braz J Med Biol Res. 1999;32:1121-6.
13. Jaya Mary Thomas, Joyamma Varkey, Bibin Baby Augustine. Association between serum cholesterol, brain serotonin, and anxiety: a study in simvastatin administered experimental animals. Int J Nutr Pharmacol Neurol Diss. 2014 Jan-Mar;4(1):69-73.
14. George I. Papakostas, Dost O¨ngu¨r, Dan V. Iosifescu, David Mischoulon, Maurizio Fava.

Cholesterol in mood and anxiety disorders: review of the literature and new hypotheses. Eur Neuropsychopharmacol. 2004;14:135-42.

15. Sjogren B, Hambline MW, Svenningsson P. Cholesterol depletion reduces serotonin binding and signalling via human 5HT(7(a)) receptors, Eur J Pharmacol. 2006 Dec;552(1-3):1-10.
16. Long-Jun Wu, Susan S. Kim, Min Zhuo. Molecular targets of anxiety: from membrane to nucleus. Neurochem Res. 2008;33:1925-32.

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