Research Article

Protective Effect of Desvenlafaxine on Ethanol-Induced Anxiety and Depression in Mice

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Abstract

Anxiety and depression like behavior induced by ethanol in mice is a consequence of changes in the CNS that are secondary to impaired serotonin and norepinephrine neurotransmitters. Treatments with selective norepinephrine or serotonin reuptake inhibitors are reported to produce beneficial effects in this model. Desvenlafaxine is reported to exhibit selective norepinephrine inhibitor. However, no report is available on the influence of desvenlafaxine on ethanol-induced anxiety and depression. Therefore, we tested its influence against anxiety and depression in ethanol-induced mice using locomotor activity, elevated plus maze and forced swim test paradigm. Fifteen days after ethanol treated mice showed decreased locomotor activity and time spend in open arm and, increased immobility time as parameter of anxiety and depression. In contrast, treatment with desvenlafaxine (20 mg/kg - 80 mg/kg, p.o.) improved anxiety and depression like behavior, and increased locomotor activity and time spend in open arm and, decreased immobility time in ethanol treated rats. In conclusion, the present study demonstrates that treatment with desvenlafaxine prevents the anxiety and depression in ethanol treated mice.

Keywords: Ethanol induced anxiety and depression; Desvenlafaxine; Forced swim test

Introduction

Depression is a chronic, relapsing, and potentially fatal disorder that affects about 20% of the population. Depression has been expected to become the second most common disorder worldwide by 2020 [1]. Antidepressant drugs often have undesirable side effects, such as cholinergic symptoms, withdrawal issues, sexual dysfunction [2], and worsening insomnia [3]. Hence, developing effective depression therapies with few side effects is essential. Depression symptoms are associated with changes in Norepinephrine (NE), Dopamine (DA) and 5-Hydroxytriptamine (5-HT) neurotransmitter levels in the CNS [1,4]. The prefrontal cortex and hippocampus which regulate emotion, motivation, learning and memory are essential in depression [5]. Nutt et al. [4] studied that NE is related to alertness, energy, and attention, and that DA is linked to pleasure, reward, and motivation in life. The 5-HT transmitter is related to compulsion, obsession, and anxiety.

Alcoholism has high co-morbid appearance with anxiety and depressive disorders [6-8]. The co-occurrence of alcoholism and depression is well documented [9]. Furthermore depressed mood may also be an important trigger of alcoholic relapse [10,11]. In addition a significant co-morbid expression of alcoholism and affective disorders (major depression, dysthymia) has been shown by clinical evidences [12-14]. The treatment becomes more complex because these patients have more physical, psychological, familial and social problems than alcoholics without co-morbid depression [7,13]. The clinical and experimental data indicated that ethanol-induced depressive-like behavior was associated with alterations in Corticotropin Releasing Factor (CRF) and Neuropeptide Y (NPY) systems in the brain [15].

Desvenlafaxine is an SNRI that increases extracellular 5-HT and NE levels by inhibiting the reuptake of 5-HT and NE. Although desvenlafaxine is used clinically, it has an insufficient antidepressant effect, a high risk of side effects and relatively low barrier permeability [16,17]. The IC50 values of desvenlafaxine inhibition of 5-HT and NE reuptake *in vitro* are 53 nM and 538 nM, respectively [16]. Desvenlafaxine Succinate (DVS) is the third serotonin-norepinephrine reuptake inhibitor approved by the Food and Drug Administration for the treatment of major depressive disorders in 2008 [18]. It increases the level of serotonin (5-HT) and Norepinephrine (NE) by blocking the presynaptic reuptake of both of these neurotransmitters [19]. Furthermore, 5-HT and NE are the key neurotransmitters in regulating gut motility. 5-HT is an important factor related to visceral sensation [20]. Therefore, the present study was designed to investigate the protective effect of desvenlafaxine on ethanol-associated anxiety and depression like effects in ethanol-induced rats.

Materials and Methods

Adult male albino Swiss mice (21 g - 25 g) born and reared in the Animal House, Agnihotri College of Pharmacy, Wardha. The animals were breed from an original stock purchased from Shree Farm, Bhandara, India. Mice were group housed (four per cage) in opaque polypropylene cages (28 cm × 21 cm × 14 cm) and maintained at 23 ± 2°C under 12:12 h light/dark cycle (0700 h - 1900 h) with free access to rodent chow and tap water. The studies were carried out in strict accordance with the guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi, India. Animals were naive to drug treatment and experimentation at the beginning of all studies. Each experimental group was comprised of six to eight mice. Testing was carried out in

a counterbalanced order with respect to the treatment conditions in noise free room between 0900 h - 1400 h.

To acclimate the subjects to housing conditions, animals arrived one week prior to testing. During this period, they were gentled once daily in order to minimize any stress effects that might result from routine handling. The animals were weighted once a week for the duration of the study. All behavioral testing were carried out in the early portion of dark phase between 09:00 A.M and 12:00 P.M using a red light as source of illumination. Different groups of mice were used for the time period exposure and the drug studies. A total of 36 mice were used. Experiment consisted of 6 groups of mice (6 mice/group).

Drugs and solutions

Desvenlafaxine was purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA), ethanol (Changshu Yangyuan Chemical, China) were used in present study. All the drugs were dissolved in Double Distilled Water (DDW). Drug solutions were prepared fresh and their doses are expressed in terms of their free bases.

Experimental Procedure

Animals were randomly divided into six groups of 6 animals in each-

Group-1, vehicle treated, received double distilled water;

Group-2, received Desvenlafaxine (80 mg/kg);

Group-3, received ethanol;

Group-4, 5, 6, received ethanol + Desvenlafaxine (20 mg/kg, 40 mg/kg & 80 mg/kg); for 14 days;

Ethanol control group mice received ethanol for 14 days at a daily dose of 8 g/kg (as a 20% solution, given as two oral gavages of 24 mL/kg at 8 a.m. & 4 p.m.). DDW Control mice received the same volume of double distilled water and treatment with desvenlafaxine (20 mg/kg, 40 mg/kg, 80 mg/kg orally) 1 hr before ethanol dosing was started from first day of experiment.

On the 15th day, 16 hours after the last ethanol exposure, the behavioral tests were performed one hour one after the other. Locomotor activity was investigated using actophotometer, anxiety was measured in the Elevated Plus Maze test (EPM). Depression-like behaviour was assessed in the Forced Swim Test (FST). At the end of each experimental session, the floor and walls of the test box and the elevated plus maze were wiped clean and dried [21].

Behavioral Parameters

Locomotor Activity (LCA) monitoring

Locomotor activity was assessed in actophotometer (VJ Instruments, Amaravati, India), having a diameter of 40 cm, equipped with three infrared beam cells pair (pair: one emitter and one receiver), 20 cm apart from each other and located on the walls of the circular arena and connected to digital counter. Locomotor activity was expressed in terms of total number of counts of beams interruptions in 30 minutes [22].

Elevated Plus-Maze (EPM)

The EPM test is one of the most widely used non-conditioned tests to evaluate anxiety-like behaviors [23-25]. EPM apparatus consists of two opposite open arms 50 cm × 10 cm and two opposite arms enclosed by 40 cm high walls and elevated 50 cm from the floor. The arms are connected by a central 10 cm × 10 cm square, and thus the maze forms a "plus" shape [26]. In this test, each rat is placed in the central square with the head facing the closed arm of the EPM and its behavior is observed for 5 minutes. Anxiety-like behaviors are defined as the decrease in the total time spent in the open arm [27]. Each animal's activity in EPM was recorded using a video camera for subsequent analysis of total time spent in the open arm.

Forced Swim Test (FST)

Forced swim test was carried out by a method described earlier [28,29]. Mice were placed for 6 minutes in a glass cylinder (height: 35 cm; diameter: 17 cm) filled with water (25 ± 1°C) to a depth of 25 cm. The water depth was adjusted so that the animals must swim or float without their hind limbs or tail touching to the bottom. During testing, an individual mouse was placed in the cylinder for 6 minutes, and the duration of immobility (the time during which mouse made only the small movements necessary to keep their heads above water) was scored. As suggested by Porsolt, only the data scored during the last 4 minutes were analyzed and presented.

Results

Effects of treatment with desvenlafaxine on locomotor activity

The effects of desvenlafaxine on locomotor activity in naive animals are illustrated in Figure 1. Two-way ANOVA revealed that treatment with desvenlafaxine (20 mg/kg, 40 mg/kg and 80 mg/kg, i.p.) was devoid of any significant effects on locomotor activity compared to vehicle treated animals [$F(5,35)=14.44$, $P=0.0001$].
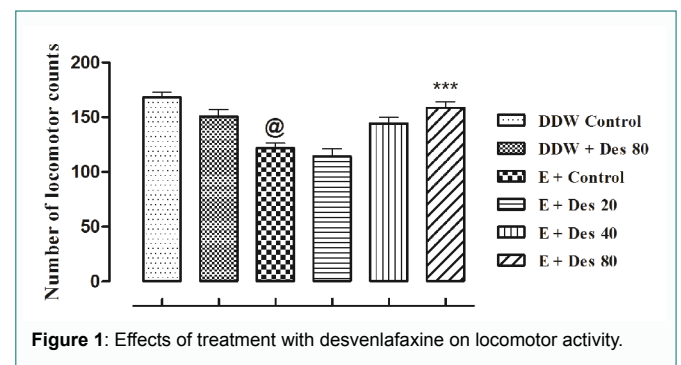

Figure 1: Effects of treatment with desvenlafaxine on locomotor activity.

Each bar represents mean ± S.E.M. of 5-6 observations. Testing was conducted 14-16 hours after the last alcohol exposure. @$P<0.001$ *vs.* DDW control group, ***$P<0.001$ *vs.* ethanol control group (One-way ANOVA followed by Tukey's post hoc test). DDW control: Double Distilled Water control; DDW + Des 80: Double Distilled Water desvenlafaxine (80 mg/kg) treated; E control: ethanol control; E + Des 20: ethanol desvenlafaxine (20 mg/kg) treated; E + Des 40: ethanol desvenlafaxine (40 mg/kg) treated; E + Des 80: ethanol desvenlafaxine (80 mg/kg) treated.

Effects of treatment with desvenlafaxine on Elevated plus maze

The influence of desvenlafaxine on time spend in open arm in elevated plus maze test is depicted in Figure 2. One-way ANOVA revealed that desvenlafaxine had significant influence on time spend in open arm [$F(5,35)=18.25$, $P=0.0001$]. Post hoc test indicated that desvenlafaxine (40 and 80 mg/kg) significantly increased time spend in open arm in elevated plus maze test as compared to ethanol treated mice. Ethanol control significantly decreased time spend in open arm in elevated plus maze test as compared to DDW control group.
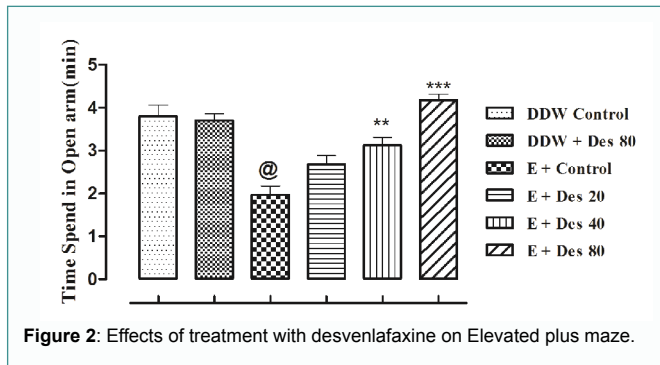
Figure 2: Effects of treatment with desvenlafaxine on Elevated plus maze.

Each bar represents mean ± S.E.M. of 5-6 observations. Testing was conducted 14-16 hrs after the last alcohol exposure. @$P<0.001$ *vs.* DDW control group, **$P<0.01$ and ***$P<0.001$ *vs.* ethanol control group (One-way ANOVA followed by Tukey's post hoc test). DDW control: Double Distilled Water control; DDW + Des 80: Double Distilled Water desvenlafaxine (80 mg/kg) treated; E control: ethanol control; E + Des 20: ethanol desvenlafaxine (20 mg/kg) treated; E + Des 40: ethanol desvenlafaxine (40 mg/kg) treated; E + Des 80: ethanol desvenlafaxine (80 mg/kg) treated.

Effects of treatment with desvenlafaxine on Forced swim test

The influence of desvenlafaxine on immobility time in forced swim test is depicted in Figure 3. One-way ANOVA revealed that desvenlafaxine had significant influence on immobility time [$F_{(5,35)}=16.01$, $P=0.0001$]. Post hoc test indicated that desvenlafaxine (20 mg/kg, 40 mg/kg & 80 mg/kg) significantly reduced immobility time in forced swim test as compared to ethanol treated mice. Ethanol control significantly increased the immobility time in forced swim test as compared to DDW control group.
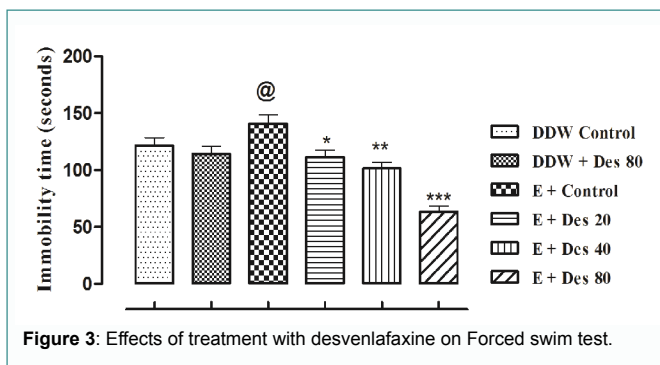

Figure 3: Effects of treatment with desvenlafaxine on Forced swim test.

Each bar represents mean ± S.E.M. of 5-6 observations. Testing was conducted 14-16 hours after the last alcohol exposure. @$P<0.001$ *vs.* DDW control group, *$P<0.05$, **$P<0.01$ and ***$P<0.001$ *vs.* ethanol control group (One-way ANOVA followed by Tukey's post hoc test). DDW control: Double Distilled Water control; DDW + Des 80: Double Distilled Water desvenlafaxine (80 mg/kg) treated; E control: ethanol control; E + Des 20: ethanol desvenlafaxine (20 mg/kg) treated; E + Des 40: ethanol desvenlafaxine (40 mg/kg) treated; E + Des 80: ethanol desvenlafaxine (80 mg/kg) treated.

Discussion

This study evaluated the influence of desvenlafaxine on the ethanol-induced anxiety and depression in mice. Ethanol-induced mice produced a marked anxiety and depression like behavior, which was associated with significant decease locomoter activity in actophotometer and time spend in open arm in elevated plus maze, and increase immobility time in forced swim test in mice. Chronic treatment with desvenlafaxine significantly reversed the behavioral activity like increase locomoter activity in actophotometer and time spend in open arm in elevated plus maze, and decrease immobility time in forced swim test in mice.

Behavioral symptoms of alcohol misuse often mimic various psychiatric disorders including anxiety and depression. Thus, co-occurrence of alcoholism with neuropsychological disorders such as anxiety and/or depression, can introduce therapeutic challenges for treatment of alcoholism. This is most readily manifested in terms of relapse prevention as such mood is orders may significantly facilitate relapse to alcohol use [30-33]. At the time of withdrawal from alcohol (16 hours after the last alcohol treatment), ethanol-treated mice had decreased locomotor activity in the actophotometer. The actophotometer is a common measure of exploratory behaviour and general activity in mice, where both the quality and quantity of the activity can be measured. This test is also commonly used to assess the sedative, toxic, or stimulant effects of compounds [34]. In the present study, the decreased locomotor activity of ethanol-treated animals might be partly due to neurotoxic effect of alcohol on neuronal cells where as desvenlafaxine treatment of ethanol treated mice increased locomoter activity in actophotometer. In the present study, elevated plus maze and forced swim test were used for assessment of anxiety and depression respectively. Increased times spend in open arm in elevated plus maze demonstrates intact anti anxiety function. Chronic ethanol treated mice show a significant decrease in time spend in open arm as compared to DDW control and per se group, whereas desvenlafaxine treatment (40 mg/kg and 80 mg/kg) of ethanol treated mice increased the time spend in open arm. The decreased time spent in the open arms of the EPM was consistent with an anxiety-like effect of ethanol [35]. In present study the forced swim test revealed that ethanol treated mice exhibited a increased immobility time as compare to DDW control group and per se group, which was significantly reversed dose dependently on treatment with the desvenlafaxine (20 mg/kg, 40 mg/kg and 80 mg/kg). Desvenlafaxine is a novel atypical antidepressant referred as 'Serotonin and Nor-Adrenaline Reuptake Inhibitor' (SNRI) as it inhibits serotonin and nor-epinephrine reuptake in the presynaptic cleft [19]. But in contrast to older tricyclic antidepressants it does not interact with cholinergic, adrenergic or histaminergic receptors or have any sedative property. Dysregulated serotonin transmission has long been implicated in depression [36]. This excludes the possibility that the activity per se may have contributed to the changes in elevated plus maze test and forced swim test in vehicle treated alcohol dependent mice and the desvenlafaxine treated alcohol dependent mice. The results of the present study revealed that treatment with desvenlafaxine dose dependently prevented anxiety and depression like behavior in ethanol-induced mice.

Conclusion

In conclusion, the findings of the present investigation suggest that desvenlafaxine exerts its beneficial effects against ethanol-induced anxiety and depression and it may be attributed to its antianxiety and antidepressant activity. Thus desvenlafaxine may be projected in the treatment of anxiety and depression associated with chronic alcoholism.

References

1. Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature. 2008;455(7215):894-902.

2. Papakostas GI. Tolerability of modern antidepressants. J Clin Psychiatry. 2008;69:8-13.

3. Pack AI, Pien GW. Update on sleep and its disorders. Annu Rev Med. 2011;62:447-60.

4. Nutt DJ. Relationship of neurotransmitters to the symptoms of major depressive disorder. J Clin Psychiatry. 2008;69:4-7.

5. Mao QQ, Xian YF, Ip SP, Che CT. Involvement of serotonergic system in the antidepressant-like effect of piperine. Prog Neuropsychopharmacol Biol Psychiatry. 2011;35(4):1144-7.

6. Schuckit MA, Tipp JE, Bergman M, Reich W, Hesselbrock VM, Smith TL. Comparison of induced and independent major depressive disorders in 2,945 alcoholics. Am J Psychiatry. 1997;154(7):948-57.

7. Hasin DS, Grant BF. Major depression in 6050 former drinkers: association with past alcohol dependence. Arch Gen Psychiatry. 2002;59(9):794-800.

8. Hasin DS, Stinson S, Ogburn E, Grant BF. Prevalence, correlates, disability, and comorbidity of DSMIV alcohol abuse and dependence in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Arch Gen Psychiatry. 2007;64(7):830-42.

9. Regier DA, Farmer ME, Rae DS, Locke BZ, Keith SJ, Judd LL, et al. Comorbidity of mental disorders with alcohol and other drug abuse. JAMA. 1990;264(19):2511-8.

10. Marlatt GA, Gordon JR. Relapse prevention. New York: Guilford Press; 1985.

11. Shiffman S. Relapse following smoking cessation: A situational analysis. J Consult Clin Psychol. 1982;50(1):71-86.

12. Brière FN, Rohde P, Seeley JR, Klein D, Lewinsohn PM. Comorbidity between major depression and alcohol use disorder from adolescence to adulthood. Compr Psychiatry. 2014;55(3):526-33.

13. Greenfield SF, Weiss RD, Muenz LR, Vagge LM, Kelly JF, Bello LR, et al. The effect of depression on return to drinking: a prospective study. Arch Gen Psychiatry. 1998;55(3):259-65.

14. Lynskey MT. The comorbidity of alcohol dependence and affective disorders: treatment implications. Drug Alcohol Depend. 1998;52(3):201-9.

15. Walker BM, Drimmer DA, Walker JL, Liu T, Mathé AA, Ehlers CL. Effects of prolonged ethanol vapor exposure on forced swim behavior, and neuropeptide Y and corticotropin-releasing factor levels in rat brains. Alcohol. 2010;44(6):487-93.

16. Tian JW, Jiang WL, Zhong Y, Meng Q, Gai Y, Zhu HB, et al. Preclinical pharmacology of TP1, a novel potent triple reuptake inhibitor with antidepressant properties. Neuroscience. 2011;196:124-30.

17. Spina E1, Trifirò G, Caraci F. Clinically significant drug interactions with newer antidepressants. CNS Drugs. 2012;26(1):39-67.

18. Lourenco MT, Kennedy SH. Desvenlafaxine in the treatment of major depressive disorder. Neuropsychiatr Dis Treat. 2009;5:127-36.

19. Deecher DC, Beyer CE, Johnston G, Bray J, Shah S, Abou-Gharbia M, et al. Desvenlafaxine succinate: A new serotonin and norepinephrine reuptake inhibitor. J Pharmacol Exp Ther. 2006;318(2):657-65.

20. Crowell MD, Shetzline MA, Moses PL, Mawe GM, Talley NJ. Enterochromaffin cells and 5 HT signaling in the pathophysiology of disorders of gastrointestinal function. Curr Opin Investig Drugs. 2004;5(1):55-60.

21. Valcheva-Kuzmanova S, Eftimov M, Kuzmanov K. An Experimental Model of Alcohol Induced Anxiety and Depressive Behaviour in Rats. Scripta Scientifica Medica. 2013;45(3):48-52.

22. Bhutada P, Mundhada Y, Bansod K, Ubgade A, Quazi M, Umathe S, et al. Reversal by quercetin of corticotrophin releasing factor induced anxiety- and depression-like effect in mice. Prog Neuropsychopharmacol Biol Psychiatry. 2010;34(6):955-60.

23. Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods. 1985;14(3):149-67.

24. Baldwin HA, Rassnick S, Rivier J, Koob GF, Britton KT. CRF antagonist reverses the "anxiogenic" response to ethanol withdrawal in the rat. Psychopharmacology (Berl). 1991;103(2):227-32.

25. Rassnick S, Heinrichs SC, Britton KT, Koob GF. Microinjection of a corticotropin-releasing factor antagonist into the central nucleus of the amygdala reverses anxiogenic-like effects of ethanol withdrawal. Brain Res. 1993;605(1):25-32.

26. File SE, Gonzalez LE, Gallant R. Role of the dorsomedial hypothalamus in mediating the response to benzodiazepines on trial 2 in the elevated plus-maze test of anxiety. Neuropsychopharmacology. 1999;21(2):312-20.

27. Cruz AP, Frei F, Graeff FG. Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol Biochem Behav. 1994;49(1):171-6.

28. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. Nature. 1977;266(5604):730-2.

29. Umathe SN, Bhutada PS, Jain NS, Shukla NR, Mundhada YR, Dixit PV. Gonadotropin-releasing hormone agonist blocks anxiogenic-like and depressant-like effect of corticotrophin-releasing hormone in mice. Neuropeptides. 2008;42(4):399-410.

30. Glenn SW, Parsons OA. Prediction of resumption of drinking in posttreatment alcoholics. Int J Addict. 1991;26(2):237-54.

31. Driessen M, Meier S, Hill A, Wetterling T, Lange W, Junghanns K. The course of anxiety, depression and drinking behaviours after completed detoxification in alcoholics with and without comorbid anxiety and depressive disorders. Alcohol Alcohol. 2001;36(3):249-55.

32. De Witte P, Pinto E, Ansseau M, Verbanck P. Alcohol and withdrawal: from animal research to clinical issues. Neurosci Biobehav Rev. 2003;27(3):189-97.

33. Saatcioglu O, Yapici A, Cakmak D. Quality of life, depression and anxiety in alcohol dependence. Drug Alcohol Rev. 2008;27(1):83-90.

34. Gould TD, Dao TD, Kavacsics CE. The open field test. In: Mood and anxiety related phenotypes in mice. T. D. Gould, ed. New York: Humana Press; 2009. p. 1-20.

35. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl). 1987;92(2):180-5.

36. Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci. 2006;7(2):137-51.