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Original Research Article

Duloxetine alleviates high light-induced anxiety-related behaviors in Wistar rats

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Abstract

Purpose: *To investigate the effect of subchronic duloxetine treatment on high light-induced anxiety-related behaviors in Wistar rats.*
Methods: *Adult male Wistar rats (n = 30) were randomly assigned to three groups of rats (10 rats/group): control group, 30 mg/kg duloxetine group, and 60 mg/kg duloxetine group. Intraperitoneal injection of duloxetine was given once a day for ten days. The anxiolytic effect of duloxetine in the rats was assessed using light/dark box (LDB) anxiety test.*
Results: *Anxiety-related behaviors were significantly reduced in duloxetine-treated rats, when compared with control group. The reductions were not dose-dependent (light zone time and latency time were significantly increased, while dark zone time decreased significantly, $p < 0.05$). The number of rearings significantly increased in 30 mg/kg duloxetine group, relative to control and 60 mg/kg duloxetine groups ($p < 0.05$). However, there were no significant differences in the number of light-to-dark entrances among the groups ($p > 0.05$).*
Conclusion: *These results show that subchronic treatment with duloxetine alleviates anxiety-related behaviors in Wistar rats.*

Keywords: *Anxiety disorders, Duloxetine, Light/dark box test, Subchronic treatment, Behavior*

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INTRODUCTION

Generalized anxiety disorder (GAD) is one of the most common psychological disorders known. The lifetime and 1-year prevalence are put at 4.0 - 6.6 and 1.9 – 5.1 %, respectively [1,2]. The prevalence of anxiety disorder is influenced by factors such as socio-demographic features and presence of certain mental or physical disorders. Recent global prevalence of anxiety disorder is about 7.3 % [3]. In Africa alone, the prevalence of anxiety ranges from 3.5 – 8.1 %, while in Europe and America, it ranges from 7.0 – 15.5 % [3]. Anxiety places a severe burden on sufferers. In the United States, the annual cost for treatment of anxiety disorders as at 1990 was approximately $42.3 billion [4].

Duloxetine is a potent selective serotonin and noradrenaline reuptake inhibitor (SSNRI) used for treatment of major depressive disorders, GAD, diabetic peripheral neuropathic pain, fibromyalgia, and chronic musculoskeletal pain [5,6]. It is effective against attention deficit hyperactivity disorder, prophylactic migraine, premature ejaculation, osteoarthritic knee pain and incontinence [6-10]. Available data show that duloxetine is also effective in the treatment of anxiety and depression in animals. Anxiety-like behaviors in animals trigger unconditioned responses such as high altitude, high light, and open area in animals. Studies have shown that duloxetine produces effective anxiolytic effect in animals with chronic treatment [11-13]. This study investigated the effect of subchronic treatment with duloxetine on high light-induced anxiety-related behaviors.

EXPERIMENTAL

Rats

Adult male Wistar rats weighing 200 - 300 g (mean weight = 250 ± 50 g) were obtained from Ankara University School of Medicine Experimental Animals and Research Laboratory and housed in metal cages. The rats were maintained under standard conditions: 12 h light/12 h dark cycle; temperature of 22 ± 2 °C, and 55 ± 5 % humidity. The study protocol was according to the Institutional Animal Care and Use Committee of Ankara University (approval no. 2016-2-10). The rats had free access to standard feed and water. The study procedures complied with the international guidelines for animal studies [14]. The rats (n = 30) were randomly assigned to three groups, each having 10 rats: control group, 30 mg/kg duloxetine group, and 60 mg/kg body weight (bwt) duloxetine group. The rats were acclimatized to laboratory conditions for 1 week before commencement of the study.

Drug administration

Duloxetine was dissolved in isotonic saline and administered intraperitoneally at doses of 30 and 60 mg/kg once a day for 10 days. The drug was prepared fresh and protected from direct light.

Light/dark box test

Light causes anxiety in rats. Therefore, the effectiveness of anxiolytic agents are evaluated using light/dark box test. This was performed as described in the literature [15,16]. Generally, anxiolytic agents increase the time spent in the light zone and decrease the time spent in the dark zone. The apparatus (40 cm width and 110 cm length) consisted of two equal compartments: light zone (850 lx illumination intensity) and dark zone. A wall with hole (7.5 x 7.5 cm^2) was placed on the floor between the light and dark zones. The rats were individually placed in the apparatus for 5 min beginning from the light zone. At the end of each procedure, the apparatus was cleaned with 70 % ethanol and allowed to dry for 15 min. A video camera was mounted to record the behaviors of the rats. The time spent in the light and dark zones, the number of light to dark entrances, latency of crossing to dark zone, number of rearings and the rearing latency were measured in each group. A rat was said to have gained entrance to either the light or dark zone when all its four paws were in one of the two compartments. The various behavioral tests were performed in the Laboratory of Behavior Physiology, Banu Ocakçıoğlu.

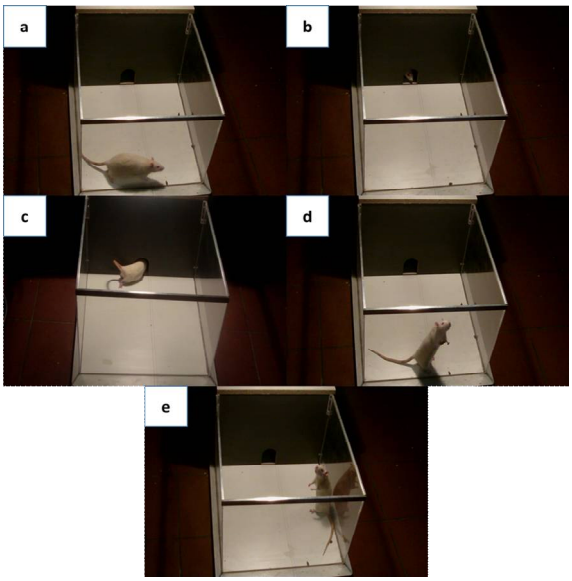

Figure 1: Procedures for light/dark box test. (a): Light zone time; **(b):** Dark zone time; **(c):** Light/dark zone entrance; **(d):** Supporting rearing; and **(e):** Non-supporting rearing

Statistical analysis

Numerical data are expressed as mean ± SEM. Statistical analysis was performed using SPSS (23.0). Normality and homogeneity of the groups were analyzed using Shapiro-Wilk test and Dunn-Bonferroni post hoc test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Light/dark box test results

In the control, 30 mg/kg duloxetine, and 60

mg/kg duloxetine groups, the time spent in the light zone was 25 s (interquartile range = 17 s), 200 sec (interquartile range = 140 s), and 189 s (interquartile range = 120 s), respectively. The latency time were 25 sec (interquartile range = 17 s), 185 s (interquartile range = 172 s), and 185 s (interquartile range = 149 s), respectively. Similarly, the time spent in dark zone were 275 sec (interquartile range = 17 sec), 100 s (interquartile range = 140 s), and 111 s (interquartile range = 120 sec), respectively. The number of rearings were 2 (interquartile range = 1), 6 (interquartile range = 11), and 2 (interquartile range = 5), respectively.

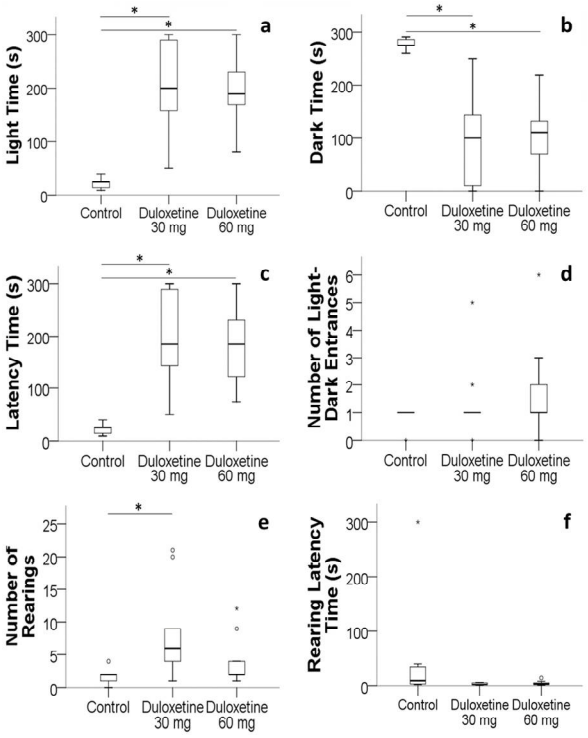

Figure 2: Results of light/dark box test. (a) Time spent in light zone; **(b)** time spent in dark zone; **(c)** latency time of crossing to dark zone; **(d)** number of light to dark entrances; **(e)** number of rearings, and **(f)** rearing latency. *$p < 0.05$, when compared with control group

The number of rearings was significantly increased in the 30 mg/kg duloxetine group, relative to the control and 60 mg/kg duloxetine groups ($p < 0.05$). However, there were no significant differences in the number of light-to-dark entrances among the groups ($p > 0.05$). The number of light-to-dark entrances were 1 (interquartile range = 0), 1 (interquartile range = 1), and 1 (interquartile range = 2) for control, 30 mg/kg duloxetine and 60 mg/kg duloxetine groups, respectively. The rearing latency were 9 (interquartile range = 35), 2 (interquartile range = 3), and 3 (interquartile range = 5), respectively.

DISCUSSION

Generalized anxiety disorder (GAD) is one of the most common psychological disorders known. This disorder is characterised by feelings of worry, anxiety or fear that are strong enough to interfere with an individual's daily activities [1].

Duloxetine is a potent selective serotonin and noradrenaline reuptake inhibitor (SSNRI) used for treatment of major depressive disorders, GAD, diabetic peripheral neuropathic pain, fibromyalgia, and chronic musculoskeletal pain [5,6]. This study investigated the effect of subchronic treatment with duloxetine on high light-induced anxiety-related behaviors.

The results showed that treatment with duloxetine significantly decreased anxiety-related behaviors, but the effect was not dose-dependent. These results indicate that subchronic duloxetine treatment may produce a potent anxiolytic effect in a rodent anxiety model.

Rearing is a sign of vertical locomotor activity and represents a decrease in anxiety-like behavior. In this study, the number of rearing was significantly increased in 30 mg/kg duloxetine group, but was significantly decreased with increasing dose of duloxetine. In a previous study, anxiety parameters in light/dark box test were significantly modified [16]. In this study, there were no significant differences in the number of light-to-dark zone entrance and rearing latency among the groups. Studies have shown that acute treatment with duloxetine is ineffective, when compared with chronic treatment.

Table 1: Data summary

Duloxetine dose	Anxiolytic	Anxiogenic	Exploratory behaviour	Locomotor activity
30 mg/kg	Light zone time * Latency time *	Dark zone time #	Light/dark zone entrance	Number of rearing behaviour *
60 mg/kg	Light zone time * Latency time *	Dark zone time #	Light/dark zone entrance	Number of rearing behaviour

*Significantly increased, when compared with control group; #significantly decreased, when compared with control group

It has also been reported that a single dose of duloxetine is not sufficient to produce an anxiolytic effect in the light/dark box test [17]. The reason for this perceived ineffectiveness may be due to acute exposure or use of low dose. Studies have shown that animal anxiety tests such as elevated plus maze, zero maze, and open field tests are not effective in assessing anxiety-like behavior in acute duloxetine treatment [11-13]. Chronic treatment with duloxetine has been shown to produce potent anxiolytic effect. It alleviates stretched-attend posture (anxiogenic parameter) and significantly increase positive head dipping behavior (exploratory behavior) [12]. The safety of duloxetine has been reported at various doses (≤ 120 mg/kg bwt/day) in placebo-controlled studies and in a 1-year, open-label, long-term study [20]. During treatment with SNRI, adverse effects such as dry mouth, nausea, dizziness and fatigue may be observed [7,21]. In order to minimize the occurrence of such adverse effects, duloxetine may be administered at a low dose and augmented with other medications. Studies have shown that treatment with duloxetine for two weeks at a dose of 60 mg/kg/day significantly increased mesolimbic dopamine system activity, and enhanced reward-related neural responses in the ventral striatum. It is likely that duloxetine attenuates anhedonia by increasing reward responsiveness [22]. Subchronic treatment with duloxetine is effective against mood disorder induced by potent serotonin and noradrenaline reuptake inhibitors. It must be noted that duloxetine has low-affinity for dopamine D2 receptor and dopamine reuptake transporters [23]. The dopaminergic system is usually taken into consideration during treatment with duloxetine.

CONCLUSION

The results obtained in this study show that subchronic treatment with duloxetine alleviates anxiety-related behaviors.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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