Research Article

Anxiolytic and Antidepressant like Profile of Repeatedly administrated Escitalopram in Behavioral Animal Models

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Abstract

Selective serotonin reuptake inhibitors (SSRIs) were initially introduced as antidepressants, and their potential as anxiolytic has been observed in the treatment of social phobia, post-traumatic stress disorder, and generalized anxiety disorder Escitalopram is therapeutically active S-enantiomer of citalopram. It is a commonly prescribed Selective serotonin reuptake inhibitor (SSRI). SSRIs are the latest generated antidepressants having the selective mechanism of action towards 5-Hydroxytryptamine (Serotonin; 5-HT) without affecting any other unwanted effects on other neurotransmitters. Escitalopram is very selective serotonin reuptake inhibitor; it blocks the serotonin transporters without producing any significant effect on other monoamines transporters. Evidences suggest that escitalopram is efficient in the treatment of major depressive disorder (MDD) and generalized anxiety disorders (GAD). The present study was designed to determine the effects of repeated administration of escitalopram on locomotor activity and anxiolytic behavior of animals in animal model. Rats were administered orally with escitalopram (5 mg/kg) daily for 7 days. This study showed anxiolytic activity produced on repeated administration of drug in light dark transition test. As compared to control animals, activity in activity box was higher and activity in open field was smaller in escitalopram administrated animals. These information support clinical discoveries that escitalopram is a powerful, very much endured SSRI with anxiolytic-like impacts.

Keywords: Antidepressants, Citalopram, Escitalopram, Selective Serotonin Reuptake Inhibitor, Serotonin

Introduction

Selective serotonin reuptake inhibitors (SSRIs) are the major and dominant class of antidepressants used over the last decade whereas ancient groups of most widely used antidepressants were Tricyclic antidepressants (TCA) and monoamine oxidase inhibitors (MAOIs) (Artigas *et al.,*2001). Pharmacological effect of antidepressants in the long term treatment of depression is due to the complex interaction between nor-adrenergic and serotoninergic receptors (Llorca *et al.,* 1993).TCA were effective in its response to reduce depression because it works at both serotonergic and nor-adrenergic receptors but its use was prohibited because TCA also blocks histaminic, cholinergic and α1-adrenergic receptors. These are the possible side effects of TCA. MAOIs were also ancient group of antidepressants produce its action by interaction with tyramine to cause potentially lethal hypertension, an adverse effect of MAOIs (Feighner, 1999). Later on SSRIs, latest generated antidepressants were discovered having the selective mechanism of action towards 5-HT without affecting any other unwanted like histaminic and cholinergic receptors (Feighner, 1999; Hyttel, 1994).

Escitalopram is S-enantiomer of citalopram, therapeutically active, and is a commonly prescribed SSRI (Waugh and Goa, 2003). But escitalopram is found more superior to citalopram in efficacy (Kennedy *et al.,* 2006;

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Moore *et al.,* 2005). Citalopram was the most commonly used antidepressants, recent studies showed that citalopram is the racemic mixture of S-enantiomer and R-enantiomer. S-enantiomer is found to be active form whereas R-enantiomer as inactive form, inactive form is thought to interrupt in the efficacy of Citalopram (Kasper *et al.,* 2009). Therefore, administration of S-enantiomer (escitalopram) shows greater efficacy (Mnie-Filali*et al.,* 2006). It has been reported that escitalopram has little or no effect on the psychomotor activities indeed it thought that it may facilitate the psychomotor performance (Rose *et al.,* 2006). Efficacy of escitalopram found superior to other classes of antidepressants like selective nor-adrenaline reuptake inhibitors (SNRIs) as well (Kennedy *et al.,* 2006). Escitalopram is the most selective and efficient serotonin reuptake inhibitor. It can use for many therapeutic purposes. It is efficiently used for the treatment of major depressive disorder (MDD) (Llorca*et al.,* 2005; Hirschfeld and Vornik, 2004), panic disorder, generalized anxiety disorder (GAD), social anxiety disorder, obsessive compulsive disorder (OCD) (Pelissolo, 2008) and alcohol dependence (Muhonen*et al.,* 2008). Escitalopram is a quite safe and well tolerated drug, unlikely to cause clinically significant drug-drug interactions (Rao, 2007; Lepola*et al.,* 2003). The possible adverse effects of escitalopram may include Nausea and ejaculatory problems, diarrhea, insomnia, dry mouth, headache and upper respiratory tract infections (Waugh and Goa, 2003).

Serotonin is the well-known neurotransmitter extensively distributed in the CNS. Mainly 5-HT and its related drugs are used in psychiatry and neurology (Berger *et al.,* 2009). In the brain, serotonin is considered as one of the most important neurotransmitter. The central serotonergic system is important in the regulation of various physiological functions, such as appetite, circadian rhythm, locomotor activity, body temperature, memory, sexual behavior, vigilance and nociception. It also involved in different disease stares, such as migraine, depression, schizophrenia, anxiety and aggressive behavior. The objective of the present study was to investigate the different behavioral consequences of escitalopram in rats on acute as well as on chronic administration.

Methods

Animals

Twenty four male locally bred albino Wistar rats with an average weight of 160 ± 10 grams were purchased from The Dow University of Health and Sciences (DUHS). They were housed individually in controlled environmental conditions such as room temperature was $22\pm2^{\circ}C$ with 12:12 hour light dark cycle. Before experiment, 3 day familiarization period was allowed with free access of food (cubes of standard rodent diet) and water. Before starting the experiment, rats were accustomed to various handling procedures. All animal experiments, approved by the Institutional Ethics and Animal Care Committee, were conducted in strict accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No.85-23, revised 1985). All treatments and

behavioral monitoring were performed in a balanced design to avoid order and time effects.

Drugs

Escitalopram was dissolved in water and administered orally at 10 mg/kg. Drug solutions were prepared daily before administration. The respective controls were administered water orally.

Experimental Protocol

Twenty four (Male Albino Wistar) Rats were divided into two equal groups i) Water administrated group ii) Escitalopram administrated group. Animals of escitalopram group were administered orally with drug (10 mg/kg) and animals of control group were administrated with water. Food intake and change in body weight were monitored on next day of 1st and 7th administration. Locomotive activity was monitored in familiar (home cage) as well as in novel (open field) environment on next day of 1st, 3rd, 5th and 7th day of administration. Anxiolytic behavior of treatment was monitored in light dark transition box on next day of 1st, 3rd, 5th and 7th day of administration.

Behavioral Assessment

Food Intake

Weighed amount of food were placed in the hopper of each cage. Food intake was monitored by weighing the amount of food left in the cage hopper on next day of 1st and 7th administration. By subtracting the weight of food left in the hopper from the weight provided on first day of experiment is the amount of food consumed by the rat.

Growth Rate

Change in body weight was monitored on next day of 1st and then a week administration. Changes in the body weight were monitored to find out the effect of specific treatment in the respective chapters. Growth rate changes were calculated as percentage of starting day weight (experiment day body weight/starting day body weight) X 100.

Activity Box

Activity box was used for the determination of locomotor activity. The duration of monitoring of activity (Number of cage crossed) is of 10 minutes Activity cage apparatus was a square Perspex cage (26 x 26 x 26 cm). The floor of the cage is covered with saw dust. For habituation, animal was placed 15 min before the monitoring of the activity (Shireen E *et al.,* 2014). All monitoring was done in balanced design.

Open Field

Open field was used to determine the exploratory activity of animals. It provides the large exploratory area in which depressive like activity of drugs can clearly observed (Haleem, 2010). The apparatus of open field was constructed of transparent Plexiglas having square area 76 x 76 cm with opaque walls 42 cm high. The floor of arena was divided into 25 equal squares. For determination of activity rat was placed in the center of the field and number of squares crossed with all four paws (exploratory activity) were monitored for 5 minutes (Farhan*et al.,* 2014; Mill *et al.,* 2002; Tang *et al.,* 2002). Time required to move from the center square (latency time) in seconds was monitored as described by (Batool*et al.,* 2011).

Light dark box

Light dark activity test is used to determine the anxiolytic effects of drugs (Maldonado

and Navarro, 2000). The light dark activity box used in this experiments was consist of two equal locally made compartments (26 x 26 x 26 cm), with an access (12 x 12 cm) between the compartments. Both the compartments were different in their sensory properties that walls of one compartment were light (transparent) and other dark (black). Time spent and number of entries in light box for 5 minutes was monitored. More the animals spent time in light box, more the drug is anxiolytic.

Statistical Analysis

All data of escitalopram administration were analyzed by two-way ANOVA (repeated measure design. Software used for the analysis was SPSS (version 17.0). Individual comparisons were made by Newman-Keuls test. Values of $p<0.05$ were considered as significant.

Results

Effects of escitalopram administration on food intake and growth rate of Albino-Wistar rats as monitored on next day of 1st and 7th administration as shown in Figure 1. Analysis of the data on food intake by two-way ANOVA (repeated measures design) showed that effects of escitalopram ($F=27.28$; df=1, 22; $p<0.01$); repeated monitoring ($F=983.84$; df=1, 22; $p<0.01$) and the interaction between escitalopram and repeated monitoring ($F=43.24$; df= 1, 22; $p<0.01$) were significant. Post-hoc analysis by Newman-Keuls test showed that escitalopram produced hypophagic condition on repeated administration ($p<0.01$) as compared to water administrated controls. Escitalopram group animals showed decreased in food intake significantly ($p<0.01$) after 7th day of

administration as compared to similarly administrated animals of 1st day.

Data on growth rate as analyzed by two-way ANOVA (repeated measures design) showed significant effects of escitalopram ($F=38.527$; df=1, 22; $p<0.01$). However, the effect of days ($F=3.01$; df=1, 22; $p>0.05$) and effects of interaction between escitalopram and repeated monitoring ($F=8.125$; df=1, 22; $p>0.05$) were non-significant. Post-hoc analysis by Newman-Keuls test showed that administration of escitalopram decreased growth rate as compared to water administrated controls. Significant decreased was found after 7th day of administration. Change in body weight was smaller in escitalopram administrated animals after 7th administration as compared to animals of similarly administrated from 1st day administration.

Effect of repeated escitalopram administration on activity (cage counts) of animals in activity box shown in Figure 2. Activity was monitored on next day of 1st, 3rd, 5th and 7th administration. Change in activity was analyzed by two-way ANOVA (repeated measures design), results showed that effects of escitalopram ($F=77.721$; df=1, 22; $p<0.01$); repeated measurements ($F=92.172$; df=3, 22; $p<0.01$) and interaction between escitalopram and repeated monitoring ($F=104.25$; df=3, 22; $p<0.01$) were significant. Post-hoc analysis by Newman-Keuls test showed that number of cage crossed was greater in escitalopram administrated animals as compared to water administrated animals. Increase in activity was significant ($p<0.01$) after 5th and 7^{th}administration. In escitalopram

administrated animals, activity was higher (p<0.01) after 3rd, 5th and 7th administration as compared to similarly administrated animals of 1st day administration.

Effect of repeated escitalopram administration on latency time to move and number of squares crossed in an open field model. Activity was monitored on next day of 1st, 3rd, 5th and 7th administration. Analysis of the data on latency time to move by two-way ANOVA (repeated measures design) showed that effects of escitalopram (F=30.102; df=1, 22; p<0.05); repeated monitoring (F=202.728; df=3, 22; p<0.01) and the interaction between escitalopram and repeated monitoring (F=50.247; df=3, 22; p<0.01) were significant. Post-hoc analysis by Newman-Keuls test showed that escitalopram decrease latency time on single administration (p<0.01) as compared to water administrated controls. Latency time was decreased (p<0.01) in escitalopram and water treated animals after 3rd, 5th and 7th administration as compared to similarly administrated animals of 1st day administration.

Analysis of the data on number of squares crossed by two-way ANOVA (repeated measures design) showed significant effects of escitalopram (F=35.842; df=1, 22; p<0.05); repeated monitoring (F=33.918; df=3, 22; p<0.01) and the interaction between escitalopram and repeated monitoring (F=36.289; df=3, 22; p<0.01) were significant. Post-hoc analysis by Newman-Keuls test showed that administration of escitalopram decreased number of square crossed as compared to water administrated controls. Significant decrease (p<0.01) was found after 3rd, 5th

and 7th administration. Decrease in number of squares crossed was higher in escitalopram administrated animals after 3rd, 5th and 7th administration as compared to animals of similarly administrated from 1st day administration.

Effect of repeated escitalopram administration on number of entries and time spent in light box in light dark transition box. Activity was monitored on next day of 1st, 3rd, 5th and 7th administration. Analysis of the data on number of entries in light box by two-way ANOVA (repeated measures design) showed that effects of escitalopram (F=62.732; df=1, 22; p<0.01); repeated monitoring (F=49.294; df=3, 22; p<0.01) and the interaction between escitalopram and repeated monitoring (F=46.325; df=3, 22; p<0.01) were significant. Post-hoc analysis by Newman-Keuls test showed that escitalopram increase (p<0.01) number of entries in light box on repeated 3rd (p<0.05), 5th (p<0.05) and 7th (p<0.01) administration as compared to water administrated controls. Activity of escitalopram was increased (p<0.01) after 3rd, 5th and 7th administration as compared to similarly administrated animals of 1st day administration. Analysis of the data on time spent in light box by two-way ANOVA (repeated measures design) showed significant effects of repeated monitoring(F=104.233; df=3, 22; p<0.01) and the interaction between escitalopram and repeated monitoring (F=60.911; df=3, 22; p<0.01) were significant. Whereas effects of escitalopram (F=15.61; df=1, 22; P>0.05) found to be non-significant.
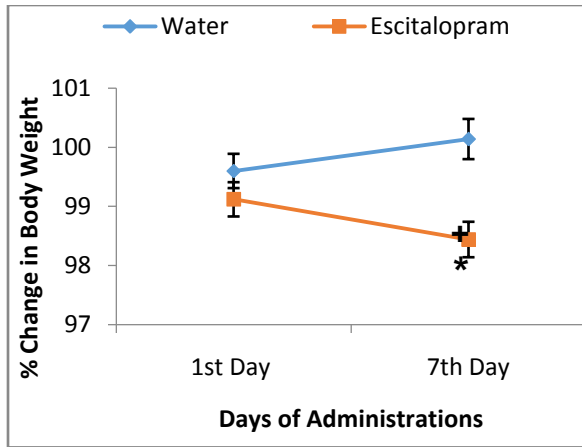
Figure 1: Effects of Escitalopram Administration on Food Intake and Growth Rate.

Values (Daily and cumulative food intake) are means $\pm$ SD (n=24) as monitored on next day of 1st and 7th administration. Significant differences by Newman-Keuls test: *p<0.01 from respective water administrated controls; +p<0.01 from similarly administrated animals from 1st day following two-way ANOVA (repeated measures design).
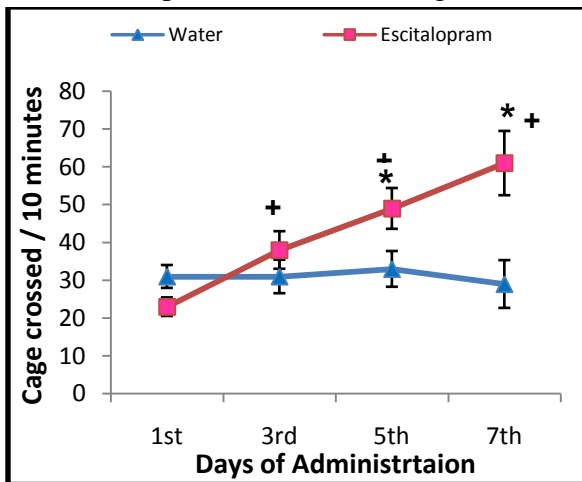

Figure 2: Effects of Escitalopram Administration on Activity of Rats in Activity Box.

Values are means $\pm$ SD (n=24) as monitored on next day of 1st, 3rd, 5th and 7thadministrations. Significant differences by Newman-Keuls test: *p<0.01 from respective water administrated controls; +p<0.01 from similarly administrated

animals from 1st day following two-way ANOVA (repeated measures design).

Post-hoc analysis by Newman-Keuls test showed that single administration of escitalopram decreased time spent in light box (p<0.01) as compared to water administrated animals. Whereas time spent in light box was increased on repeated administration as compared to water administrated animals. Significant increase (p<0.01) was found after 7thadministration. Increase in time spent (p<0.01) was higher in escitalopram administrated animals after 3rd, 5thand 7th administration as compared to animals of similarly administrated from 1st day   administration.
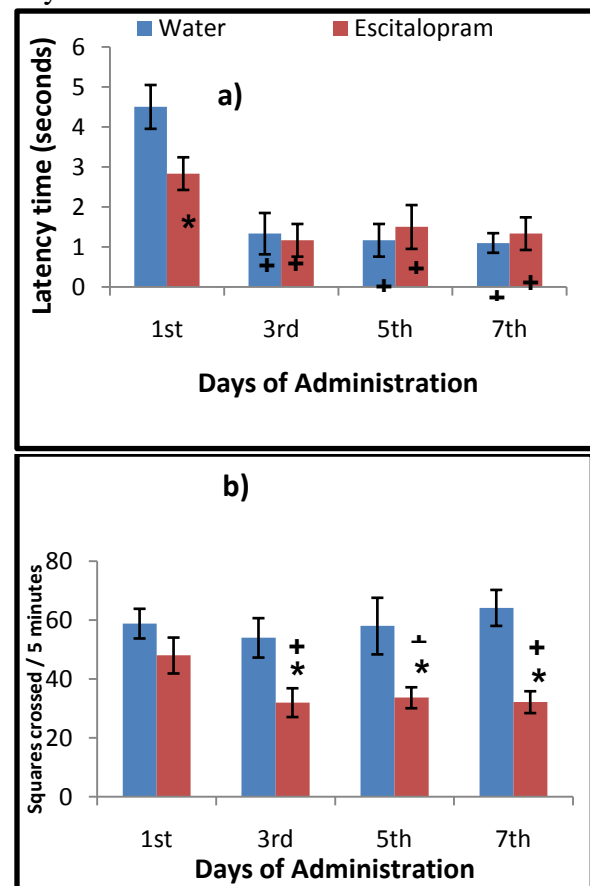

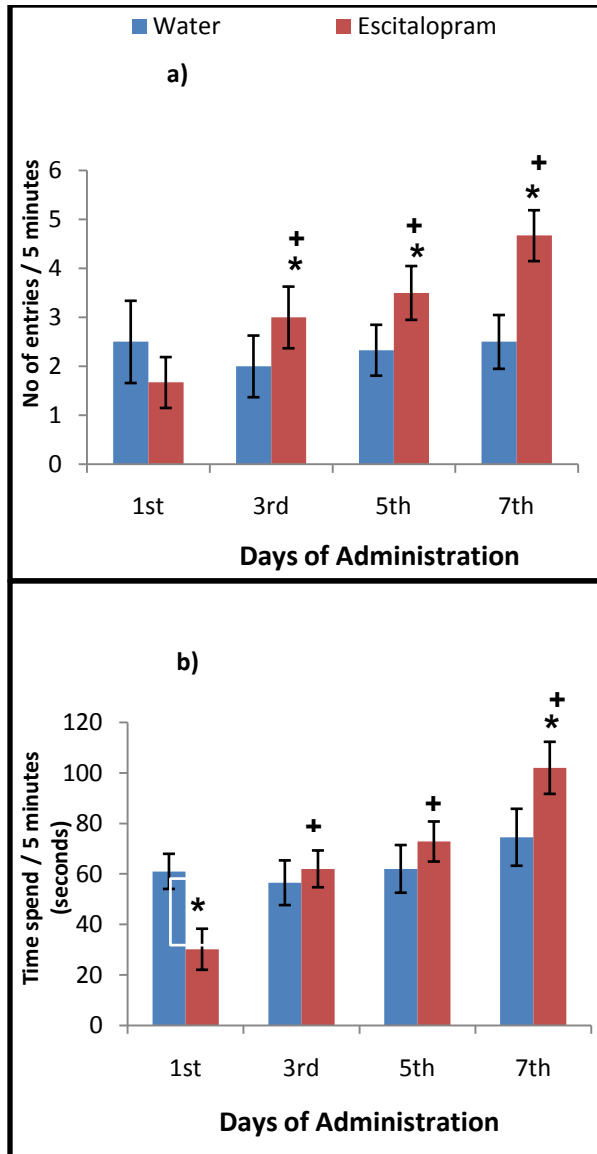**Figure 3:** Graphical representation of Escitalopram with (a) latency time and (b) Squares crossed.

Figure 4: Effects of Escitalopram Administration on Activity on (a) no. of entries and (b) time spent of Rats in Light Dark Activity Box.

Values are means $\pm$ SD (n=12) as monitored on next day of 1st, 3rd, 5th and 7^{th}administrations. Significant differences by Newman-Keuls test: *p<0.01, **p<0.01 from respective water administrated controls; +p<0.01 from similarly administrated animals from 1st day following two-way ANOVA (repeated measures design).

DISCUSSION

Particularly serotonin reuptake inhibitors (SSRIs) were at first presented as antidepressants, and their potential as anxiolytic has been seen in the treatment of social fear, post-traumatic anxiety issue, and summed up uneasiness issue (e.g., Nutt et al., 1999). Albeit each of the SSRIs increments extracellular serotonin (5-hydroxytryptamine, 5-HT) in the mind by hindering the 5-HT transporter SERT, they contrast generously as far as their selectivity (Nutt *et al*., 1999; Owens et al., 2001; Sánchez et al., 2003). Activities at other tying destinations, for example, the muscarinic, histamine, adrenergic, and 5-HT2 receptors, may add to a portion of the symptoms normally connected with SSRI treatment. In spite of the fact that when all is said in done, these symptoms are preferable endured over those from tricyclics and benzodiazepines; they are a restriction in the utilization of SSRIs. The motivation behind the present study was to focus the locomotor impacts and anxiolytic movement of escitalopram in animal model of rats. Results from the present study showed that escitalopram administration induced hypophagic condition in rats on repeated administration.

Serotonin is believed to involve in the regulation of many vital activities like food intake, sleep and mood. Therefore alterations in the serotonin levels may leads to disturbance in mood like aggressive and depressive behaviors, increased food craving and insomnia (Costagliola *et al.,* 2008). Repeated escitalopram administration showed increased food intake SSRIs therapy usually reduces the bodyweight. In the

present study, it has been observed that repeated administration of escitalopram to rats at a dose of 10 mg/kg showed significant decreased in body weight. Findings from this study showed escitalopram, showed hyperactivity in home cage. Time required to start movement in open field was increased after repeated administration and also the number of cage crossed is decreased indicating that escitalopram is able to produce hyperactivity in familiar environment but not able to induce exploratory activity in novel (open field) environment. As shown in Figure 3 and 4. Escitalopram is a SSRI and best known for the treatment of GAD (Bielski *et al.,* 2005). In the present study, increased in number of entries and time spent in light box in light dark anxiety model indicates that escitalopram at dose 1mg/ml/kg is sufficient to produce anxiolytic behavior in rats.

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