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ORIGINAL ARTICLE

Anxiolytic profile of fluoxetine as monitored following repeated administration in animal rat model of chronic mild stress

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KEYWORDS

Chronic mild stress (CMS);
Selective serotonin re-uptake inhibitors (SSRIs);
Depression;
Exploratory activity

Abstract *Background:* Fluoxetine, a selective serotonin re-uptake inhibitor (SSRI), has been proposed to be more effective as an antidepressive drug as compared to other SSRIs. After chronic SSRI administration, the increase in synaptic levels of 5-HT leads to desensitization of somatodendritic 5-HT autoreceptors in the raphe nuclei. Chronic stress may alter behavioral, neurochemical and physiological responses to drug challenges and novel stressors. *Methods:* Twenty four male rats were used in this study. Animals of CMS group were exposed to CMS. Animals of stressed and unstressed group were administrated with fluoxetine at dose of 1.0 mg/kg s well as 5.0 mg/kg repeatedly for 07 days 1 h before exposed to CMS. The objective of the present study was to evaluate that repeated treatment with fluoxetine could attenuate CMS-induced behavioral deficits. *Results:* Treatment with fluoxetine attenuated CMS-induced behavioral deficits. Fluoxetine administration induced hypophagia in unstressed as well as CMS rats. Acute and repeated administration of fluoxetine increased motor activity in familiar environment but only repeated administration increased exploratory activity in open field. Anxiolytic effects of fluoxetine were greater in unstressed rats. These anxiolytic effects were produced as result of repeated administration not on acute administration of fluoxetine at 1.0 mg/kg as well as 5.0 mg/kg. *Conclusion:* The present study demonstrated that CMS exposure resulted into behavioral deficits and produced depressive-like symptoms.

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Fluoxetine, an SSRI, administration attenuated behavioral deficits induced by CMS. Anxiolytic effects of repeated fluoxetine administration were greater in unstressed than CMS animals.

1. Introduction

The term stress was originally defined by Hans Selye, in the 1940's (Selye and Fortier, 1949), as the nonspecific reaction of an organism to adverse stimuli. Stress is characterized as an adaptive response (physical, mental or emotional) toward events capable of causing shifts on the homeostasis in the organism, allowing it to maximize its chances of survival when facing a challenge. Berger (1980) defined stress as the total sum of the bodily responses which occurs in response of adaptation of changes by the organisms (Berger, 1980). There is increasing evidence that stress affects health not only through its direct biological effects but also through changes in health behavior that they influence health (Siperoe, 1991; Alder and Mathews, 1994). Stress exposure caused activation of sympathetic nervous system (Dunn and Welch, 1991). Stress can lead to a number of diseases such as as hypertension, anxiety, headache, gastritis, ulcerative colitis, migraine, asthma and depression (Gardner, 1975; Tortora and Anagnostakos, 1990).

It has been reported that chronic mild stress models are comparatively more suitable than acute stress models for investigating depression in experimental models (Katz et al., 1981; Willner et al., 1987). A previous study has reported that exposure to unpredictable stressors induces significant changes in behavioral parameters (Farhan et al., 2014) such as altered locomotive and explorative behavior, a decline in food intake, water intake and sexual activity (Willner et al., 1991). It has also been suggested that chronic mild stress-induced behavioral deficits in experimental animals could be used effectively as an animal model of depression (D'Aquila et al., 2000). In addition to anhedonia, CMS has shown to decrease aggressive and male sexual behavior in rats (D'Aquila and Brain, 1994).

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, ancient groups of antidepressants. (Artigas et al., 2001). Fluoxetine, a selective serotonin re-uptake inhibitor (SSRI), has been proposed as more effective as an antiaggressive drug when compared with other SSRIs (Detke et al., 1995; Contreras et al., 2001). A number of studies have reported that fluoxetine as well effective in treating a wide spectrum of mood disorders including depression, panic disorder and anxiety (Kindler et al., 1997; Mancini and Ameringen, 1996).

After chronic SSRI administration, the increase in synaptic levels of 5-HT leads to desensitization of somatodentritic 5-HT autoreceptors in the raphe nuclei. Both SSRIs and anxiolytic 5-HT receptor agonists can desensitize the somatodentritic 5-HT-1A autoreceptors in the raphe nuclei, and subsequently induce a sustained elevation of 5-HT in the synaptic cleft. However, this desensitization occurs within 3 days of drug administration, a time-course that is shorter than the delayed onset of therapeutic improvement and may correlate with an initial aggravation of anxiety (Boyer and Feighner, 1992; Kahn et al., 1988a,b). Most of the effects induced by CMS can also be reversed by selective serotonin reuptake inhibitors (SSRIs) Willner et al., 1987; Willner, 1997; Isingrini et al., 2010, illustrating a strong predictive validity. Fluoxetine, a SSRI also exhibits antidepressant activity in experimental models (Detke et al., 1995; Contreras et al., 2001) and clinical trials (Stoke and Holtz, 1997; Vaswani et al., 2003). It has been reported that fluoxetine increases serotonergic transmission in synaptic cleft (Stahl, 1996). The present study was designed to evaluate the ability of fluoxetine to reverse CMS-induced depression-like behavior in rats.

2. Materials and methods

2.1. Animals

Locally bred male (180–220 g) albino-Wistar rats purchased from Aga Khan University, Karachi, Pakistan were housed individually under 12-h light and dark cycle and controlled room temperature (25 ± 2 °C) with free access to cubes of standard rodent diet and water, for a period of three days before experimentation.

2.2. Drugs and doses

Fluoxetine, purchased from Merck Company was dissolved in distilled water and administrated orally at a dose of 1 mg/kg as well as 5 mg/kg and control animals were administrated with water by using stainless steel feeding tubes.

2.3. Experimental protocol

Thirty-six animals were randomly divided into two equal groups (i) Unstressed and (ii) CMS. Animals of both groups were further divided into three groups (i) Unstressed-Water (ii) Unstressed-Fluoxetine (1.0 mg/kg), (iii) Unstressed-Fluoxetine (5.0 mg/kg), (iv) CMS-Water (v) CMS-Fluoxetine (1.0 mg/kg) and (vi) CMS-Fluoxetine (5.0 mg/kg). Animals of the CMS group were exposed to a schedule of chronic mild stress shown below over a period of 14 days (Table 1) while animals of unstressed groups remained in their home cages.

Table 1 Chronic mild stress (CMS) schedule.

S.#	Day	CMS	Time
1	Day 1	Exposed to 4 °C for 50 min	11:00 am
2	Day 2	60 min cage agitation (60 rpm)	11:00 am
3	Day 3	60 min restrained stress (wire grid)	11:00 am
4	Day 4	12 h water deprivation	11:00 am–11:00 pm
5	Day 5	3 h light off day time	11:00 am–02:00 pm
6	Day 6	60 min noise stress	11:00 am
7	Day 7	60 min restraint stress (tube)	11:00 am

Water or respective dose of fluoxetine (1.0 mg/kg and 5.0 mg/kg) was given orally to animals each day 1 h before exposing to daily schedule of CMS (Table 1). Locomotor activity was monitored in familiar environment (activity box) and in novel environment (open field) on next day of 1st and 7th stress. Activities in light compartment of light dark activity box and in open arm of elevated plus maze were monitored on next day of 1st and 7th stress.

2.4. Behavioral assessment

2.4.1. Activity box

The assessment of locomotor activity in a familiar environment was done in activity box. Apparatus used in this study was made up of transparent perspex (26 × 26 × 26 cm) with saw dust covered floor. Testing was done in a quiet room under white light as described by Haleem et al, (2007); Ikram et al. (2011), 15 min before monitoring the activity animals were placed in the home cage for habituation. Numbers of cage crossings were monitored for 10 min.

2.4.2. Open field activity

The assessment of exploratory activity in a novel environment was done in an open field apparatus. Open field apparatus used in present investigation consisted of a square area (76 × 76 cm) with walls 42 cm high. The floor was divided by lines into 25 equal squares. To determine the activity rats were placed in the center squarer of the open field. Numbers of square crossed with all four paws were recorded for 5 min.

2.4.3. Light dark box activity

Activity in a light–dark box is used as animal model of anxiety (Shimada et al., 1995). The test was conducted in a locally made compartment box. The compartment of equal size (26 × 26 × 26 cm), with an access (12 × 12 cm) between the compartments, differed in their sensory properties. The coverings and walls of one compartment were light (transparent) and other dark (black). To determine the activity a rat was introduced in the middle of the light compartment of the box. Entries and time spent in the light compartment were monitored for a cut off time of 5 min. Entry into a compartment of the box is defined as the placement of all four paws in the compartment of the activity box (Bourin and Hascoet, 2003).

2.4.4. Elevated plus maze test

The elevated plus maze is also widely used as animal model of anxiety (Pellow et al., 1985). The plus maze apparatus used in the present investigation was specially designed in our laboratory and it consists of four arms in which two were open and two were closed. The arms were of identical length (50 cm) and width (10 cm). Arms were joined by central area of 5 cm^2. The maze was elevated from the floor as a height of 60 cm. To determine the activity a rat was placed in the center of the plus maze and time spent and the entries in the open arm were determined for 5 min.

2.5. Statistical analysis

Values are means ± SD. Data were analyzed by three-way ANOVA (repeated measures design). Software used for the analysis was SPSS (version 17). Post-hoc comparison was done by Newman–Keuls test. Values of $p < 0.05$ were considered as significant.

3. Results

Fig. 1 shows effects of repeated fluoxetine administration on activity in familiar environment (activity box) of rats exposed to CMS as monitored on next day of 1st and 7th stress. Data on number of cage crossing as analyzed by three-way ANOVA (repeated measures design) showed that effects of stress ($F = 120.30$; df = 1, 32; $p < 0.01$), fluoxetine ($F = 52.02$; df = 2, 32; $p < 0.01$), repeated monitoring ($F = 42.45$; df = 3, 32; $p < 0.01$) and the interaction among all the factors ($F = 22.20$; df = 6, 64; $p < 0.01$) were significant. Post-hoc analysis by Newman–Keuls test showed that exposure to CMS decreased number of cage crossed in water administered animals after 7th day of stress. Fluoxetine administration

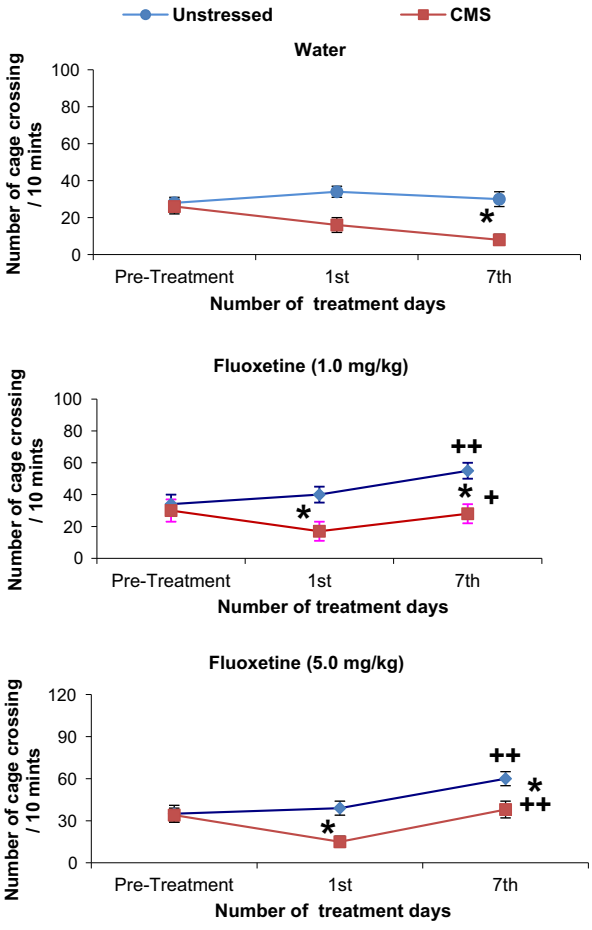

Figure 1 Effects of administration of fluoxetine (1.0 mg/kg and 5.0 mg/kg) of activity in familiar environment in unstressed and CMS rats. Values are means + SD ($n = 6$) as monitored on next day of the administration. Significant differences by Newman–Keuls test: $^{*}p < 0.05$, $^{**}p < 0.01$ from respective unstressed animals; $^{+}p < 0.05$, $^{++}p < 0.01$ from respective water treated unstressed or CMS animals following three-way ANOVA (repeated measure design).

increased activity in activity box of unstressed animals and values were significantly higher after 7th day of administration of 1.0 mg/kg as well as 5.0 mg/kg fluoxetine treated animals. Exposure of fluoxetine administered animals to CMS, resulted to decrease in activity and difference were significant after a week of stress exposure in 1.0 mg/kg as well as 5.0 mg/kg fluoxetine administered animals.

Fig. 2 shows effects of repeated fluoxetine administration on activity in light dark transition box of rats exposed to CMS as monitored on next day of 1stand 7th stress. Data (Fig. 2a) on number of entries in light box as analyzed by three-way ANOVA (repeated measures design) showed that effects of stress ($F = 152.25$; df = 1, 32; $p < 0.01$), fluoxetine ($F = 8.70$; df = 2, 32; $p < 0.01$), repeated monitoring ($F = 21.51$; df = 3, 32; $p < 0.01$) and the interaction ($F = 8.31$; df = 6, 64; $p < 0.01$) were significant. Post-hoc analysis by Newman–Keuls test showed that exposure to CMS decreased number of entries in light box in water treated animals after 7th day of stress. Fluoxetine administration increased activity of unstressed as well as CMS animals as compared to similarly treated water administrated animals but values were not significant.

Data (Fig. 2b) on time spent in light box as analyzed by three-way ANOVA (repeated measures design) showed that effects of administration of fluoxetine ($F = 40.24$; df = 2, 32; $p < 0.01$), effects of repeated monitoring ($F = 28.22$; df = 3, 32; $p < 0.01$), effects of CMS ($F = 113.78$; df = 1, 32; $p < 0.01$) and the interaction among all the factors ($F = 27.16$; df = 6, 64; $p < 0.01$) were significant. Post-hoc analysis by Newman–Keuls test showed that CMS decreased activity in water treated animals after 7th day of stress than unstressed animals. Administration of fluoxetine increased activity in unstressed as well as CMS animals as compared to water administrated unstressed or CMS animals respectively. Values were significant after 7th administration in 1.0 mg/kg as well as 5.0 mg/kg fluoxetine administrated animals. Exposure to CMS decreased activity in fluoxetine administrated animals as compared to similarly administrated unstressed animals and values were significant after 1st and 7th day in 1.0 mg/kg fluoxetine treated animals.

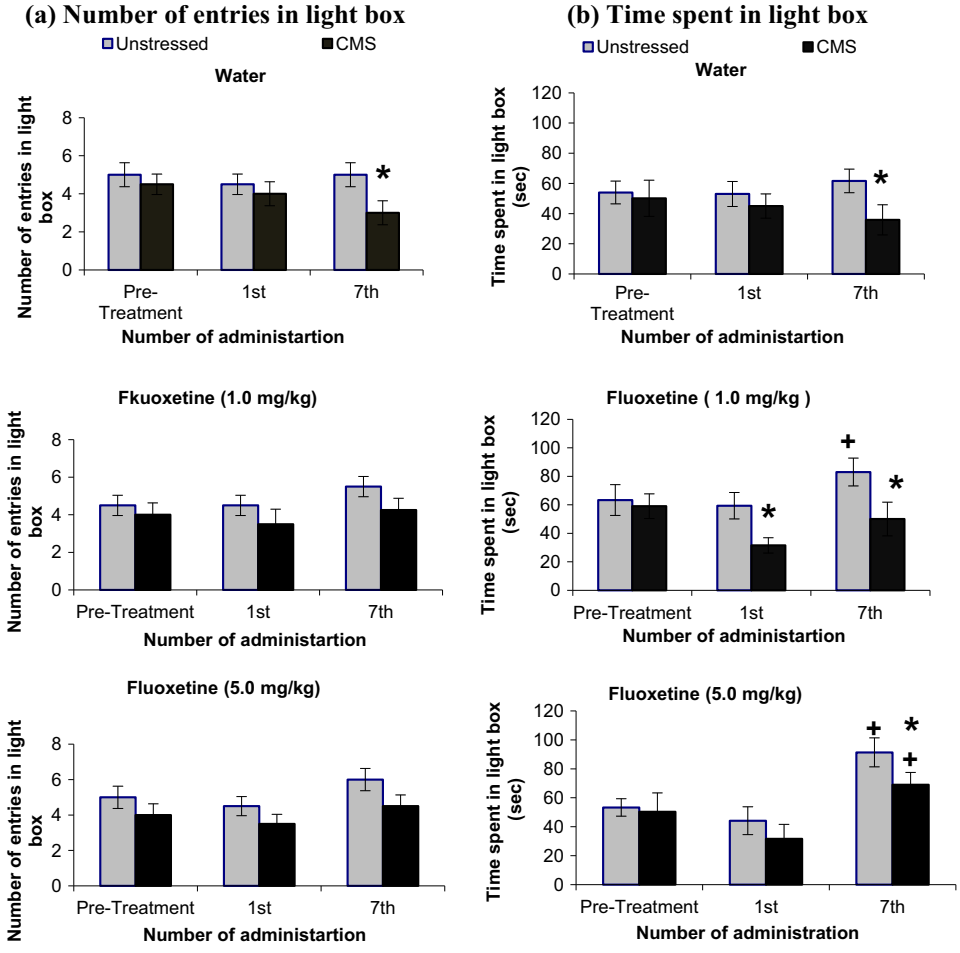

Figure 2 Effects of administration of fluoxetine (1.0 mg/kg and 5.0 mg/kg) on activities in light dark box in unstressed and CMS rats. Values are means + SD ($n = 6$) as monitored on next day of the administration. Significant differences by Newman–Keuls test: $^{*}p < 0.01$ from respective unstressed animals; $^{+}p < 0.01$ from respective water treated unstressed or CMS animals following three-way ANOVA (repeated measure design).

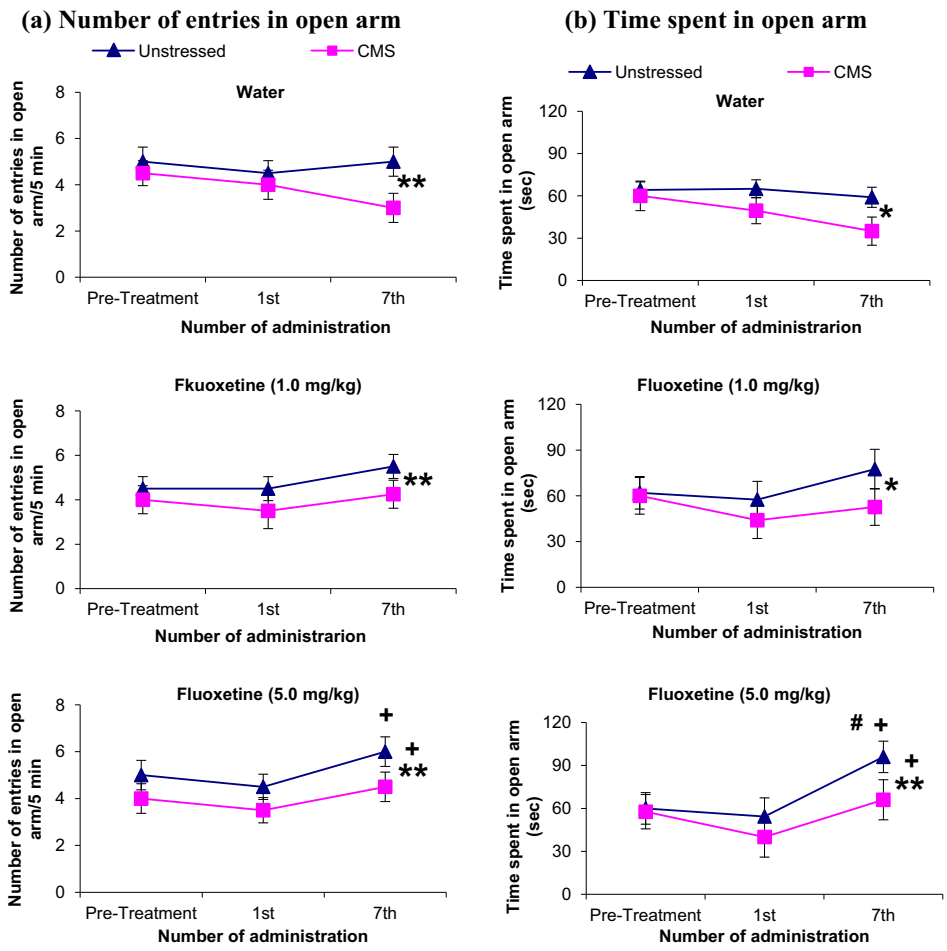

Figure 3 Effects of administration of fluoxetine (1.0 mg/kg and 5.0 mg/kg) on activities in elevated plus maze of unstressed and CMS rats. Values are means + SD ($n = 6$) as monitored on next day of the administration. Significant differences by Newman–Keuls test: $^{*}p < 0.05$, $^{**}p < 0.01$ from respective unstressed animals; $^{+}p < 0.01$ from respective water treated unstressed or CMS animals; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ from respective day 1.0 mg/kg fluoxetine treated unstressed or CMS animals following three-way ANOVA (repeated measure design).

Administration of fluoxetine increased activity in unstressed and CMS animals' then water treated animals and values were significantly higher after one week treatment in unstressed and CMS groups of 1.0 mg/kg as well as 5.0 mg/kg fluoxetine treated animals.

Fig. 3 shows effects of repeated fluoxetine administration on activity in light dark box of rats exposed to CMS as monitored on next day of 1st and 7th stress. Data (Fig. 3a) on number of entries in open arm as analyzed by three-way ANOVA (repeated measures design) showed that effects of repeated monitoring ($F = 20.26$; df = 3, 32; $p < 0.01$), effect of CMS ($F = 114.89$; df = 1, 32; $p < 0.01$), effects of fluoxetine ($F = 51.43$; df = 2, 32; $p < 0.01$) were significant. Interaction among repeated monitoring, fluoxetine administration and CMS ($F = 7.47$; df = 6, 64; $p < 0.01$) were also significant on counts of entries in open arm. Post-hoc analysis by Newman–Keuls test showed that exposure to CMS decreased activity (number of entries in open arm) in water treated animals than unstressed animals after 7th day of stress.

Administration of fluoxetine increased activity of unstressed as well as CMS animals as compared to water administrated unstressed or CMS animals respectively. Values were significantly higher after 7th day of administration in 5.0 mg/kg fluoxetine administrated animals. CMS decreased activity in fluoxetine administrated animals as compared to similarly administrated unstressed animals and values were significant after 7th day of CMS in 1.0 mg/kg as well as 5.0 mg/kg fluoxetine administrated animals.

Data (Fig. 3b) on time spent in open arm as analyzed by three- way ANOVA (repeated measures design) showed that effects of fluoxetine ($F = 30.07$; df = 2, 32; $p < 0.01$), repeated monitoring ($F = 21.09$ df = 3, 32; $p < 0.01$), CMS ($F = 80.74$; df = 1, 32; $p < 0.01$) and the interaction among all the factors ($F = 25.89$; df = 6, 64; $p < 0.01$) were significant. Post-hoc analysis by Newman–Keuls test showed that exposure to CMS decreased activity in water treated animals after 7th day of stress as compared to similarly administrated unstressed animals on the same respective days. Fluoxetine administration increased activity in unstressed and CMS animals than water treated unstressed and CMS animals. Values were significantly higher after 7th of 5.0 mg/kg fluoxetine administrated animals but not significant change was found in 1.0 mg/kg fluoxetine administrated animals. Exposure to CMS decreased activity in fluoxetine administrated animals

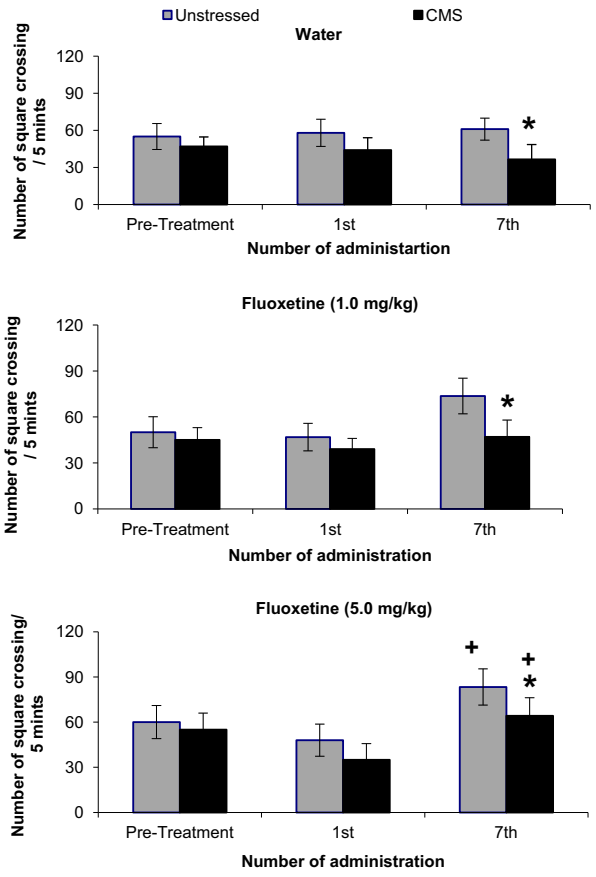

Figure 4 Effects of administration of fluoxetine (1.0 mg/kg and 5.0 mg/kg) on activity in open field in unstressed and CMS rats. Values are means + SD ($n = 6$) as monitored on next day of the administration. Significant differences by Newman–Keuls test: $^{*}p < 0.05$, $^{**}p < 0.01$ from respective unstressed animals; $^{+}p < 0.05$, $^{++}p < 0.01$ from respective water treated unstressed or CMS animals; following three-way ANOVA (repeated measure design).

as compared to similarly administrated unstressed animals and values were significantly smaller after 7th day of CMS schedule in 1.0 mg/kg and 5.0 mg/kg fluoxetine animals. Fluoxetine at dose 5.0 mg/kg increased activity in unstressed animals than similarly treated 1.0 mg/kg fluoxetine administrated animals and activity were significant after a one week administration.

Fig. 4 shows effects of repeated fluoxetine administration on activity in novel environment (open field) of rats exposed to CMS as monitored on next day of 1st and 7th stress. Data on number of square crossing as analyzed by three-way ANOVA (repeated measures design) showed that effects of repeated monitoring ($F = 42.79$; df = 3, 32; $p < 0.01$), fluoxetine ($F = 25.62$; df = 2, 32; $p < 0.01$) and stress ($F = 92.154$; df = 1, 32; $p < 0.01$) were significant. Interaction among CMS, fluoxetine and repeated monitoring ($F = 21.10$; df = 6, 64; $p < 0.01$) were also significant. Post-hoc analysis by Newman–Keuls test showed that exposure to CMS decreased activity in water administrated animals after 7th day of stress. Administration of fluoxetine increased activity in unstressed animals and values were significant after 7th day of administration at dose 5.0 mg/kg. Exposure of fluoxetine administrated animals (1.0 mg/kg as well as 5.0 mg/kg) to CMS decreased activity after 7th day of stress.

4. Discussion

The aim of the present study was to investigate that whether fluoxetine administration could reverse the behavioral deficits induced by chronic mild stress (CMS). In this experiment we used CMS to produce behavioral deficits which are considered to be a valid and useful experimental model of depression (van Eldik and Wainwright, 2003; Surget et al., 2008). Results from the present study show that exposure to CMS reduces food intake, growth rate and locomotor activity as compared to unstressed animals indicating a behavioral consequence of CMS as predicted for an animal model of depression. Willner et al. (1991) have reported that exposure to stressors induced significant changes in behavioral parameters, such as decreased locomotive and explorative activity, a decline in food intake, water intake and sexual activity (Willner et al., 1991). Joca and his colleagues have reported that CMS-induced hypolocomotive effects could be due to the decrease in serotonergic function resulting in the development of depressive symptoms (Joca et al., 2003). In the present study, group of stressed rats showed significant decreases in locomotor and exploratory activities as compared with the control group. In stressed but untreated animals, we observed a decrease in time spent in light box of light dark transition box as well as in open arm of elevated plus maze ant after but difference was significant after 7th day of stress compared with unstressed animal.

A number of studies have reported that fluoxetine and other selective serotonin reuptake inhibitors (SSRIs) produce anorexia in human and experimental animals (Caccia et al., 1992; Clifton et al., 1989; Clifton and Lee, 1997; Currie et al., 1998; Halford et al., 2007; Heisler et al., 1999). SSRI-induced anorexia in thought to result, at least in part, from blockage of the reuptake of serotonin (5-HT) into nerve terminals and a subsequent elevation of extracellular 5-HT in the somatodentritic region which desensitize somatodentritic receptors to increase 5-HT availability in terminal region (Clifton et al., 1989; Heisler et al., 1999; Gobert et al., 1997; Hernandez et al., 1991; Lee and Clifton, 1992; Malagie et al., 1995; Tao et al., 2002; Trillat et al., 1998; Wong et al., 1995).

Serotonergic mechanisms play an important role in the modulation of locomotor activity at a number of levels in the neuroaxis including the spinal cord, the basal ganglia, limbic structures, and in the frontal cortex (Brocco et al., 2002; Geyer, 1996; Wallis, 1994). Results from the present study showed that fluoxetine induced higher activity was more significant in familiar and novel environment at both doses that is low (1.0 mg/kg) as well as high (5.0 mg/kg) in unstressed than CMS animals. SSRIs administered acutely or sub-chronically are known to have limited beneficial effects or even adverse effects on anxiety and depression (Griebel, 1995; Dulawa et al., 2004). However, chronic SSRIs treatments are effective in depressed or anxious patients (Barr et al., 1997; Gelfin et al., 1998) as well as in highly emotional animal models (Dulawa et al., 2004; Popa et al., 2008). Unstressed as well as CMS group animals showed an anxiolytic effect in open field followed fluoxetine administration than saline injected animals. An increase in activity or time spent in the center of

the open field indicates reductions in anxiety and / or increases in exploration (Dulawa et al., 1999). Fluoxetine is devoid of affinity for serotonin receptors (Beasly et al., 1992; Wong et al., 1983), but it acts as an indirect agonist, stimulating multiple 5-HT receptors. Because serotonergic neurotransmission is based on multiple 5-HT receptors types and subtypes, 5-HT-1A-1F, 5-HT-2A-2C AND 5-HT-3-7 (Gothert, 1992; Gothert and Schlicker, 1987; Hoyer et al., 1994; Peroutka, 1991), the study of the specific blockade of 5-HT receptors could be useful to explain the mechanisms of action of this monoamine on learning and memory.

Anxiolytic effects of fluoxetine were monitored in light dark transition box and an elevated plus maze test. We find that repeated administration of fluoxetine produced anxiolytic effects but not on single administration in both unstressed as well as CMS group animals as compared to water administrated control animals. A number of studies have reported that repeated fluoxetine administration leads to a decrease in spontaneous firing activity of serotonergic neurons (Blier et al., 1988; Chaput et al., 1991; Fuller, 1994; Perry and Fuller, 1992).

In conclusion, the present study demonstrated that CMS exposure resulted into behavioral deficits and produced depressive-like symptoms. Fluoxetine, an SSRI, administration attenuated behavioral deficits induced by CMS. Anxiolytic effects of repeated fluoxetine administration were greater in unstressed than CMS animals.

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