



Paroxetine: to meliorate the behavioral deficits-induced by repeated exposure to chronic mild stress in rats

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General Note

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ABSTARCT

Background: Stress is an important precipitant factor for depression. Changes in various body systems that occur in depression are similar to those observed in response to stress. Chronic stress may alter behavioral, neurochemical and physiological responses to drug challenges and novel stressors. Chronic mild stress (CMS) could be used as an animal model of depression. Paroxetine is a

novel phenylpiperidine compound that acts as a selective serotonin reuptake inhibitor (SSRI). It is a more selective and potent SSRI than fluoxetine, sertraline, or fluvoxamine. Paroxetine pharmacokinetics is well suited to clinical use. Stressful conditions possess a complex relationship with brain and body reaction to stress and depression as well. There is some stress evolved depression.

Method: Male rats were divided into two groups, animal of stressed group were exposed to CMS. Animals of unstressed and stressed group were administrated with paroxetine at dose 10 mg/kg/day for 14 days 1 hour before the animals exposed to CMS. The purpose of the present study was to investigate whether paroxetine might be act as antidepressant to reduce the depressive like behavior in animal model of depression.

Results: Chronic mild stress induced behavioral deficits which can be attenuated by repeated administration of paroxetine. CMS exposed animals showed depressive like behavior, which observed to be attenuated by the paroxetine treatment repeatedly. Growth rate was decreased as resultant of paroxetine treatment. Behavioral sensitization monitored in novel and familiar environment as well as anxiolytic activity in light dark and elevated plus maze were higher significantly in unstressed then stressed animals.

Conclusion: Clinical and preclinical studies consistently show that paroxetine alleviates moderate or severe depression and associated anxiety. It begins to act at least as rapidly as the tricyclic antidepressants.

Keywords: Chronic mild stress (CMS), Depression, SSRIs, Paroxetine Locomotor activity, Exploratory activity, Anxiolytic activity.

1. INTRODUCTION

Distressing life occasions may assume a critical part in the etiology of depression (Kenneth *et al.*, 2000). It is accepted that introduction to wild stressors affects a sentiment loss of control which may bring about a depressive behavioral state (Tracey and Benedetta, 2003). The principal and the first chronic mild stress model of depression was developed by Katz (1981). In 1987, Willner and associates built up the ceaseless mellow stretch convention, which incorporates an assortment of low – grade stressors directed over a drawn out stretch of time. Eccentric incessant gentle anxiety models are relatively more appropriate than intense anxiety models for exploring melancholy in trial models (Katz *et al.*, 1981; Willner *et al.*, 1987). Chronic exposure to stressful life events is an established risk factor for the development of many psychological conditions in humans, including major depression. Various animal models of depression are used to study behavioral and neurochemical responses to challenge drugs (Kronfeld-Schor N, Einat H. 2012; Song C, Wang H. 2011). Contrary to repeated exposure to 'similar' stress, chronic exposure to 'unpredictable' mild stress prevents the adaptation to stress and could lead to depression. The first chronic stress model (UCMS) of depression was developed by Kaltz in 1981. In 1987, Willner and colleagues built up the CMS convention which incorporates an assortment of poor quality stressors managed over a drawn out stretch of time (Willner P. *et al.*, 1987). Flighty perpetual gentle anxiety is utilized as a creature model of dejection. The greater part of the impacts can be turned around by particular serotonin reuptake inhibitors (SSRIs) (Willner P. *et al.*, 1987; Willner P. 1997), representing a solid prescient legitimacy. In rodents, CMS (flighty) likewise has great face legitimacy as it can evoke sadness like indications, for example, an absence of sucrose inclination (Willner P. *et al.*, 1987; Pothion S. *et al.*, 2004).

Selective serotonin reuptake inhibitors (SSRIs) are the major and predominant class of antidepressants utilized throughout the most recent decade though antiquated gatherings of most broadly utilized antidepressants were Tricyclic antidepressants (TCA) and monoamine oxidase inhibitors (MAOIs) (Artigas F. *et al.*, 2001). Paroxetine is a novel phenylpiperidine intensify that goes about as a specific serotonin reuptake inhibitor (SSRI). It is a more particular and intense SSRI than fluoxetine, sertraline, or fluvoxamine. Paroxetine pharmacokinetics is appropriate to clinical use. Its half-life is roughly 24 hours, and it has no dynamic metabolites. Likewise with different SSRIs, there are few clinically critical medication connections with paroxetine. Upsetting conditions have an unpredictable association with cerebrum and body response to stretch and despondency also. There are some stress developed depressions. Stress may bring about sadness has been idea in numerous exploration papers, incessant gentle anxiety instigated behavioral shortfalls which can be constricted by rehashed organization of paroxetine. Clinical and preclinical concentrates reliably demonstrate that paroxetine lightens moderate or extreme misery and related nervousness. It starts to act in any event as quickly as the tricyclic antidepressants. Creature information proposes relative wellbeing in overdose and no confirmation of teratogenicity. Likewise with different SSRIs, the most widely recognized symptom of paroxetine is queasiness, which is normally very much endured. The sickness infrequently prompts drug stopping or even measurement diminishment. Little weight reduction or weight pick up happens with paroxetine at dosages used to treat sorrow, and the medication has low impact on the seizure limit. Not at all like different SSRIs, has paroxetine had a moderately low occurrence of nervousness and tumult. There is no proof that paroxetine increments self-destructive ideation.

Stress-instigated changes in brain reward circuits build the affectability to the strengthening properties of medications; in this way expanding the inspiration to utilize tranquilizers impulsively (Koob GF. *et al.*, 1997). Presentation to electric foot-stun stretch additionally expanded the resulting fortifying viability of heroin (Shaham, Stewart. 1994) and morphine (Will MJ. *et al.*, 1998) in rats. Past works (Haleem, Parveen. 1994; Haleem. 1999; Haque Z. *et al.*, 2013; Kennett. *et al.*, 1985; Calvez J. *et al.*, 2011; Valles A. *et al.*, 2000) have reported that introduction to a scene of 2-hr immobilization stress diminished 24-hr combined sustenance admission and body weight in rats. The creatures displayed nervousness/discouragement like conduct in light dull move test, hoisted in addition to labyrinth test (Haque Z. *et al.*, 2013; Haleem. 2011a; Suvrathan A. *et al.*, 2010) and constrained swimming test (Suvrathan A. *et al.*, 2010; Synder JS. *et al.*, 2011). The deficiencies in nourishment admission and different practices no more continued upon rehashed immobilization (Haleem, Parveen. 1994; Kennett G. *et al.*, 1985; Valles A. *et al.*, 2000; Gil M. *et al.*, 1992). It was proposed that rehashed presentation to the same stressor produces versatile changes that prompted behavioral resilience (Haleem 2011a). Drug-affected excessive touchiness of motivational hardware is recommended to intervene an expansion in medication "needing," moving recreational medication use to obsessive misuse showed by addicts (Berridge KC. *et al.*, 2009). Despite the fact that refinement and restoration include covering neuronal hardware, neurotransmitters and their receptors yet the association of sharpening in reestablished drug looking for conduct stays dubious (Vanderschuren, Pierce. 2010; Kalivas PW. *et al.*, 2006). Behavioral sharpening be that as it may remains a valuable model for deciding neural premise of fixation and neuro-adjustments connected with the behavioral refinement are considered as introductory stride in the medication dependence.

The present study will comprehend the cooperation of stress and fixation at behavioral and neurochemical level to comprehend whether wild life occasion stress prompts enslavement and utilization of addictive medications produces discouragement or anxiety actuated melancholy prompts compulsion.

2. METHODS AND MATERIALS

Animals

Locally bred male albino- Wister rats of weight 180-220 g purchased from the Dow University of Health and Sciences, Karachi, Pakistan, were housed individually under 12- h light and dark cycle and controlled room temperature (25 ± 2) with free access to cubes of standard rodent diet and water for at least 3 days before experimentation.

Drugs and Doses

Paroxetine (Sigma, St. Louis, USA) was dissolved in saline (0.9% NaCl) and administrated orally through a stainless steel feeding tube at dose of 10 mg/kg /day to the respective group animals. Drug was freshly prepared before starting the experiment. Saline (0.9% NaCl solution; 1ml/kg) was administrated to control animals.

Experiment Protocol

Twenty four male rats were randomly divided into two groups: (i) Unstressed and (ii) chronic mild stress (CMS) groups. Animals of the stress group were exposed a schedule of CMS (table 1) shown below over a period of 14 days, while animals of unstressed groups remained in their home cages. After exposure to CMS the animals were again subdivided into two groups each i.e. Saline- and Paroxetine administrated. This resulted in a total of four groups: (i) Unstressed- Saline, (ii) Unstressed- Paroxetine, (iii) CMS- Saline and (iv) CMS- Paroxetine administrated animals. Animals were administrated accordingly with paroxetine (10 mg/kg/day) or saline (1.0 mg/ml) 1 hr before exposed to CMS schedule for next 14 days. Food intake and activity in familiar and novel environment were monitored on next day of 1st, 7th and 14th day of stress. Anxiolytic behavior were monitored in light dark transition box on next day of 1st and then weekly of paroxetine administration.

Behavioral Assessment

1. Growth Rate

Body weight changes were monitored to find out the effect of treatments. Daily or weekly growth rate changes were calculated as percentage of starting day weight (experiment day body weight/starting day body weight) X 100.

2. Home cage activity

The assessment of locomotor activity and exploration in a familiar environment as it may be altered by Unpredictable chronic mild stress and by apomorphine repeated administration was done by home cage activity test. The apparatus used in this study is a

rectangular perspex activity cage consists of small square area (26x26x26 cm) with a saw dust covered floor. Testing was done in a quiet room under weight light as described by Haleem (Haleem DJ. *et al.*, 2007).

3. Open field activity

The assessment of locomotor activity and exploration in a novel environment was done by open field activity test. The test consists of measuring the activity of rats in an open novel space, from which escapee is prevented by a surrounding wall (Haleem, Batool, 1996).

4. Light Dark Box

The light/dark transition test or anxiolytic activity was developed by Crawley and Colleagues (Leng G and Russel JA, 1998). It is used as measure of anxiety (Shimada et al, 1995). Apparatus consist of two equal compartments with size (26x26x26 cm), floored with the saw dust, one of the compartment is exposed into the light and second compartment is in dark. To monitor the activity a rat is place in the centre of the light compartment of the box. Entries and time spent with all four paws in the light compartment were monitored for a cut off time of 5 minutes (Bourin and Hascoet, 2003). Increased number of entries and time spent in light compartment are used as indicator of reduced anxiety states (Imaizumi et al, 1994).

Statistical Analysis

Values are presented as means \pm SD. Data on drug administration of unstressed and stressed rats 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.

Table 1 Chronic mild stress schedule (CMS)

S. No	Chronic Mild Stress	Days	Duration
1.	Cold Stress (4 C)	Day 01 & Day 08	09:00 am -11:45 am
2.	Cage Agitation	Day 02 & Day 09	10:00 am - 01:30 pm
3.	Noise Stress	Day 03 & Day 10	09:45 am -12:35 pm
4.	Tilted Cage	Day 04 & Day 11	11:25 am -02:25 pm
5.	Wet Cage	Day 05 & Day 12	09:05 am -12-15 pm
6.	Crowding	Day 06 & Day 13	11:30 am -01:45 pm
7.	Repeated Light Dark Cycle	Day 07 & Day 14	09:30 am - 12:30 pm

3. RESULT

Figure 1 showed the effect of administration of Paroxetine (100 mg/kg/day) administration on animals exposed to chronic stress on growth rate as monitored on next day of 1st, 7th and 14th day of stress. Analysis of data on %change in body weight done by three – way ANOVA (repeated measures design) revealed that the effects of repeated monitoring ($F=41.59$; $df=2, 21$; $p < 0.01$), the effects of drug administration ($F=52.74$; $df=2, 21$; $p < 0.01$) were significant. However, the effects of stress ($F=20.54$; $df=2, 21$) and the interaction between drug treatment, days and stress ($F=0.97$; $df=2, 21$) were found to be non significant. Post-hoc analysis by Newman-Keuls test shows that paroxetine decreased growth rate on repeated administration in unstressed as well as stressed animals as compared to saline administrated unstressed and CMS animals. Significant difference was found in unstressed animals after 7th * ($p < 0.05$) and 14th ** ($p < 0.01$) day of paroxetine administration.

Figure 2 showed the effect of repeated administration of Paroxetine (100 mg/kg/day) on animals exposed to chronic stress on locomotor activity (number of cage crossed) in familiar environment as monitored on next day of 1st, 7th and 14th day of stress. As the data on number of cage crossed analyzed by three – way ANOVA (repeated measures design) revealed significant effects of repeated monitoring ($F=117.78$; $df=13, 21$; $p < 0.05$), paroxetine ($F=97.11$; $df=1, 21$; $p < 0.05$), CMS ($F=130.96$; $df=1, 21$; $p < 0.01$) and the interaction among the all variables ($F=110.15$; $df=13, 21$; $p < 0.05$). Post-hoc analysis by Newman-Keuls test shows that in unstressed animals, administration of paroxetine increased the locomotor activity. Significant increased in locomotor activity was found after 7th day ** ($p < 0.01$) as well as 14th day ** ($p < 0.01$) of administration, while in stressed animals administration of paroxetine also increased locomotor activity, significant increases in locomotor activity was found after 7th day ** ($p < 0.01$) and 14th day ** ($p < 0.01$) of administration. As compared to unstressed animals administration of paroxetine decreased the locomotor activity of stressed animals, significant decreased in motor activity was found after 7th day + ($p < 0.05$) and 14th day ++ ($p < 0.01$).

Figure 3 revealed the effect of paroxetine administration for 14 days at dose 100 mg/kg/day on animals exposed to chronic mild stress on exploratory activity as monitored in novel environment on next day of 1st, 7th and 14th day of stress. Analysis of data on number of square crossed was done by three – way ANOVA (repeated measures design), showed significant effects of days ($F=88.30$; $df=2, 21$; $p < 0.05$), stress ($F=113.67$; $df=1, 21$; $p < 0.05$) and the interaction between stress, days and drug ($F=127.75$; $df=2, 21$; $p < 0.05$), while the effects of drug ($F=58.51$; $df=1, 21$) was found to be non significant. Post-hoc analysis by Newman-Keuls test shows that in unstressed animal administration of paroxetine increased the activity (number of square crossed). Significant increased in activity (number of square crossed) was found after 14th day ** ($p < 0.01$) of administration, while in stressed animals administration of paroxetine also increased the activity (number of square crossed), increased significantly ** ($p < 0.01$) was found after 7th day and 14th ay of administration. As compared to unstressed animals, administration of paroxetine decreased the activity (number of square crossed) of stressed animals, significant decreased in activity (number of square crossed) was monitored after 1st day ++ ($p < 0.01$), 14th day + ($p < 0.05$) as well as 14th day ++ ($p < 0.01$), also in comparison with saline treated unstressed animal, number of square crossed activity were decreased in saline treated stressed animals, significant decreased in activity was found after 1st day ++ ($p < 0.01$), 7th day ++ ($p = 0.01$) and 14th day ++ ($p < 0.01$).

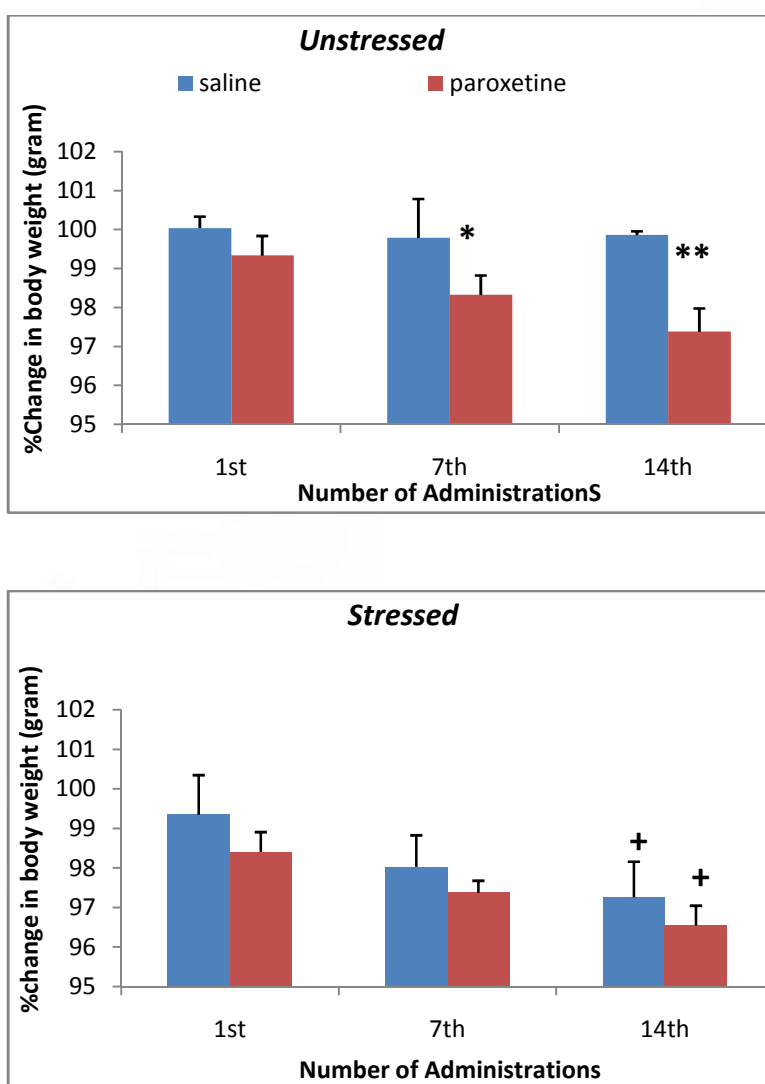


Figure 1

Effects of administration of paroxetine (10 mg/kg/day) on growth rate in unstressed and CMS animals. Values are means \pm SD ($n=06$) as monitored on next day of 1st, 7th and 14th day of administration. Significant differences by Newman-Keuls test: * $P < 0.05$, ** $p < 0.01$ from respective saline administrated unstressed or stressed animals; + $p < 0.05$, ++ $p < 0.01$ from similarly saline or paroxetine administrated unstressed animals of same day following three-way ANOVA (repeated measures designs)

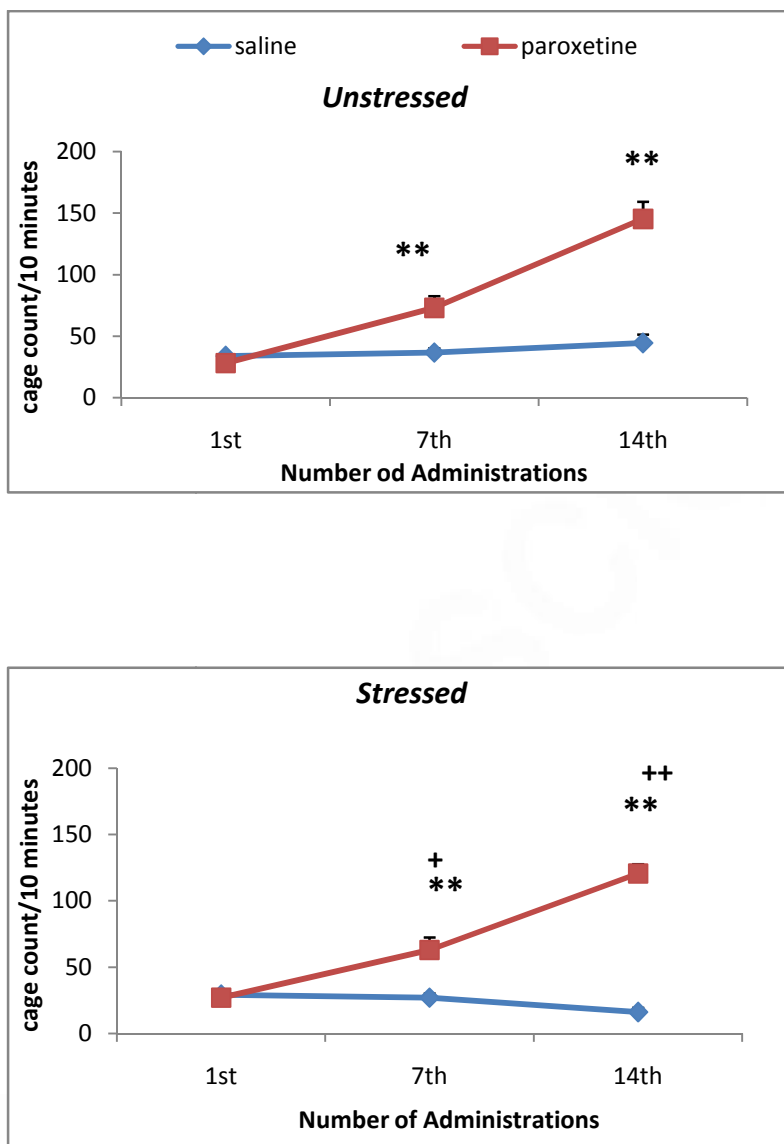


Figure 2

Effects of administration of paroxetine (10 mg/kg/day) on number of cage crossing in the activity box of unstressed and CMS animals. Values are means \pm SD (n=06) as monitored on next day of 1st, 7th and 14th day of administration. Significant differences by Newman-Keuls test: *P<0.05, **p<0.01 from respective saline administered unstressed or stressed animals. +p<0.05, ++p<0.01 from similarly saline or paroxetine administered unstressed animals of same day following three-way ANOVA (repeated measures designs).

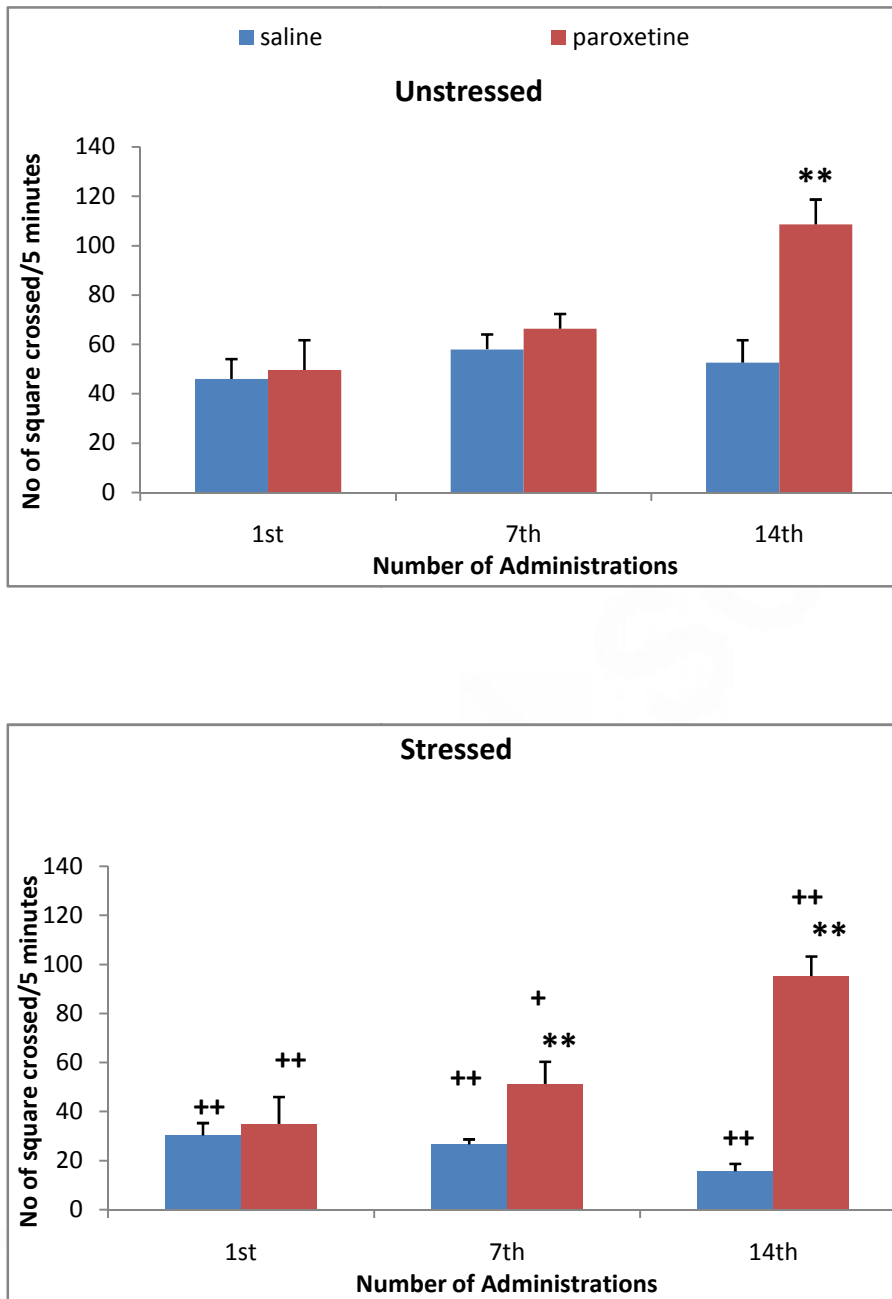


Figure 3

Effects of paroxetine administration (10 mg/kg/day) on activity (number of square counts) in open field of unstressed and CMS animals. Values are means \pm SD (n=6) as monitored on next day of 1st, 7th and 14th day of administration. Significant differences by Newman-Keuls test: *P<0.05, **p<0.01 from respective saline administered unstressed or stressed animals. +p<0.05, ++p<0.01 from similarly saline or paroxetine administered unstressed animals of same day following three-way ANOVA (repeated measures designs).

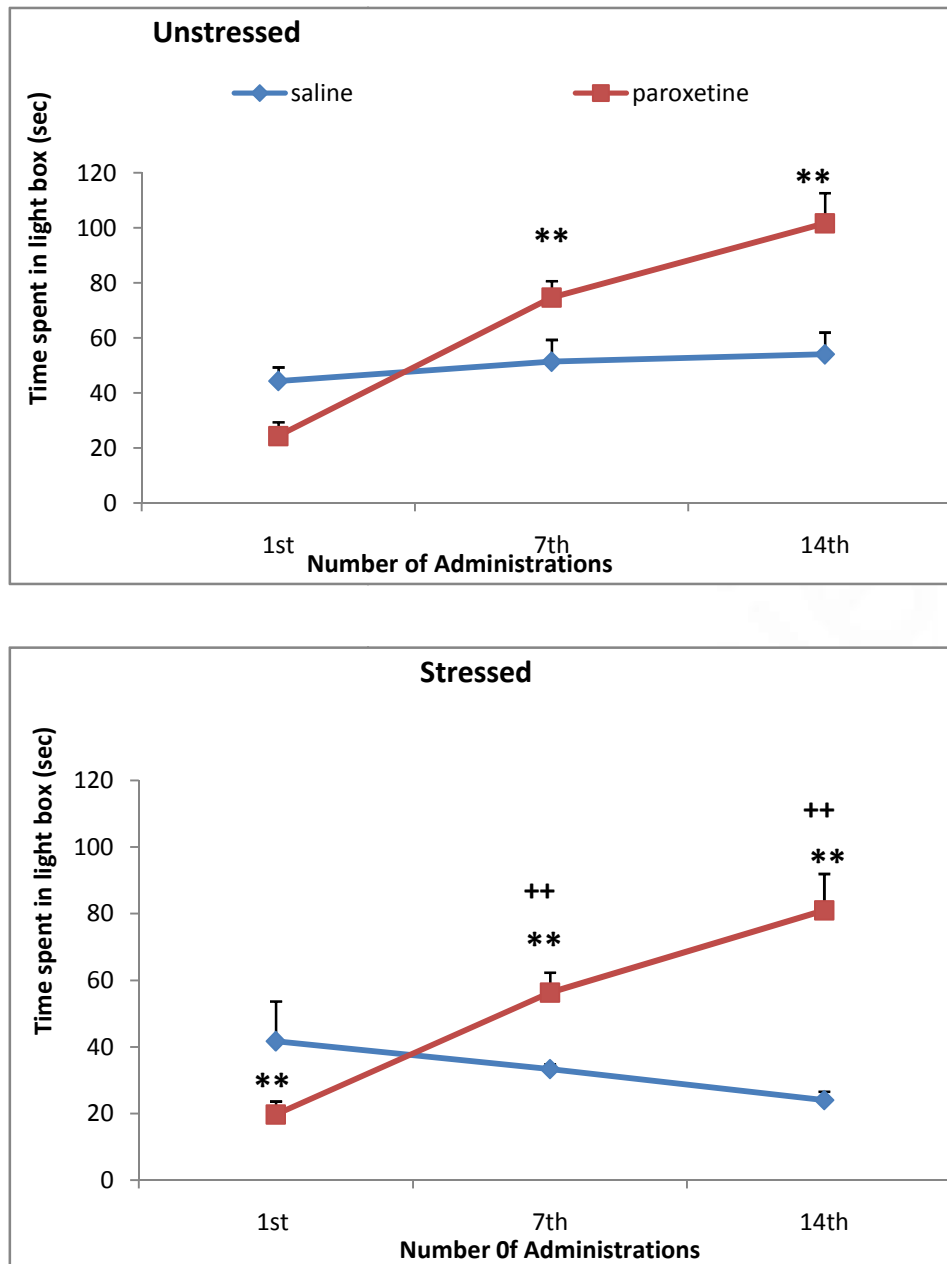


Figure 4

Effects of administration of paroxetine on activity (time spent in light box) in light dark transition box of unstressed and stressed animals. Values are means \pm SD ($n=6$) as monitored on next day of 1st, 7th and 14th day of administration. Significant differences by Newman-Keuls test: * $P<0.05$, ** $p<0.01$ from respective saline administered unstressed or stressed animals. + $p<0.05$, ++ $p<0.01$ from similarly saline or paroxetine administered unstressed animals of same day following three-way ANOVA (repeated measures designs).

Figure 4 determined the effect of administration of Paroxetine (100 mg/kg/day) on animals exposed to chronic stress on activity in light dark box as monitored on next day of 1st, 7th and 14th day of stress. Analysis of data on time spent in light compartment was done by three-way ANOVA (repeated measures design). The significant effects of days ($F=128.39$; $df=2, 21$; $p<0.05$), drug ($F=73.53$; $df=1, 21$; $p<0.05$), stress ($F=98.37$; $df=1, 21$; $p<0.05$) and the interaction between stress, days and drug ($F=158.88$; $df=13, 21$; $p<0.05$) were found. Post-hoc analysis by Newman-Keuls test shows that in unstressed animals, administration of paroxetine increased the time spent in light box. Significant increased in activity was found after 7th day ** ($p<0.01$) as well as 14th day **

($p < 0.01$) of administration, while in stressed animals administration of paroxetine also increased activity (time spent in light compartment), significant increases in activity was found after 1st day ** ($p < 0.01$), 7th day ** ($p < 0.01$) and 14th day ** ($p = 0.01$) of administration. As compared to unstressed animals administration of paroxetine decreased the activity of stressed animals, significant decreased in motor activity was found after 7th day ++ ($p < 0.01$) and 14th day ++ ($p < 0.01$), no significant result was monitored in comparison with unstressed saline to stressed saline.

4. DISCUSSION

The current analysis was done to investigate whether repeated administrations of Paroxetine at dose 10 mg/kg/day could reverse the behavioral deficits induced by chronic mild stress in rat model of depression. Stressful conditions possess a complex relationship with brain and body reaction to stress and depression as well. There are some stress evolved depressions (Kioukia-Fougia *et al.*, 2002). Stress may cause depression has been concept in many research papers. It is well known that Serotonergic and Noradrenergic neurons originating from the cell bodies in the brain stem and expended to many brain regions may be involved in some of the symptoms associated with depression. Antidepressant drugs are used for depression its acting on Serotonin that are involved in the beneficial effects on anxiety and moods in depressed patients (Montgomery, 1995, 1997). Previous study has revealed that 5-HT reuptake inhibitors are now considered as the most prescribed antidepressant (Gordon M *et al.*, 2013). Paroxetine is an antidepressant compound through its inhibiting effect on uptake of the 5-hydroxy-triptamine neurotransmitter. Paroxetine usually prescribed to treat major depression and other psychological pathologies that include panic disorder, obsessive-compulsive disorder, social anxiety, generalized anxiety disorder and posttraumatic stress syndrome. This drug also used for chronic headaches, tingling in the hands and feet caused by diabetes and bipolar disorder. Paroxetine is a potent serotonin (5-HT) reuptake inhibitor (Petersen *et al.*, 2002).

Activity in familiar environment determined the loco motor pattern of rats in familiar environment under the influence of stressful and stress free conditions (K. Ganea *et al.*, 2007). The present study shows that increased in loco motor activity of paroxetine treated animals in both, stress free as well as stressful conditions as compared with saline (Figure 3). Stressed rats shows less activity in activity box than unstressed because stress cause fatigue, depression and hopelessness etc. Whereas by the treatment of antidepressant particularly SSRIs, the distress can be reversed back. Paroxetine acts on serotonin receptor 5HT1A, 5HT1B AND 5HT2C. 5HT1A act on anxiety and mood behavior (Park CL *et al.*, 1998). It was observed that the loco motor activity in both stressed as well as unstressed animals was increased in paroxetine treated animals as compared to saline treated animals. Furthermore a decreased in paroxetine treated stressed rats locomotion was observed than in unstressed paroxetine treated rats.

Hall in 1934 described the open field test for the emotionality in rats. A rodent is placed in novel environment from which escape is prevented (Walsh and Cummins 1976). This study shows the antidepressant effect of SSRIs. In open field (Figure 4) number of square crossed were monitored, increased in square count in paroxetine treated unstressed rats as compared to saline treated unstressed animals as well as increases in square count in paroxetine administrated stressed rats. This is due to the effect of paroxetine as an antidepressant in novel environment which increases number of count in stressed rats.

The light and dark box consists on the inborn aversion of rats to the brighter area and on the exploratory behavior in response to light and novelty (Crawley and Goodwin, 1980). Our study shows that, the time spent in light area was observed clearly more in paroxetine treated unstressed and stressed rats than unstressed and stressed saline treated rats (Figure 5) because of antidepressant effects of paroxetine, its increases the time duration of rats in illuminated novel area. The exploration and present time spent seems to be best measure in each compartment. The mood, anxiety and memory are improved by the agonist of 5-HT 1A, 5HT 1B and 5-HT 2C receptors and Paroxetine is found to be the best candidate as a SSRIs antidepressant.

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