Vol 1| Issue 1 | Jul –Dec 2010 |54-63.

Research Article

# Chronic Unpredictable Stress: Possible Animal Model of Comorbid Depression

**[*]DILIP KUMAR PANDEY, [1]DIPANWITA PATI, [1]ABHAYRAJ JOSHI, [2]RADHAKRISHNAN MAHESH**

[*,1]Pharmacy Group, FD-III, Birla Institute of Technology & Science, Pilani, Rajasthan-333031, India.
[2]Professor and Group Leader, Pharmacy Group, FD-III, Birla Institute of Technology & Science, Pilani, Rajasthan-333031, India

**Abstract**

Chronic stress is a causative for the development of many psychopathological syndromes in humans, including major depression and anxiety disorders. There is a high degree of comorbidity of depression and anxiety. Therefore, the current study investigated whether the CUS can produce comorbid depression and anxieties like behaviour in rats.Rats were subjected to an experimental setting of CUS for 14 days. Escitalopram (5-20 mg/kg per oral, p.o) was administered to stressed and sham rats for 14 days during stress period. Following the CUS and treatment, rats were subjected to a rodent's behavioural test battery (depression/anxiety). The results revealed that, CUS rats significantly exhibited depression-like behaviour in open field exploration, socio-sexual interaction tests. In addition, an anxiety-like behaviour was observed in neophobic situation like elevated plus maze and social interaction test. Further chronic treatment with escitalopram prevented the CUS induced depression and anxiety like behaviour in rats. CUS induced behavioural deficits in rats were comparable to the element of comorbid depression and anxiety in humans. The present findings provide the information that, CUS can be the model for comorbid depression associated anxiety.

**Key words**: Escitalopram, Chronic stress, comorbid, depression, anxiety, behavioural test battery

## INTRODUCTION

Chronic stress is generally considered as a key risk factor for the development of a variety of human ailments. Specifically anxiety and depressive disorders have been frequently associated with stressful life events [1,2]. Activation of the stress system leads to behavioural and peripheral changes that adjust homeostasis and improve coping with stress situations. On the other hand, a lack of adaptation to excessive demands can lead to the development of pathological syndromes, such as depression and anxiety. Chronic stress affects brain areas such as hippocampus, amygdala and prefrontal cortex, involved in anxiety and affective disorder, evidenced in postmortem and brain imaging studies of depressed and anxious patient [3,4]. The neurochemical pathways in central nervous system have been reported to play a vital role in the regulation of stress responses [5] such as diminish the serotonergic transmission in the prefrontal cortex [6] postulated to involved in pathogenesis of depression and anxiety. The increased depression and anxiety like behaviour following CUS may be related to the dysfunction of serotonergic neurotransmission in the prefrontal cortex and limbic system, although involvement for glutamatergic system have also been reported [7,8]. Several lines of evidence suggest that, depletion of monoamines : serotonin [9], noradrenalin [10] and dopamine [11] sustained stress could be the reason for anxiety and behavioural depression [12,13].

In support of the behavioural consequences post chronic stress, animal studies have revealed that, chronic exposure to acute or chronic can modify the activity of neuroendocrine and neurotransmitters system that affect behaviour profile indicative of human psychopathology [14]. Previous studies reported

[*]**Corresponding Author Mr.Dilip Kumar Pandey E mail:** pandeysdl1408@gmail.com

reported that, chronic stress procedure using variable stressor, 1-2 times per day for 7-54 days can model long term human stress exposure (Buwalda et al., 2005; Zurita et al., 2000). A rodent exposed to unpredictable stress procedure induces emotional or psychic states including anxiety, anhedonia, enhanced fear and depression [15].

Chronic stress is the causative for the development of comorbid depression and anxiety disorders. Comorbid depression and anxiety disorders [16] are very commonly associated with more impairment than either pure anxiety or depression [17]. The traditional view postulates anxiety and depression as separate and distinct disorders. However, several clinical studies have shown a great overlapping between depression and anxiety states. In particular, strong evidence highlights high anxiety trait as an important risk factor for the development (onset, severity and outcome) of depression [18]. Although several preclinical studies attempting to model aspect of depression and anxiety [19] was done using chronic stress.

A critical link in successful translational research in the development of animal models of traumatic brain injury is required that can provide a platform to correlate meaningful functional outcome measures with well-characterized behaviour viz depression and anxiety is envisaged. In the light of preceding information, the study was devised to describe more completely, the nature of the relationship between particular neuro-behavioural symptoms in post-CUS Earlier attempt to design comorbid condition using forced swim test (FST) followed by elevated plus maze (EPM) and in reverse EPM followed by FST was inadequate, how ever the best methodology to induce co-morbidity may be to perform the tests after repeated stress period [20]. Thus the prime objective of the present experiment was to design a model that can simulate comorbid depression and anxiety in rodents using chronic unpredictable stress. Escitalopram, a selective serotonin reuptake inhibitor (SSRI), with potential antidepressant and anxiolytic activity in both humans [21] and rodent's [22] was selected for reversal of CUS induced behavioural anomalies. In order to probe the behavioural similarity between CUS rat model and depression, symptomatology-based research was performed by reconstructing the symptoms of depression (psychomotor agitation and loss of interest and the symptoms of anxiety in anxiety-comorbid depression. Another goal of this work is to provide additional information regarding the neuro-psychological and functional out come of escitalopram in a rodent test battery involving exposure to novel stimuli following CUS.

## MATERIALS AND METHODS

### *Drugs and Chemicals*

Escitalopram was procured form Glenmark research centre Mumbai, India as generous gift sample. Estradiol Valerate and Progesterone was purchased from German Remedies, Mumbai and Tetragon Chemie Pvt .Ltd Bangalore, India respectively. Escitalopram (5, 10 and 20 mg/kg) was freshly prepared in distil water before administration. The control group was administered distilled water (10ml/kg per oral, p.o.) daily.

*Animals housing and Grouping*

Male Wistar rats (approximately 200–240 g weighing at the time of study) were obtained from Hissar Agricultural University, Haryana, India and maintained in standard laboratory conditions. The animals were housed in colony cages under standard light (lights on from 7:00A.M. to 7.00P.M.), temperature (22 ±2ºC), and room humidity (60% ± 5%) conditions for at least two weeks before the experimental sessions. The experimental procedures were in compliance with the Institutional Animal Ethics Committee (IAEC) of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/4/1, dated 13.08.08).The rats were used only once for each experiment. All efforts were made to minimize animal pain, suffering or discomfort. Forty eight rats were used in the present study. Rats were divided into the following eight experimental groups : Sham group, sham+ ESC (5), Sham+ ESC (10), Sham+ ESC (20) and CUS group, CUS + ESC (5), CUS + ESC (10), CUS + ESC (20) , (n=6/group).

### Experimental Design for Rats

***Stress Procedure***

Exposure to a single severe or repetitive, uncontrollable stressor may trigger or facilitate the development of psychopathologies. Major depressive disorder is one among these illnesses known to result from an interaction between environmental stressors and genetic/developmental predispositions [23]. There fore the CUS model has been adopted for this study. The stress procedure was performed as described [24] with substantial modification. This animal model of stress consists of chronic exposure to variable unpredictable stressors, none of which is sufficient alone to induce long-lasting effects. The stressors used vary and they were applied in a different sequence each week to avoid any habituation. Each animal received one stress per day individually. The CUS procedure was then applied for 14 consecutive days (Detail in table 1). This paradigm was devised to maximize unpredictability, in that the stressors were applied in apparently random order and at varying times. The procedures were carried out in an isolated room adjacent to the rat housing unit, requiring minimal handling or transport of the rats. After each stressor, animals were kept in a recovery room for 1 h, following which they were placed in clean cages with fresh bedding and returned

to the housing facility. Sham rats were individually housed for the same period of time, and were handled daily for 30 s in the housing room, but were not stressed. Details of stressor are as follows: for 1 hr restraint, rats were placed in a restraining device made of plexiglass and flexible nylon, thus restricting movement but allowing free respiration and air circulation. Rotation procedure was carried out by placing rats in rotating spinner (50 rpm) for 1hr. Four hour of wetting was done by keeping rats in cage with wet husk (5 cm high). Warm swim and cold swim were accomplished by placing the rat in a cylindrical tank (60 cm height x 30 cm diameter) filled with water to a 30 cm depth at 45 and 8°C, respectively. Electric footshock consisted of 1.5 mA scrambled shock delivered through the grid floor of a chamber enclosed within a skinner box (30 s on, 120 s off, for 10min). Inversions of light and dark cycle were performed by placing the rats in dark in day time and light in night time. Finally, tail pinch involved placing the rat in the previously described restraining device, and applying a clothespin 2 cm from the base of the tail for 20 min. Isolation housing was done by keeping each rats in different home cage individually (4h). Behavioural tests were started after one day of chronic stress protocol to pass up the acute effect of CUS.

*Schedule for Drug administration and Behavioural tests*

The schedule drug administration and behavioural test is shown in Table. 2 [25]. The administration of escitalopram (5-20mg/kg/10ml p.o, daily) was started from day 1- 14. After the rats were treated with escitalopram or vehicle for 14 days, the first behavioural test was performed. One hour before behavioural assessment, the rats were acclimatized to testing room, which is free form any disturbance. The behavioural testing was done at 20h after the last dose in order to avoid acute effect of treatment. Drug treatment was continued till 17$^{th}$ day of the study. Behavioural models, incorporating repeated exposure to stress have been widely used as experimental models for depression because stress is thought to play an important role in the etiology of depression and anxiety.

**Behavioural Assays**

***Open field Test***

The open field exploration was performed as described [26]. The apparatus consisted of a circular (90-cm diameter) arena with 75-cm high aluminum walls and white floor equally divided into 10 cm squares. A 60 W light bulb was positioned 90 cm above the base of the arena, which was the only source of illumination in the testing room. During the test each animal was individually placed in the center of the open field apparatus and the following parameters were recorded for 5 min by trained observer unaware of the specific treatments. Ambulation scores (number of squares crossed), number of rearing episodes and fecal pellets were recorded. Each rat was transported, one hour before to the testing room using the home cage. After each test, the apparatus was sprayed with dilute alcohol and wiped thoroughly to eliminate the residual odor. Testing was performed in a temperature, noise and light controlled room.

*Socio-Sexual interaction test*

The present protocol was adapted from that of Breigeiron et al. (2002), Meyerson and Hoglund (1981) [27,28], performed with slight modification. The apparatus for the socio-sexual interaction test consisted of a transparent Plexiglas box [45 cm (L) ×30 cm (W) ×40 cm (H)] with a black plastic base, illuminated with a red lamp (10 W). On the 18$^{th}$ day post CUS, socio-sexual behaviour was observed from 22:00 to 3:00 h in the dark phase of the illumination cycle. A male rat was first placed into the plexiglas box to be habituated to the environment for 5 min. Then, a sexually receptive female rat which had received subcutaneous injections of 0.14mg of estradiol 72 and 48 h before the test and 0.7mg of progesterone 4 h before the test. The sexual behaviour of the male rats was observed for 20min following 5 min of male rat acclimatization. The following parameters of sexual behaviour were recorded: starting latency of genital-probing (licking and grooming female rats) and thrusting, number of genital-probing and thrusting, and pursuit (duration of male rat following female rats) by male rat. After each test, the apparatus was sprayed with dilute alcohol and wiped thoroughly to eliminate the olfactory cue.

***Elevated plus-maze test***

The procedure was adopted same as described by Yamada et al., 2000 [29]. The plus-maze consisted of two open (50 cmx10 cm) and two enclosed (50 cm x10 cm) arms surrounded by 30-cm high walls. The four arms were joined by a central platform (10 cm x 10 cm) open to all the arms, to form a plus shape. The entire apparatus was elevated to a height of 60 cm above the floor. The apparatus was indirectly illuminated with a ceiling-fronting lamp (60 W) which was placed 90 cm above the apparatus. At the beginning of the test, the animal was placed in the central platform facing an open arm. The number of time rats enter open and closed arm and time spent in open arm was recorded for 5 min**.** (1) open arm duration, i.e., the total amount of time spent by the rat in an open arm; (2) closed arm duration, i.e., the total amount of time spent by the rat in a closed arm; (3) open arm entries, i.e., the total number of entries with all four paws into the open unprotected arms; (4) closed arm entries, i.e., the total number of entries with all four paws into the closed protected arms. After each test, the apparatus was sprayed with alcohol and wiped thoroughly to eliminate the residual odor.

***Social interaction test***

The protocol was adapted (with slight modification as mentioned below) from elsewhere [30]. The same apparatus and testing environment as those of the open field test were used for the social interaction test, except that the illumination was milder (15 W) than that in the open field test. On the day of operation, pairs of rats of the same group housed in different cages were put into two different corners of the open field arena. The social interaction behaviour including the passive interaction (number of crossing to each other) and social interaction time (s) (grooming, mounting and crawling under the other rat) were recorded for 5 min after placement of the rats into the apparatus. After each test, the apparatus was sprayed with dilute alcohol and wiped thoroughly.

***Statistical analysis***

All analysis was performed using graph pad prism 5 for windows. All the results of experiments are expressed as mean ± S.E.M. Statistical differences were evaluated with a one-way analysis of variance (ANOVA) followed by the bonferroni for multiple comparisons. The criterion for a statistically significant difference was fixed to $p < 0.05$.

**RESULTS**

***Modified Open field test***

CUS rats exhibited decreased ambulation ($F_{7, 40} = 3.55$, $p < 0.05$) and rearing ($F_{7, 40} = 3.39$, $p < 0.05$) and increased fecal pellets ($F_{7, 40} = 0.54$, $p < 0.05$), behaviour for 5 min after being put into the open field test (Table. 3). The results of this study demonstrated that, both decreased frequencies of ambulation and rearing were dose-dependently reversed by chronic escitalopram treatment (10 and 20mg/kg). However, the ambulation and rearing in the sham group were not significantly affected by the treatment with escitalopram.

***Socio-Sexual interaction test***

The loss of interest shown by depressive patients is one of the core symptoms of depression as depicted in the DSM-IV. We examined the socio-sexual behaviour and the effect of escitalopram on socio-sexual behaviour in CUS rats. The starting latency of genital probing ($F_{7, 40} = 11.84$, $p < 0.05$) and thrusting ($F_{7, 40} = 3.66$, $p < 0.05$) in CUS rats was significantly increased, whereas the number of genital probing ($F_{7, 40} = 3.88$, $p < 0.05$), thrusting events ($F_{7, 40} = 3.44$, $p < 0.05$) and pursuit ($F_{7, 40} = 8.28$, $p < 0.05$) were decreased as compared to sham rats (Table 4.). CUS rats showed significant sexual deficits in the socio sexual interaction test resembling core symptom of depression. Chronic treatment with escitalopram (10 and 20 mg/kg) significantly ($p < 0.05$) improved the deficit of genital-probing behaviour and pursuit in CUS rats, without affecting the behaviour in sham rats.

***Elevated plus-maze test***

Fig.1. displays the time spent and number of entries to open arm by sham and CUS rats, cumulated over the 5-min test. As shown in Fig (3.A-B) CUS and escitalopram treated rats demonstrate the variable response in EPM task. CUS rats exhibited a significant ($p < 0.05$) reduction in the percent entries and time spent in the open arms as compared to sham rats. These results are indicative of an enhanced anxiety response in rats exposed to stress compared with control animals. Drug induced changes was significantly observed in CUS rats. Chronic escitalopram treatment (10 and 20 mg/kg) significantly increased the number of entry ($F_{7, 40} = 8.4$, $p<0.05$) and time spent ($F_{7, 40} = 7.6$, $p < 0.05$) in open arm compared to vehicle treated CUS rats in EPM task (Fig.1.A-B).

***Social interaction test***

An attempt to design a model that simulates the changes in human social behaviour, social interaction test between two adult animals of the stressed group was performed. As demonstrated in Fig.2.A, unfamiliar pairs of CUS rats showed decreased social interactive time compared with sham rats ($F_{7, 40} = 8.03$, $p < 0.05$). In addition CUS rats showed increased passive interaction ($F_{7, 40} = 3.84$, $p < 0.05$) compared to sham rats in social interaction test (Fig. 2.B). The social impairment in CUS rats was significantly ($p < 0.05$) ameliorated by the chronic escitalopram treatment.

**DISCUSSION**

The use of animal model of human mental disorder, despite of their oblivious limitation have proved to be of value in the pre-clinical behavioral analysis for experimental validation of psycho-pharmacological assessment. The present study demonstrates that, chronic exposure to unpredictable stress augmented the expression of depression-like and anxiety-like behaviours in rats. A variety of stress situations have been employed to investigate the consequences of stress and to evaluate the depression-like and anxiety-like behaviour in rats. The experimental design of the current study fulfils these criteria and has been shown in earlier studies [31] to induce significant behavioural deficits in rats.

*Behavioural features in CUS rats resembling the symptoms of depression*

In the present study, we tried to establish a CUS-induced rat model with depression-like behavioural changes by measuring, open field exploration, hyperemotionality and socio-sexual interaction tests. Persistent exposure to uncontrollable and dissimilar aversive situations leads to behavioural alterations in response to subsequent novel stressors [24]. CUS is generally thought to be the

most promising and valuable rodent model with high face, predictive and construct validity [32] indicated by behavioural depression, cognitive deficits, male sexual dysfunction [33].

Modified open field exploration investigates the stress induced behaviour [26] characterized by an overall decrease in locomotor activity (including the movement distance and the number of rearings) as reported in previous studies [34]. When the rats were placed in a novel environment, similar paradigm was observed in CUS rats. CUS rats exhibited reduced locomotor activity of rats in open field test, which may mimic some aspects of human psychomotor retardation an accompanying symptom of major depression in humans [12]. Normal rats generally show increased ambulation and rearing in a novel open field. Whereas, CUS rats display decreased ambulation and rearing in a novel open field. This deficit can be effectively reversed by chronic treatment with the traditional antidepressant drug escitalopram in accordance with the past research that antidepressants were effective in reversal of inescapable stress-induced hypolocomotion in an open field [35].

Chronic stress can lead to diminished sexual desire [33] which is considered as a one of the core symptom of depression [36] that can be successfully modeled in rats [28]. The presence of impaired socio-sexual behaviour in CUS rats adds to the rationality of using this model of depression in the current study. The results revealed that, chronic sequential exposure to a variety of stressors causes a substantial decrease in socio-sexual interaction (sexual anhedonia) as characterized by increased latency in genital probing and thrusting and decreased episodes of genital probing, thrusting and pursuits, which resembles the loss of interest and this deficit can be effectively normalized by chronic treatment with anti-depressant drug escitalopram.

*Behavioural features in CUS rats resembling the symptoms of Anxiety*

Previous research has found that, exposure to chronic unpredictable stress can augment anxiety in humans and animals [37]. The appearance of anxiety symptoms in humans frequently develop after stress exposure has terminated, but few rodent studies have systematically examined the delayed anxiogenic effects of unpredictable stress. The tests used in this study can be differentiated and described as a model of "social anxiety" (assessed in the social exploration test in rats), a model of "novelty-induced" anxiety (assessed in the marble burying test), a model of anxiety in an "approach-avoidance conflict" (assessed in the elevated plus maze). Past research with chronic predictable or single stress exposure reported an increase in anxiety measures 1–3 weeks following the last stress exposure [38].

The elevated plus-maze test has been widely used for screening of anxiolytic drugs and for exploring neurobiological bases of anxiety and the classical indices of anxiety-related behaviour is the number of open arm entries and the time spent on the open arms [39]. Anti-anxiety drugs and SSRIs (selective serotonin reuptake inhibitors) have a reduction effect on anxiety reaction in an elevated plus maze [40]. The current study displayed that, CUS prolonged the time spent in closed arm and decreased the number of entries to open arm in stressed rats compared to sham group. Elevated plus-maze results suggest that chronic stressed rats, behave more anxiously than sham control rats. These results are in agreement with previous reports using these signs as stress markers and the results confirms the anxiogenic effect of chronic unpredictable stress in EPM. CUS induced anxiogenic behaviour was significantly reversed by chronic escitalopram treatment.

The social interaction tests have long been established as a valid model of anxiety [41]. According to File (1980) [30], decreased social interaction time in this test is indicative of a heightened level of anxiety. Chronic stressor produces limitation in the ability to perform social roles and may interfere with social interaction. Decreased social interaction among the rat's pairs indicates the anxiety-like [30] and depression-like symptoms. The most important finding of the current study is impaired social interaction as indicated by diminished social interaction time and increased passive interaction in CUS rats. The results suggested that, these animals are more anxious/fearful due to anticipated stressful event. Chronic escitalopram treatment, dose dependently increased social interaction and decreased passive interaction in CUS rats similar to that seen in sham group.

This study leads to several predictions with important implications for the development of therapeutic intervention (treatment) that could be applied at the different steps of trajectory from high anxiety traits to depression. Although the precise mechanism CUS induced behaviour deficits remain unclear, the involvement of serotonergic and dopaminergic dysfunction in prefrontal cortex and hippocampus following chronic stress are implicated in depression and anxiety. The current data do not directly address the question of which particular neural areas involve in CUS. However, limbic areas like the hippocampal subfields, lateral septum and central amygdala are particularly vulnerable to damage [42].

**CONCLUSION**

Though the availability of several types of anti-depressant and anxiolytic drugs are more, but their is steady rise in the prevalence of depression and anxiety (Andrews et al., 2000). As the several patients suffering from depression also have comorbid anxiety (Kessler et al.,

1997), it became crucial to design a comorbid model as well as to evaluate the drug with equipotent anxiolytic and anti-depressant like activity. The present study constitutes an extensive behavioural data characterizing the effect of CUS and action of escitalopram, on behavioural features resembling the sequelae of psychiatric comorbidity following CUS. The present study suggested that, the CUS can be useful model of depression-comorbid anxiety as was evidenced in behavioural assays, rather than only a model of major depression/ anxiety and hypothesis was strengthens with the potential role of escitalopram reversing the symptoms of comorbid depression with anxiety. Future studies will be aimed at the molecular and neurochemical substrate mediating the effects of CUS as well as the effect of CUS on neurogenesis relevant for depression and anxiety.

**ACKNOWLEDGMENTS**

This work was partly supported by the Council of Scientific and Industrial Research, Human resources development group, India. The authors would like to thanks Glenmark Pharmaceutical Ltd. India for providing Escitalopram as a gift sample in very short notice.

**Table 1. Experimental schedule for the chronic unpredictable stress procedure**

| Days | Schedule Stressor |
|---|---|
| | **Phase –I** |
| 1 | 1 hour restraint |
| 2 | 1 hour rotation |
| 3 | 4 hour of wet wedding |
| 4 | 10-min warm water swim (45°C) |
| 5 | 10 min of mild shock |
| 6 | 5-min cold water swim (8 °C) |
| 7 | 4 hour of isolation |
| 8 | 20 min tail pinch in restrainer |
| 9 | 1 hour rotation |
| 10 | 4 hour of wet wedding |
| 11 | 10-min warm water swim (45°C) |
| 12 | 10 min of mild shock |
| 13 | 5-min cold water swim (8 °C) |
| 14 | Inversion of light and dark cycle |

**Table 2. Schedule of treatments and behavioural assessments on stressed /sham rats in chronic study**

| Day 1- 14 | Behavioural Tests Schedule | | |
|---|---|---|---|
| | **16th** | **17th** | **18th** |
| Chronic Stress and Drug/vehicle Treatment (once a day for 14 days | Open field test<br>Elevated Plus maze test | Social Interaction Test<br>Hyperemotionality test | Marble Burying test<br>Socio -sexual Interaction test |

**Table 3. Effect of escitalopram on the behaviour on stressed /sham rats in modified open field test.**

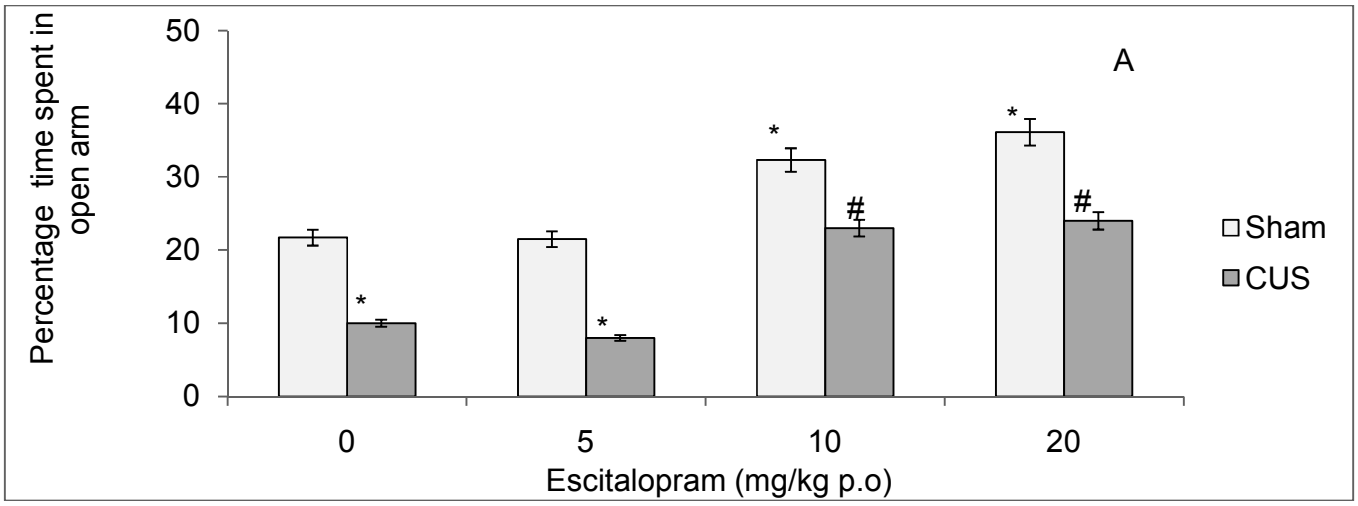
| Treatment (mg/kg) | Ambulation | Rearing | Pellets |
|---|---|---|---|
| **Sham Group** Vehicle Control | 96±4.44 | 11.33±1.62 | 2.16±0.79 |
| Escitalopram (5) | 99.83± 6.93 | 13±2.08 | 3±0.81 |
| Escitalopram (10) | 100.66±10.28 | 13.66±1.12 | 2.33±0.71 |
| Escitalopram (20) | 110.66±7.70 | 13.5±1.80 | 2.5±0.43 |
| **CUS Group** Vehicle Control | 69.83±8.08* | 5.16±1.78* | 4±0.82* |
| Escitalopram (5) | 71±8.59 | 6.5±1.52 | 3.5±0.76 |
| Escitalopram (10) | 94.83±8.26# | 12±2.13# | 2.5±1.12# |
| Escitalopram (20) | 105.16±8.24# | 12.83±1.9# | 2.66±1.09# |

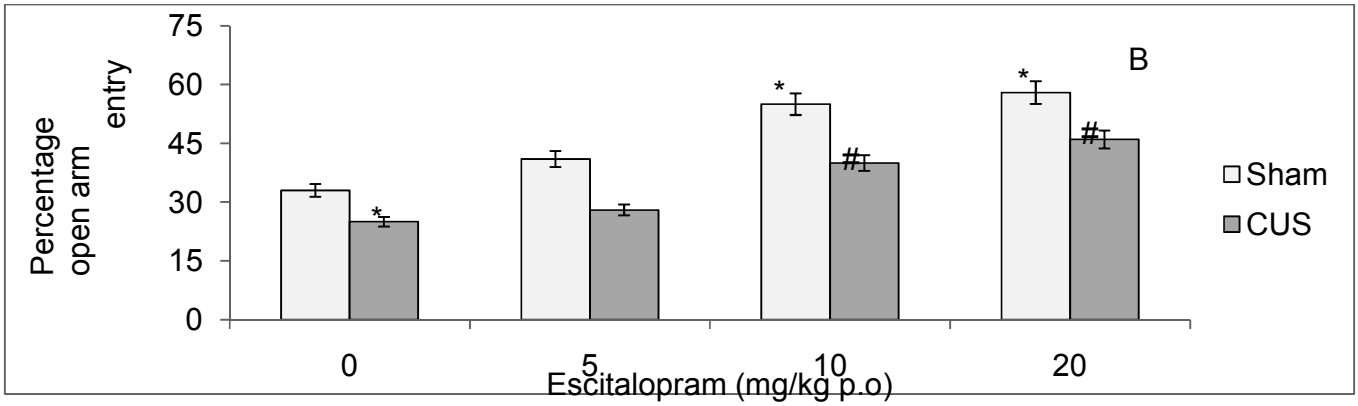
**Table 4. Effect of escitalopram on the behaviour of on stressed /sham rats in socio-sexual interaction test.**

Values represent mean ± S.E.M. $n = 6$ in each group. Escitalopram/vehicle was administered (p.o.) once day for 14 days. *$P <$

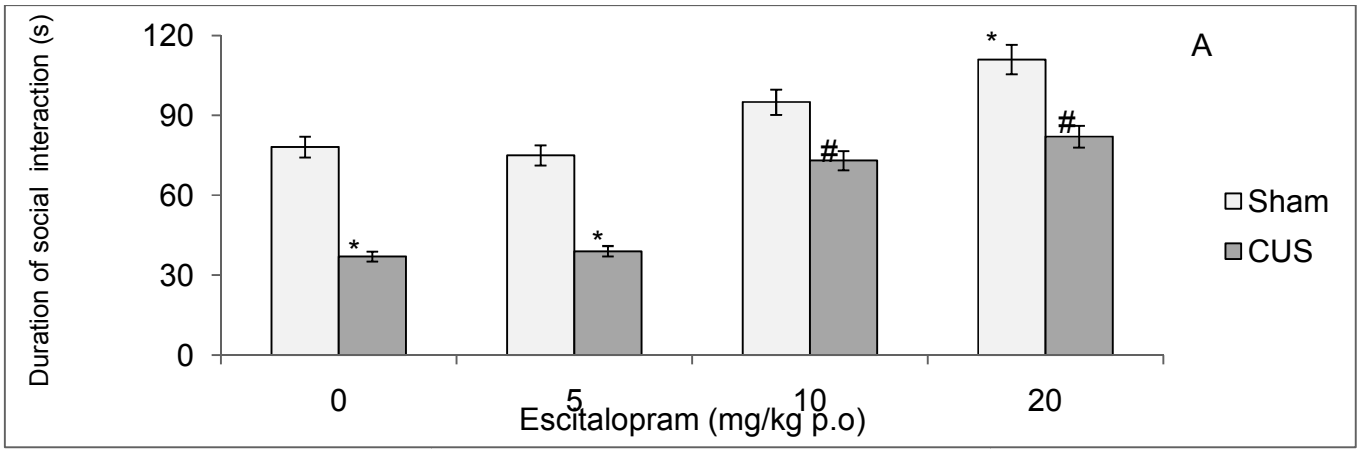
| Treatment (mg/kg) | Genital Probing Latency (Sec) | Number of Genital Probing | Thrusting Latency (Sec) | Number of Thrusting | Pursuit (sec) |
|---|---|---|---|---|---|
| **Sham Group** Vehicle Control | 5.33±1.63 | 35.00±5.26 | 18.67±6.63 | 36.83±4.55 | 146.33±15.55 |
| Escitalopram (5) | 5.67±1.31 | 35.67±4.80 | 20.33±7.41 | 37±3.86 | 150.67±15.06 |
| Escitalopram (10) | 6.67±0.95 | 36.33±4.57 | 21.33±5.99 | 36.67±4.67 | 148.00±16.77 |
| Escitalopram (20) | 6.50±1.23 | 34.67±4.02 | 18.36±3.46 | 38.83±3.88 | 164.33±17.69 |
| **CUS Group** Vehicle Control | 24.00±2.67* | 12.33±2.33* | 84.00±29.33* | 19.09±3.99* | 38.83±14.04* |
| Escitalopram (5) | 23.17±3.69 | 14.23±2.65 | 83.83±24.11 | 20.00±6.02 | 37.67±4.57 |
| Escitalopram (10) | 12±2.32# | 27±5.93# | 34.67±9.14# | 31.67±4.11# | 132.17±25.46# |
| Escitalopram (20) | 10.83±2.63# | 29.00±7.57# | 31.17±9.28# | 34.67±3.04# | 144.50±24.96# |

*0.05* compared with vehicle treated sham rats, # $P <$ *0.05* compared with vehicle treated CUS rats.






1. Effect of escitalopram on the open arm activity of sham and CUS rats in the elevated plus-maze exploration paradigm. (A) Percentage ratio of open arm to total arm entries; (B) percentage of total time spent in open arms. Columns represent mean of values. Escitalopram/vehicle was administered (p.o.) once day for 14 days. Error bars represent S.E.M. $n = 6$ per group. $*P < 0.05$ compared with vehicle treated sham group , $\# P < 0.05$ compared with vehicle treated CUS group.



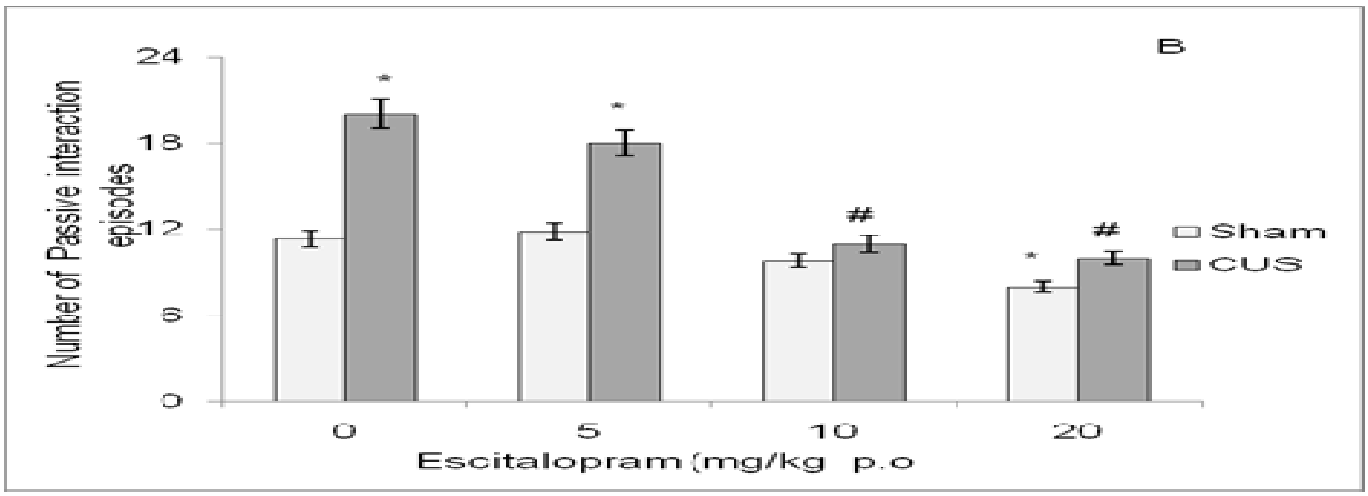



Fig. 2 Effect of escitalopram on the behaviour of sham-operated and CUS rats in social interaction test. (A) Social interaction time; (B) number of passive interactions. Columns represent mean of values. Escitalopram/vehicle was administered (p.o.) once day for 14 days. Error bars represent S.E.M. $n = 6$ per group. $*P < 0.05$ compared with vehicle treated sham group, $\#P < 0.05$ compared with vehicle treated CUS group.

**REFERENCES**


1. Ehlert U, Gaab J, Heinrichs M. Psychoneuroendocrinological contributions to the etiology of depression, posttraumatic stress disorder, and stress-related bodily disorders: The role of the hypothalamus-pituitary-adrenal axis. *Biological Psychology.* 57, 2001, 141–152.
2. Lopez JF, Akil H, Watson SJ. Neural circuits mediating stress. *Biological Psychiatry* 46, 1999, 1461–1471.
3. Shah PJ, Ebmeier KP, Glabus MF, Goodwin GM. Cortical gray matter reductions associated with treatment-resistant chronic unipolar depression. Controlled magnetic resonance imaging study. *British Journal of Psychiatry.* 172, 1998, 527–532.
4. Sheline YI, Wang PW, Gado MH, Csernansky JG. Hippocampal atrophy in recurrent major depression. Proceeding of National Academy of Science U. S. A. 93, 1996, 3908–3913.
5. Ray A, Henke PG, Sullivan RM. Effects of intra-amygdalar dopamine agonists and antagonist on gastric stress ulcer formation in rats. *Neuroscience Letter.* 84, 1988, 302–306.
6. Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Tabira T. Chronic stress impairs rotarod performance in rats: implications for depressive state. Pharmacology *Biochemistry and Behaviour.* 71, 2002, 79–84.
7. Brown TA, Chorpita BF, Barlow DH. Structural relationships among dimensions of the DSM-IV anxiety and mood disorders and dimensions of negative affect, positive affect, and autonomic arousal. *Journal of Abnormal Psychology.* 107, 1998, 179–192.
8. Mineka S, Watson D, Clark LA. Comorbidity of anxiety and unipolar mood disorders. *Annual Review of Psychology.* 49, 1998, 377–412.
9. Djavadian RL. Serotonin and neurogenesis in the hippocampal dentate gyrus of adult mammals. *Acta Neurobiol. Exp.(Wars).* 64, 2004, 189–200.
10. Joca SR, Ferreira FR, Guimaraes FS. Modulation of stress consequences by hippocampal monoaminergic, glutamatergicnand nitrergic neurotransmitter systems. *Stress.* 10, 2007, 227–249.
11. Domínguez-Escriba L, Hernández-Rabaza V, Soriano-Navarro M, Barcia JA, Romero, FJ, García-Verdugo JM, Canales JJ. Chronic cocaine exposure impairs progenitor proliferation but spares survival and maturation of neural precursors in adult rat dentate gyrus. *Eur. J. Neurosci*. 24, 2006, 586–594.
12. Anisman H, Kokkinidis L, Sklar LS. Neurochemical consequences of stress. In: Burchfield SR, editor. Stress. *Psychological and Physiological interactions*. 1984, 67–98.
13. Bhattacharya A, Muruganandam AV, Kumar V, Bhattacharya SK. Effect of polyherbal formulation, Eumil, on neurochemical perturbations induced by chronic stress. *Indian Journal of Experimental Biology*. 40, 2002, 1161– 1163.
14. Zurita A, Marinelli M, Cuadra G, Brandao ML, Molina VA. Early exposure to chronic variate stress facilitates the occurrence of anhedonia and enhanced emotional reactions to novel stressors: reversal by naltrexone pretreatment. *Behaviour Brain Research.* 117, 2000, 163–171.
15. Bekris S, Antoniou K, Daskas S, Papadopoulou-Daifoti Z. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. *Behavioural Brain Research.* 161, 2005, 45–59.
16. Kessler RC. The epidemiology of psychiatric comorbidity, in Textbook of Psychiatric Epidemiology. Edited by Tsuang M, Tohen M, Zahner G. New York, John Wiley & Sons, 1995, 179–197.
17. Wittchen HU, Zhao S, Kessler RC, Eaton WW. DSM-III-R generalized anxiety disorder in the National Comorbidity Survey. *Archives of General Psychiatry.* 51, 1994, 355–364.
18. Hettema JM. What is the genetic relationship between anxiety and depression? American Journal of Medical Genetics *.Seminar in Medical Genetics*. 148, 2008, 140–146.
19. Lu XY, Kim CS, Frazer A, Zhang W. Leptin: a potential novel antidepressant. *Proceeding of National Academy of Sciences* USA 103, 2006, 1593–1598.
20. Mcblane J, Handley SL. Effects of two stressors on behaviour in the elevated x-Maze preliminary investigation with 8-OH DPAT. Psychopharmacology. 83, 1994, 173-182.
21. Wade A, Michael LO, Bang HK. Escitalopram 10mg/day is effective and well tolerated in a placebo-controlled study in depression in primary care. *International Clinical Psychopharmacology.* 17, 2002, 95–102.

22. Sánchez C, Bergqvist PB, Brennum LT, Gupta S, Hogg S, Larsen A. Escitalopram, the S-(+)-enantiomer of citalopram, is a selective serotonin reuptake inhibitor with potent effects in animal models predictive of antidepressant and anxiolytic activities. *Psychopharmacology.* 167, 2003, 353–362.
23. Kendler KS, Karkowsk LM, Prescott CA. Causal relationship between stressful life events and the onset of major depression. *Am. J. Psychiatry.* 156, 1999, 837–841.
24. Katz RJ, Roth KA, Carroll BJ. Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression. *Neuroscience Biobehavioural Reviews.* 5, 1981, 247–251.
25. Pandey DK, Yadav SK, Ramamoorthy R, Mahesh R. Depression-like and anxiety-like behavioural aftermaths of impact accelerated traumatic brain injury in rats: A model of comorbid depression and anxiety? *Behavioural brain Research.* 205, 2009, 43-442.
26. Redmond AM, Kelly JP, Leonard BE. Behavioural and neurochemical effects of dizocilpine in the olfactory bulbectomized rat model of depression. *Pharmacology Biochemistry and Behaviour.* 58, 1997, 355—359.
27. Breigeiron MK, Morris M, Lucion AB, Sanvitto GL. Effects of angiotensin II microinjected into medial amygdala on male sexual behaviour in rats. *Hormone and Behaviour.* 41, 2002, 267–274.
28. Meyerson BJ, Hoglund AU. Exploratory and sociosexual behaviour in the male laboratory rat: A methodological approach for the investigation of drug action. *Acta Pharmacology Toxicology.* 48, 1981, 168–180.
29. Yamada K, Iida R, Miyamoto Y, Saito K, Sekikawa K, Seishima M. Neuro- behavioural alterations in mice with a targeted deletion of the tumor necrosis factor-α gene: implications for emotional behaviour. *Journal of Neuroimmunology.* 111, 2000, 131–138.
30. File SE, Hyde JR. Can social interaction be used to measure anxiety? *British Journal of Pharmacology.* 62, 1978, 19–24.
31. Bhattacharya A, Ghosal S, Bhattacharya SK. Antioxidant effect of Withania somnifera glycowithanolides in chronic foot shock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *Journal of Ethanopharmacology.* 74, 2001, 1–6.
32. Forbes NF, Stewart CA, Matthews K, Reid IC. Chronic mild stress and sucrose consumption: validity as a model of depression. *Physiology and Behaviour* 60, 1996, 1481-1484.
33. Muruganandam AV, Kumar V, Bhattacharya SK. Effect of polyherbal formulation, Eumil, on chronic stress-induced homoeostatic perturbations in rats. *Indian Journal of Experimental Biology.* 40, 2002, 1151– 1160.
34. Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-years review and evaluation. *Psychopharmacology (Berl).* 134, 1997, 319–329.
35. Pal SN, Dandiya PC. Glutathione as a cerebral substrate in depressive behavior. *Pharmacol. Biochem. Behav.* 48, 1994, 845–851.
36. Kennedy SH. Core symptoms of major depressive disorder: relevance to diagnosis and treatment. *Dialogues Clinical Neuroscience.* 10, 2008, 271–277.
37. McEwen BS. Allostasis and allostatic load: implications for neuropsychopharmacology. *Neuropsychopharmacology.* 22, 2000, 108–24.
38. Adamec RE, Blundell J, Collins A. Neural plasticity and stress induced changes in defense in the rat. *Neuroscience Biobehavioural Reviews.* 25, 2001, 721–744.
39. Rodgers RJ, Cole JC. Anxiety enhancement in the murine elevated plus maze by immediate prior exposure to social stressors. *Physiology and Behaviour.* 53, 1993, 383– 388.
40. Hogg S. A review of the validity and variability of the elevated plus maze as an animal model of anxiety. *Pharmacology Biochemistry and Behaviour.* 54, 1996, 21–30.
41. Rex A, Voigt JP, Gustedt C, Beckett S, Fink H. Anxiolytic-like profile in Wistar, but not Sprague–Dawley rats in the social interaction test. *Psychopharmacology.* 177, 2004, 23–34.
42. Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T. Chronic stress differentially regulates glucocorticoid negative feedback response in rats. *Psycho-neuroendocrinology.* 26, 2001, 443–459.