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Research Article

Dapoxetine: An Innovative Approach in the Therapeutic Management in Animal Model of Depression

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ABSTRACT

Stress is a complicated condition that effects on person's mental and physical health, and it is the precursor of other psychological disorders mainly depression. Serotonin (5-Hydroxytryptamine; 5-HT) is well known to have hypo function in unpredictable chronic mild stress whereas, unpredictable chronic mild stress (UCMS) has produced the most steady and continuous results of anhedonia and learned helplessness particularly in rats. The stress-induced depressive like behavior can be reversed by many antidepressants such as SSRIs. Selective serotonin [5-hydroxytryptamine (5-HT)] reuptake inhibitors (SSRIs) is mostly prescribed antidepressant that can deplete neurochemical and behavioral deficits. The present study was designed to investigate whether repeated administration of dapoxetine at dose (1.0 mg/kg) could reverse the behavioral deficits induced by UCMS in rat model of depression. UCMS induced behavioral deficits. Locomotive activity in familiar environment (home cage), novel (open field) environment and anxiolytic behavior in light/dark activity box were greater in unstressed group than stressed group. The inhibition of serotonin reuptake at pre-synaptic receptors by repeated dapoxetine administration is mainly the mechanism involved and discussed. This particular study may assist in novel approach for understanding the interaction between stress and behavioral functions and extending the therapeutic use of dapoxetine.

Keywords:SSRIs, Dapoxetine, Unpredictable chronic mild stress, 5-hydroxytryptamine (5-HT), Depression, Locomotive activity.

INTRODUCTION:

The concept of stress was originally developed by Walter B. Cannon (Cannon, 1914) which defines stress as the behavioral reactions due to stimuli (stressor). Stressful conditions possess a complex relationship with brain and body's reaction to stress and beginning of depression. There are some stress evoked interferences in normal body function which seem to be linked with pathophysiology of depression (Kioukia-Fougia *et al.*, 2002). Stress is a state of disharmony or threatened homeostasis and is confronted by a complex standard of behavioral and physiological responses of that particular organism. (Chrousos *et al.*, 1998). Stressed life events have been connected to digressive symptoms as well as to MDD (Constance Hammen *et al.*, 1992). Dapoxetine ((+)-(S)-N, N-dimethyl-(a)-[2-(1-naphthalenyloxy) ethyl]-benzenemethanamine hydrochloride) is a unique effective serotonin transport inhibitor which has a matchless pharmacokinetic profile (Dresser *et al.*, 2004; Peter J. Gengo *et al.*, 2005). Dapoxetine is a highly effective compound that after dosing attains its peak plasma concentration in about 1.5 hours, which is more rapidly than fluoxetine, paroxetine, or sertraline (Strassberg *et al.*, 1999). Dapoxetine is an effective antidepressant and has a similar mechanism of action with other SSRIs. It inhibits the serotonin reuptake transporter, with less inhibitory effects at the nor-epinephrine and dopamine reuptake transporters (Peter J. Gengo *et al.*, 2005). Dapoxetine is readily absorbed,

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afterwards a rapid decay in plasma concentrations after oral administration. . By primarily inhibiting the reuptake of the serotonin transporter, dapoxetine exhibits its efficacy (Peter J. Gengo *et al.,* 2005).

Most of the animal models possess the common characteristics of stress which were various stress procedures or avoiding events and chronic stress models observed to be more suitable for the experimental determination of depression as compare to acute stress models (Rebecca J. Katz *et al.,*1981; Paul Willner, 1991).Models which involve chronic stress, such as the sustained social stress (Eberhard Fuchs and Gabriele Flügge, 2005) or unpredictable chronic mild stress (UCMS) (Porsolt, 2000; Willner and Mitchell, 2002) have produced the most steady and continuous results of anhedonia and learned helplessness, particularly in rats. A rat model of unpredictable chronic mild stress (UCMS) induced a cognitive defect in extra dimensional set shifting capability in an intentional set shifting test, which suggests an alteration in function of the medial prefrontal cortex (Corina O Bondi *et al*.,2008). Possessing variety of stressors is a vital feature of the UCMS model, as repetition of single or few stressors cause habituation of behaviors rapidly (Muscat and Willner, 1992).The present study was designed to evaluate the efficiency of dapoxetine to deplete UCMS induced behavioral deficiencies in rats.

MATERIALS AND METHODS

Animals

Albino-Wistar rats (weighing 180-220 grams) provided by The Dow University of health and sciences Ojha campus, Karachi. All animals were housed individually in perspex cages under 12-hrs light and dark cycle and controlled room temperature (25±2 ^{o}C) with free access to cubes of standard rodent diet and water, for a period of three days before experimentation to familiarize them with surrounding. 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.

Drug

Dapoxetine was acquired from Sigma (St Louis, Missouri, USA) and dissolved in distilled water, 1:1 v/v and was administered orally (to stressed and unstressed rats at a dose of 1mg/kg /ml /body weight for 28 days) by using stainless steel feeding tubes.

Experimental protocol

Twenty four male Albino-Wistar rats were randomly divided into two groups: (i) Unstressed and (ii) UCMS groups. The animals of each group were again subdivided into two further groups each i.e., saline and dapoxetine administrated. This resulted in a total of four groups: (i) Unstressed- Saline, (ii) Unstressed-Dapoxetine, (iii) UCMS-Saline and (iv) UCMS-Dapoxetine injected animals. Animals were administrated accordingly with Dapoxetine (1.0 mg/kg) or saline (1.0 mg/ml) 1 hour before of each stress. Stressed group rats were exposed to unpredictable chronic mild stress for 4 weeks (Table 1) while animals of unstressed groups remained in their home cages. Locomotive activity was monitored in activity box and exploratory activity in open field on next day of 1st

Table 1: Chronic Mild Stress (CMS) Schedule Exposed To Stressed Rats.

DAYS				*UCMS*	*DISCRIPTION*
Day 1	Day8	Day 15	Day 22	Cage agitation(12 rpm)	2 hours
Day 2	Day 9	Day 16	Day 23	Water Deprivation stress	12 hours
Day 3	Day 10	Day 17	Day 24	Noise stress (12 dB)	Alternative noise stimuli pattern for 1 hour
Day 4	Day 11	Day 18	Day 25	Light/Dark inversion cycle	Alternatively for 2 hours
Day 5	Day 12	Day 19	Day 26	Overcrowding stress	Stressed rats in a single cage for 1 hour
Day 6	Day 13	Day 20	Day 27	Cage tilting stress	1 hour (right side) 1 hour (left side)
Day 7	Day 14	Day 21	Day 28	Cold stress	1 hour at 4 ^{o}C

and 28th day of administration. To monitor the anxiolytic behavior of treatment, light dark transition box was used. Activity was monitored on next day of 1st and last (28th) administration.

Behavioral assessment

Activity Box

Home cage test is used for the determination of locomotive activity in a familiar environment. Using the home cage activity test, the duration of monitoring is of 10 minutes for the study of stress or drug induced activity. 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 (Erum Shireen e*t al.*, 2014). All monitoring was done in balanced design. Observations were recorded simultaneously

Open Field Activity

Open field activity test is used for the determination of exploratory activity of rats in a novel environment (Frye and Rhods, 2008). The open field activity test is used to estimate locomotives activity to the novelty. In this test, the open field apparatus is much larger than that of the home cage and is unfamiliar to the rodent. Rat is taken out from their cages and placed into an open field arena. The open field apparatus was consisted of opaque plastic walls of 42cm high and 76 x 76 balanced design. The floor of apparatus consists of 25 equal squares. The rat was placed in the center square of the field. The parameters observed were

cm square area. All rats were monitored in a the latency time which was taken by the animal for exploring from the center square of arena and numbers of square crossed by the animal with all four paws in 5 minutes.

Light-Dark Transition Box

Rats tend to stay in darker place and feel safer over there. If a choice is given between bright open area and a dark place, rats preferred the dark, enclosed environment. Avoidance of lit space is considered as the reflection of anxiety provoking characteristic of rodents. The apparatus was consisted of two compartments. The boxes are of equal size (26 x 26 x 26 cm), with an opening of (12 x 12 cm) between the boxes .Walls of one compartment were made up of Plexiglas (transparent) and other were painted dark and enclosed (with top).The light dark exploration activity assess the anxiolytic and anxiogenic response of a drug (Martine Hascoët *et al.*, 2001).Rat was placed in the middle of the light box. The procedure was done in balanced design. The parameter observed was time spent in light compartment (the time spent by the animal exploring the light area in 5 minutes).

Statistical analysisResults are represented as mean ± S.D. Behaviors were analyzed by three-way ANOVA repeated measure designs (SPSS).

Software used for the analysis was SPSS (version 17). Individual comparisons were made by Newman- Keuls test. Values of $p<0.05$ were considered as significant.

RESULTS

1. *Effects of Dapoxetine on activity in activity box of rats exposed to UCMS*

As the data (figure 1) analyzed by three-way ANOVA (repeated measured designing) showed that the effects of repeated monitoring ($F=32.572$; df=1, 20: $p<0.01$), dapoxetine ($F=340.492$; df=1, 20: $p<0.01$) and stress ($F=203.100$; df=1, 20: $p<0.01$) were found to be significant. Whereas, the interaction between drug, day and stress ($F=1.767$; df=1, 20) was non-significant. Post-hoc analysis by Newman-Keuls test showed that administration of dapoxetine increased activity in unstressed as well as stressed animals as compared to saline administrated controls. Significant value ($p<0.01$) found after 28^{th} day of administration. In stressed animals, activity found greater ($p<0.01$) on repeated administration of dapoxetine as compared to single administration. Dapoxetine induced hyper locomotion was decreased ($p<0.01$) in stressed animals after 28^{th} day of administration as compared to unstressed animals.

2. *Effects of Dapoxetine on square crossed in open field of rats exposed to UCMS*

Data (figure 2) analyzed by three-way ANOVA (repeated measured designing) showing that the effect of days ($F=30.812$; df=1, 20: $p<0.01$) was significant whereas, the effects of dapoxetine ($F=2.430$; df= 1, 20), stress ($F=0.001$; df=1, 20) and the interaction between drug, days and stress ($F=2.830$; df=1, 20) were found non-significant. Post-hoc analysis by Newman Keuls test showed that administration of dapoxetine at dose 1.0 mg/kg decreased activity in unstressed animals but increased in stressed animals as compared to saline administrated unstressed or stressed animals. Significant value ($p<0.01$) found after 28^{th} day of administration. Greater activity was found after 28^{th} administration in stressed animals as compared to similarly treated animals of 1^{st} day dapoxetine administration ($p<0.01$).Exposure to UCMS resultant into hypo locomotive behavior ($p<0.01$) of animals of saline administrated group after 28^{th} day of stress as compared to unstressed saline controls. Dapoxetine induced hyper locomotion was clearly observed in stressed animals but not in unstressed animals. Significant value ($p<0.01$) was found after 28^{th} day of administration.

3. *Effects of Dapoxetine on latency time in open field of rats exposed to UCMS*

As the data analyzed by three-way ANOVA (repeated measured designing) the effect of days ($F=92.658$; df=1, 20: $p<0.01$), the effect of dapoxetine ($F=365.890$; df= 1, 20: $p<0.01$) ,the effect of stress ($F=83.031$; df=1, 20; $p<0.01$) and the interaction between drug, days and stress ($F=17.360$; df=1, 20: $p<0.01$) were significant. Post-hoc analysis by Newman Keuls test showed that time required to move in open field increased with the administration of dapoxetine in unstressed as well as stressed animals as compared to similarly treated saline controls. Significant value ($p<0.01$) was found after 28^{th} day of administration in unstressed and stressed animals. Greater latency time was found on repeated administration ($p<0.01$) in unstressed and stressed animals as compared to 1^{st} day administration. Stressed animals repeatedly administrated with dapoxetine required higher ($p<0.01$) time to move in open field as compared to single administrated animals.

4. *Effects of Dapoxetine on activity in light dark box of rats exposed to UCMS*

Data (figure 4a) as analyzed by three-way ANOVA (repeated measured designing) revealed that the effect of days ($F=61.037$; df=1, 20: $p<0.01$), dapoxetine ($F=256.077$; df= 1, 20: $p<0.01$) and the interaction between all factors ($F=5.623$; df=1, 20: $p<0.01$) were significant. The

effect of stress (F=0.445; df=1, 20) was found non-significant. Post-hoc analysis by Newman-Keuls test showed that administration induced anxiolytic behavior in unstressed and stressed animals on repeated administration not on single as compared to saline administrated unstressed or stressed animals. Greater increase in time spent in light box was seen in animals of stressed group administrated with dapoxetine as compared to similarly administrated animals of unstressed group ($p<0.01$).

As the data (figure 4b) analyzed by three-way ANOVA (repeated measured designing) the effect of days (F=20.345; df=1, 20: $p<0.01$), the effect of dapoxetine (F=132.500; df= 1, 20: $p<0.01$), the effect of stress (F=5.708; df=1, 20: $p<0.01$) and the interaction between drug, days and stress (F=4.629; df=1, 20: $p<0.01$) were found to be significant. Post- hoc analysis by Newman Keuls test showed that number of entries in light box was decreased with the administration of dapoxetine in unstressed as well as stressed animals as compared to saline administrated controls. Significant value ($p<0.01$) was found

after 28th day of administration in unstressed and after 1st as well as 28th day of administration in stressed animals. In stressed animals, activity was smaller ($p<0.01$) in dapoxetine administrated animals after 1st and 28th day of administration as compared to similarly administrated unstressed animals

DISSCUSION

The aim of the present study was to investigate whether repeated administration of dapoxetine at dose 1.0 mg/kg could reverse the behavioral deficits induced by UCMS in rat model of depression. Stressful conditions possess a complex relationship with brain and body's reaction to stress and beginning of depression. The present study reveals that the long term stress and depression can be attenuate and behavioral deficits due to stressful situations can be inverse by the treatment of antidepressants particularly SSRIs. Activity box (home cage) test determines

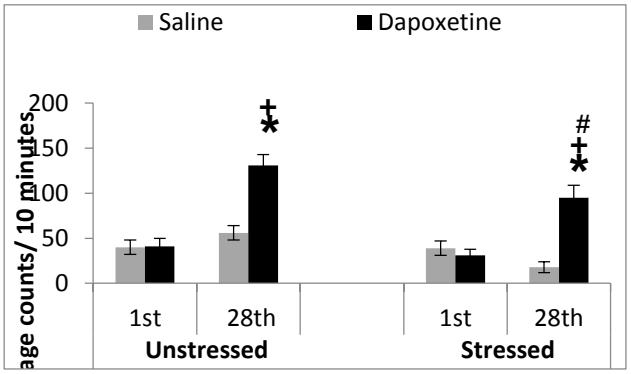

Figure: 1 Effects of Dapoxetine on activity in activity box of rats exposed to UCMS*.

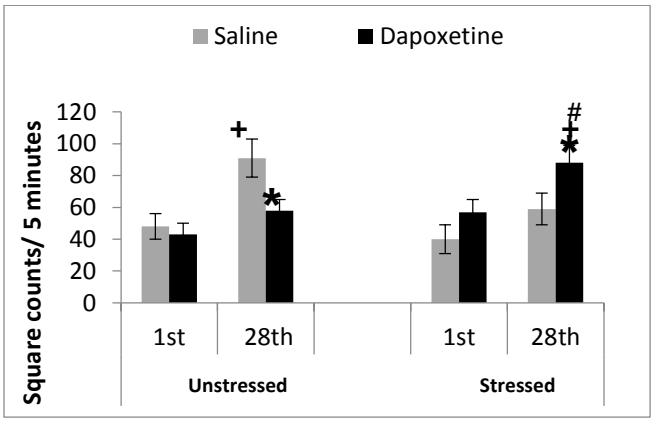

Figure 2:*Effects of Dapoxetine on number of square crossed in open field of rats exposed to UCMS*.*

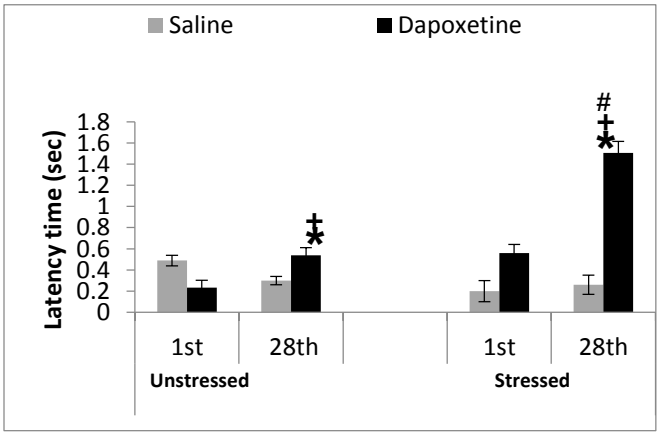

Figure 3 *Effects of Dapoxetine on latency time in open field of rats exposed to UCMS*.*

* Values are means + SD (n=6) as monitored on next day of 1st and 28th day of stress. Significant differences by Newman-Keuls test:

*p<0.01 from similarly treated saline administrated controls; +p<0.01 from respective 1st activity monitored controls; #p<0.01 from respective unstressed controls following three-way ANOVA (repeated measures design)

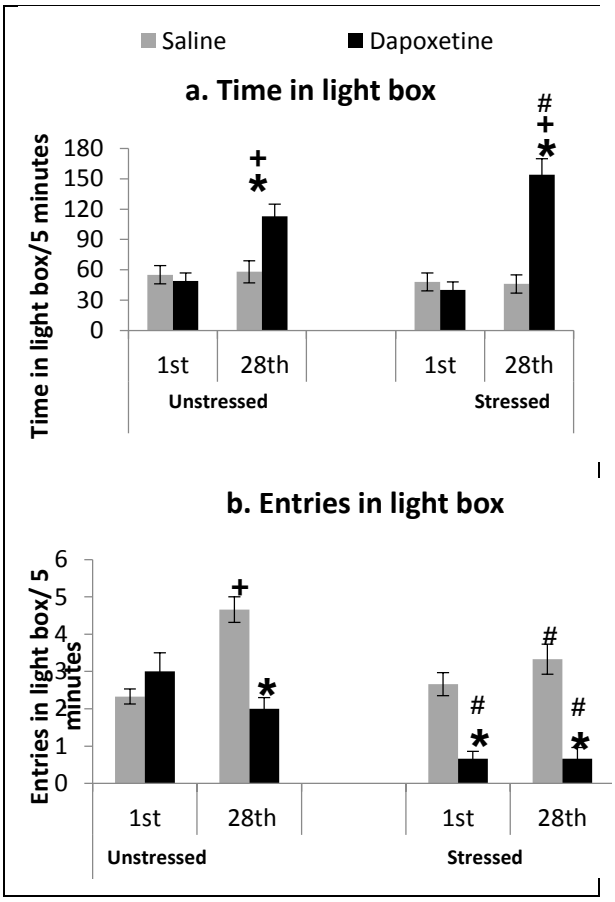

Figure: 4 Effects of Dapoxetine on activity in light/dark box of rats exposed to UCMS.

*Values are means + SD (n=6) as monitored on next day of 1st and 28th day of stress. Significant differences by Newman-Keuls test: *p<0.01 from similarly treated saline administrated controls; #p<0.01from respective unstressed controls following three-way ANOVA (repeated measures design).*

the locomotive pattern of rats in familiar environment under the influence of stressful or stress-free conditions (Karin Ganea *et al.*, 2007). The present study shows the decrease in locomotive activity in both dapoxetine and saline treated stressed rats (figure 1). Depression and stress cause fatigue, desperation and hopelessness. Stressed rats showed less activity in activity box than unstressed although home cage was a familiar environment for the animal. Whereas, by the treatment of antidepressants particularly SSRIs, the distress can be reverse back. Dapoxetine mainly acts on serotonin receptors 5-HT-1A, 5-HT-1B and 5-HT-2C. 5-HT-1A acts on anxiety and mood behavior (Guy A. Kennett *et al.,* 1987) whereas, 5-HT-1B acts on anxiety and loco motor behavior (Ewa Chojnacka-Wójcik *et al.,* 2005) and 5HT-2C acts on mood anxiety and locomotion (Mark J. Millan *et al.,* 2005, 2008). It was clearly observed that locomotion in both stressed and unstressed rats was increased in dapoxetine treated rats as compare to saline treated animals. Furthermore, a decrease in dapoxetine treated stressed rats locomotion was observed than in unstressed rats.

Hall in 1934 described the open field test for the study of emotionality in rats. A rodent is placed in a novel environment from which escape is prevented (Walsh and Cummins 1976). This study shows the antidepressant effect of SSRIs dapoxetine in an open field arena by two parameters i.e. latency time and number of square crossed. Figure 2 presents an increase in square counts in dapoxetine administrated stressed rats. Whereas, less number of square counts in saline treated stressed rats. This is due to the effect of dapoxetine as an antidepressant in novel environment which increases number of counts in stressed rats.

Furthermore, Dapoxetine treated unstressed rats showed less square counts than saline treated rats. Novelty and unfamiliarity to the environment in spite of stress-free state caused less activity in dapoxetine treated rats.

Increase in the time spent in the central part and ratio of central to the total locomotion or decrease of the latency to the central part are the indications of anxiolytic (Laetitia Prut *et al.,* 2003). The particular study shows the increase in latency in dapoxetine treated unstressed rats till 14th day where as it started decreasing till 28th day

of administration (figure 3) due to the antidepressant effect of the drug. Moreover, latency increased in SSRIs treated stressed rats due to the stress and depression in open field and the observed behavior is avoidance of threatening places also can be observed in humans (Laetitia Prut *et al* ., 2003). Forced hostility in rodents with novelty is stressful (Misslin and Cigrang, 1986).

The light/dark box test 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 has revealed that entries in light/dark box in unstressed saline and dapoxetine treated rats as compare to stressed rats whereas, dapoxetine treated rats showed less entries in light box than saline treated rats (figure 4b) whereas, time spent in light area was observed clearly more in dapoxetine treated unstressed and stressed rats than saline administrated animals. Moreover, dapoxetine treated stressed rats showed most activity than unstressed rats (figure 4a). The antidepressants effect of dapoxetine increased the time duration of rats in illuminated novel area although the entries of SSRIs treated rats were less than saline treated rats. Due to the habituation and adaptations over long time, transformations have found to be an index of exploration activity and the time spent in each compartment is due to aversion (Belzung *et al.*, 1987). The explorations and percent time spent seems to be best measure in each compartment. The mood, anxiety locomotion and memory are improved by agonist of 5-HT-1A, 5-HT-1B and 5-HT-2C receptors and dapoxetine is found to be one of the best candidates as a SSRIs antidepressant. Present study reveals that long term exposure to stressful situation results into several behavioral deficits which can be attenuated by repeated administration of dapoxetine.

Administration of dapoxetine produced continuously increase in activity but this increase was smaller in animals exposed to stress.

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