RESEARCH

21(88), November - December, 2017

ISSN
2321–7359
EISSN
2321–7367

Medical Science

Inhibition of unpredictable chronic mild stress induced anxiogenic condition by the repeated administration of methylphenidate in male rats

Muhammad Farhan☼, Safia Habib, Fatima Riaz, Sana Wali

Neurochemistry and Biochemical Neuropharmacology Research Unit,
Department of Biochemistry,
University of Karachi, Karachi,
Pakistan

☼**Corresponding author:**
Muhammad Farhan
Neurochemistry and Biochemical Neuropharmacology Research Unit,
Department of Biochemistry,
University of Karachi, Karachi,
Pakistan
Email: farhankamali@uok.edu.pk

Article History
Received: 22 June 2017
Reviewed by: 3 Reviewers
Accepted: 19 August 2017
Published: November-December 2017

Citation
Muhammad Farhan, Safia Habib, Fatima Riaz, Sana Wali. Inhibition of unpredictable chronic mild stress induced anxiogenic condition by the repeated administration of methylphenidate in male rats. *Medical Science*, 2017, 21(88), 280-286

Publication License
This work is licensed under a Creative Commons Attribution 4.0 International License.

General Note
Article is recommended to print as color digital version in recycled paper.

© 2017 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

ABSTARCT

Many preclinical trails on laboratory rats have well demonstrated the effects of unpredictable stressors that they produce behavioral and neurochemical alterations. Chronic stress produces the alteration in serotonergic transmission it has potential to increase the responsiveness to drug challenges and novel stressors to behavioral and neurochemical alteration. The present study was performed to determine the antidepressant potential of methylphenidate in animal model of chronic mild stress. The experimental case-control study was conducted in April 2015 at the Department of Biochemistry, University of Karachi and comprised male rats. Average weight rats were randomly divided into two equal groups, animal of stressed group were exposed to CMS. Animals of unstressed and stressed group were administrated with methylphenidate at dose 1.0 mg/kg/day for 14 days 1 hour before the animals exposed to CMS. Behavioral anxiolytic assessments were done by using light/dark transition and elevated plus maze apparatus models. CMS exposed animals showed behavioral deficits, which recovered by the repeated administration of methylphenidate treatment. Animals showed anxiogenic deficit on repeatedly exposure to stress as compared to unstressed animals which were attenuated by repeated administration of methylphenidate. It has concluded from present study that methylphenidate possesses anti-depressive effects and it can potentially suppress the behavioral deficits induced by chronic mild stress. Chronic mild stress showed the behavioral disturbance in laboratory rats which can be successfully decreased by the repeated treatment of methylphenidate.

Keywords: Behavioral Sensitization Chronic Mild Stress (CMS), Depression, Methylphenidate Locomotor Activity, Exploratory Activity.

1. INTRODUCTION

Stress is an alarming situation or homeostasis; it can disbalance the physical and mental equilibrium. Once disbalance, it can interrupt all the underlying processes inside the body which is accompanied by behavioral and physiological responses (Chrousos *et al.,* 1998).

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. Kaltz was the first who presents chronic unpredictable stress model in rats in 1981. A variety of stressors over a long period are required for the development of UCMS protocol (Willer *et al.,* 1987). If single or few stressor are used, it results in the habituation of behaviors (Muscat and willner 1992). Depression which is 4th reading cause overloaded among all the diseases (Murray and lopez 1996), produced by UCMS (Belzoni *et al.,* 2006), repeated stress increases ihe duration of corticoreleasing factor, glucocorticoid and catecholaminergic signaling , due to this disruption, mood and anxiety disorder occurred.(Porasko 2006). It results in behavioral deficits (Willner 1991; Muhammad Farhan *et al.* 2017), altered the sympathetic cardiac regulation (Grippo *et al.,* 2003), reduces the locomotive and explorative behavior (Willner 1991) which is due to serotonergic function results into the depression (Joca *et al.,* 2013) and used as a model for depression (D'Aquila *et al.,* 2000).

Behavioral sensitization is the consequence of repeated exposure of psychostimulants. It potentiates by the combine action of stressors and drug in somatodendritic region o dopamine neurons, changes allow the stimulation of D1 receptors. The activation of D1 thereby causes behavioral sensitization. Eric candle was the first to describe the neural basis of sensitization. Psychostimulants causing neurochemical and behavioral sensitization. Methylphenidate acts as a psychostimulants, first synthesized in 1944. The action of drug is mainly appear by hyperactive behavior and control impulse due to the responsiveness of neurotransmitters particularly dopamine and nor epinephrine. The effects of methylphenidate are produced by the binding and blocking the transporter proteins of dopamine and nor epinephrine (Solanto 1998). Significant dopamine transporter blockade(50-75%) induced by the therapeutic doses of oral methylphenidate (0.256-1mg/kg) as dopamine transporter is the main process for extracellular dopamine removal from brain(Volkow *et al.,* 1998) it is also reported that, if methylphenidate give orally it increases the extra cellular dopamine. It works by elevating level of dopamine, which inturns activate the dopamine auto receptor, increasing dopamine release in response to its activation (Seeman and Madareas 1998). Serotonergic system is known to respond the stress induced alteration (Maes and Meltzer, 1995). Serotonin precursor and agonists has influence on the level of serotonin through their effects on serotonergic receptors they also has significant impact on HPA activation.

Work has been presented which was aimed to observe the effects of psychostimulants on depressive behavior. Depressive people are more often seen to become habituated with psychostimulants; therefore present work is design in order to find out the possible effects of methylphenidate on uncontrollable stress induced depressive behavior.

© 2017 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

2. METHODS AND MATERIALS

Twenty four (Albino Wister) adult male rats (weighing 150-200 gm), locally bred in the central animal house of DUHS (Dow University of health and sciences). All animals were kept under 12-h light and dark conditions at 25 2^{o} with free access to tap water and standard rodent diet for familiarization for 3 days before the experimentation.

Methylphenidate (MPD) purchased from Sigma, St. Louis (USA). MPD at dose of 1 mg/kg/day was prepared in saline (0.9% NaCl) and administrated orally through a stainless steel feeding tube respective group animals. Control group animals were administrated with Saline.

Rats (n=24) were equally divided into two groups: (i) Unstressed and (ii) CMS groups. Animals of sCMS group were exposed to different stresses i.e. Cold stress (4`c), Cage agitation, Noise stress, Water deprivation stress, Day light off stress, Cage tiled stress, Repeated light/dark cycle, Crowding stress for a period of 14 days, while animals of unstressed groups remained in their home cages. After two weeks of stress schedule, each group animal was divided into two groups i.e. saline and methylphenidate injected. (i) Unstressed-saline,(ii) Unstressed-methylphenidate, (iii) CMS-Saline and (iv) CMS-Methylphenidate administrated group. Animals were administrated accordingly with methylphenidate at dose 1.0 mg/kg/day or saline 1 hour before of each stress. On next day activities in familiar and novel environment were monitored. The experimental case-control study was conducted in April 2015 at the Department of Biochemistry, University of Karachi and comprised male albino-wistar rats.

2.1. Behavioral assessment

Light Dark Transition Box: Assessment of anxiolytic profile of methylphenidate was done in a light dark transition box. In this box, number of entries and time spent by animals in light box was monitored. Cut off time is 5 minutes. This locally made transition box consists of two equal size compartments (26 x 26x 26) with an access (12 x 12 cm) between the compartments, differed in their sensory properties. Number of entry made by animals into a compartment of the box is defined as the placement of all four paws in the compartment of the activity box (Bourin M. Hascoet, 2003).

Elevated Plus Maze: The anxiolytic activity was assessed by elevated plus maze. The elevated plus maze was made up of white acrylic with four arms. Two of the arms were closed and two were open For the determination of activity animal was placed in the central area, facing the corner between an open and a closed arms. Entry was defined as all four paws being positioned within one of the open arms. Activity was determined in terms of number of entries in open arms and time spend in the open arms were monitored for 5 minutes.

2.2. Statistical analysis

SPSS 17 was used statistical analysis. Values were presented as means ± standard deviation (SD). Data on MPD administration of unstressed and CMS rats was analyzed by three-way analysis of variance (ANOVA) (repeated measures design). Post-hoc comparison was done by Newman-Keuls test. Values of $p<0.05$ were considered significant.

3. RESULTS

Data from the figure 1 demonstrate the behavioral effects of chronic stress on 1st,7th and 14th day on number of entries in male rats administered with methylphenidate (1.0 mg/kg/ml). The effect of days ($F=25.523,df=2,21;p<0.05$) was found significant. The effect of methylphenidate ($F=12.192,df=1,21$),the effect of stress($F=12.197,df=1,21$) and the effects of interaction among all the associated factors ($F=2.908,df=1,21$) were found non-significant. Individual group comparison done by Newman-Keul Test showed that methylphenidate administration repeatedly significantly decreased the entries in light box on 7th day($p<0.01$) of administration in unstressed group while the administration of methylphenidate increased the entries in light box from the 1st day ($p<0.01$) as compared to their respective saline control. Here saline treated unstressed rats shows the increase in activity on 7thy day ($p<0.01$) and 14h day($p<0.05$) of administration as compared to their control. In comparison of stressed group with their respective unstressed group, Methylphenidate showed to increase number of entries on 1st day ($p<0.01$) from their respective unstressed control.

Figure 2 shows the effect of daily stress on 1st, 7th and 14th day on food intake in male rats administered with methylphenidate (1.0 mg/kg/ml). analysis of data showed that the effect of days ($F=714.737,df=2,21;P<0.01$) and interaction of stress, drug and days($F=89.574,df=2,21;P<0.05$) were found significant. The effect of methylphenidate ($F=179.119,df=1,21$)and the effect of stress($F=61.356,df=1,21$) were found non-significant. Individual comparison done by the Newman-Keuls test revealed that Methylphenidate increased he time spent in light box of unstressed and stressed group animals. In unstressed animals

© 2017 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

Methylphenidate significantly increased ($p<0.05$) time spent on 7th and 14h day of administration s compared to saline control. Whereas in stressed animals Methylphenidate increased time spent only on 14th day ($p<0.05$) of administration. Repeated administration of methylphenidate decreased the time spent in stressed group as compared to their respective unstressed group on 7th and 14 day($p<0.01$) of administration whereas in saline treated animals of stressed group, Time spent first decreased after 1st day($p<0.01$) and then increased after 7th day($p<0.01$) of administration with respect to their respective control.

Data from the figure 3 as analyzed shows the behavioral effect of CMS on 1st, 7th and 14th day on number of entries/5minutes in male rats administered with methylphenidate at the dose of 100 mg/kg/ml. The effect of repeated monitoring ($F=61.791$, $df=2,21$;$p<0.05$) was found significant. The effect of methylphenidate ($F=9.947$,$df=1,21$),the effect of stress ($F=70.737$,$df=1,21$) and interaction of stress, drug and days ($F=4.683$,$df=2,21$) were found non-significant. Individual group comparison showed that number of entries increased in methylphenidate administrated rats on 14th day ($p<0.01$) as compared to saline rats in unstressed group. Methylphenidate is shown to increase the number of entries in stressed group on 7th day ($p<0.01$) whereas decreases the entries on 14th day ($p<0.05$) of administration. Saline treated unstressed rats shows the significant increase in entries on 7th day ($p<0.01$) and decrease the entries on 14thday ($p<0.01$) of administration; in comparison of stressed group with their respective unstressed group.

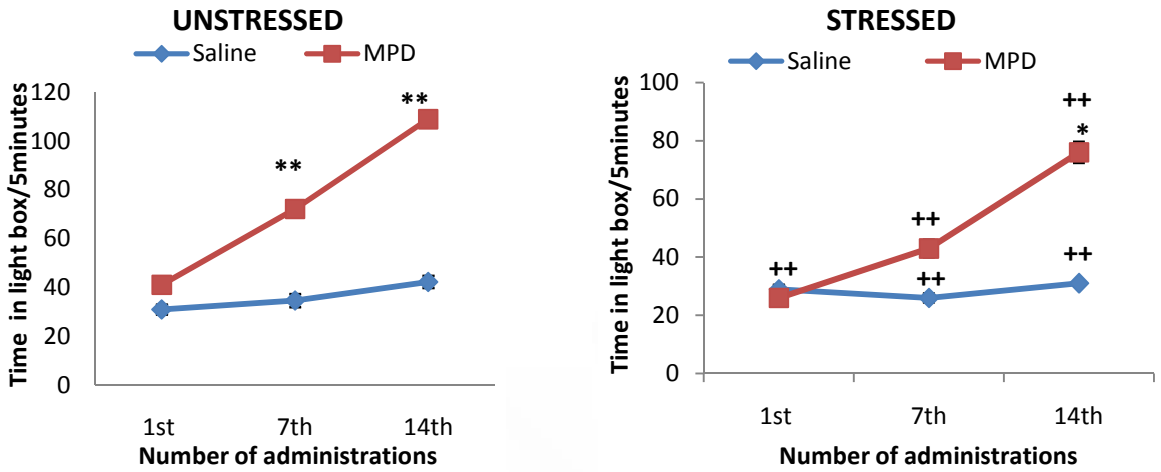

Figure 1 Effect of methylphenidate administration on activity in Light/Dark box of rats exposed to UCMS. Values are means + SD (n=6) as monitored on 1st, 7th and 14th day of drug administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from similarly treated saline control; +p<0.05, ++p<0.01 from saline and drug unstressed control of same day following three way ANOVA (repeated measure design).

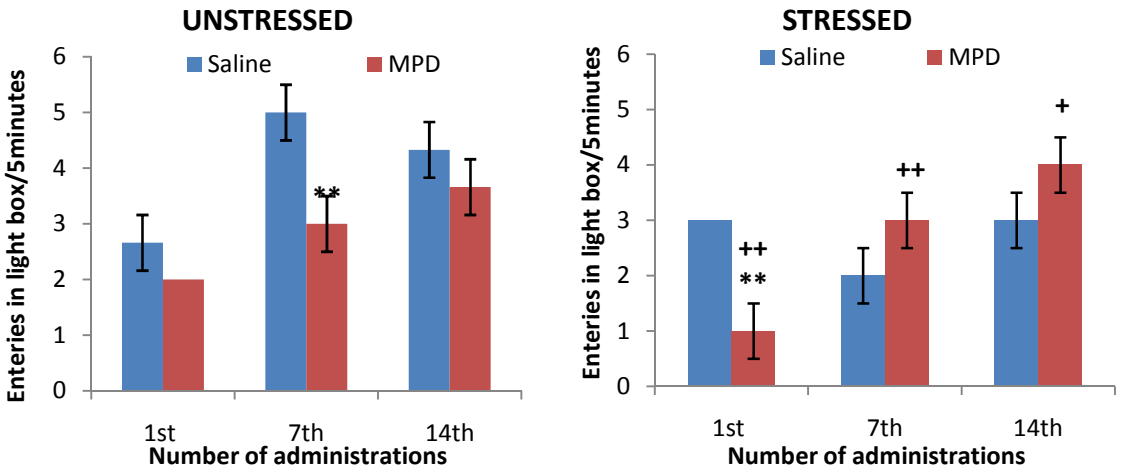

Figure 2 Effect of methylphenidate administration on activity in Light/Dark boxof rats exposed to UCMS. Values are means + SD (n=6) as monitored on 1st, 7th and 14th day of drug administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01

© 2017 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

from similarly treated saline control; +p<0.05, ++p<0.01 from unstressed control of same day following three way ANOVA (repeated measure design).

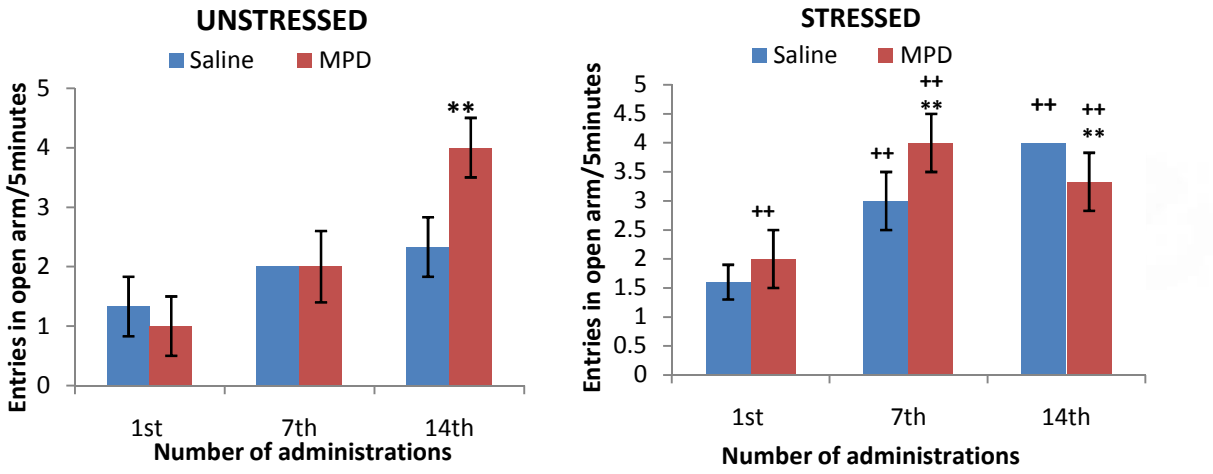

Figure 3 Effect of methylphenidate administration on activity in Elevated Plus Maze of rats exposed to UCMS. Values are means+SD (n=6) as monitored on 1st, 7th and 14th day of drug administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from similarly treated saline control; +p<0.05, ++p<0.01 from unstressed control of same day following three way ANOVA (repeated measure design).

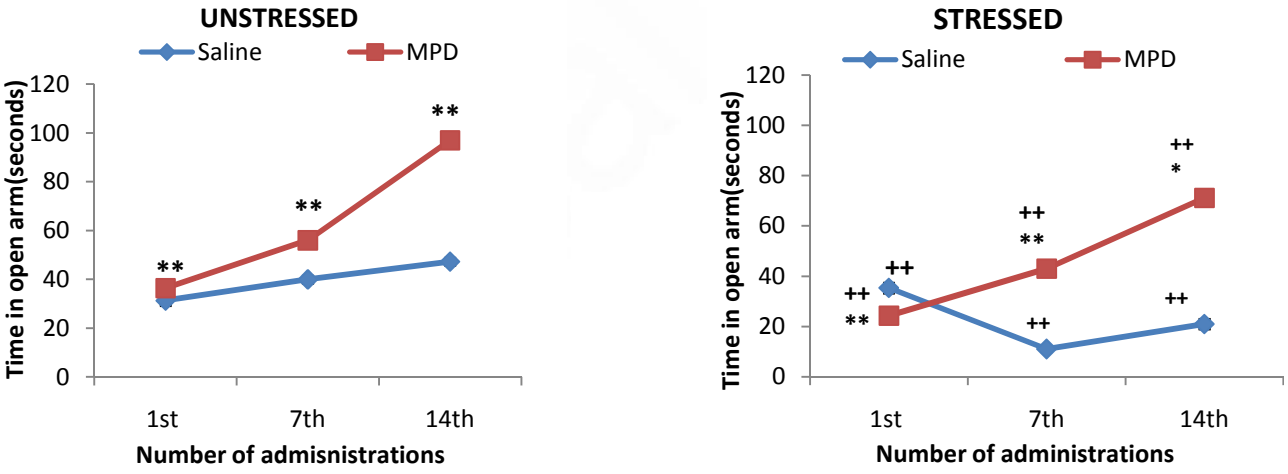

Figure 4 Effect of methylphenidate administration on activity in Elevated plus Maze of rats exposed to UCMS. Values are means + SD (n=6) as monitored on 1st, 7th and 14th day of drug administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from similarly treated saline control; +p<0.05, ++p<0.01 from unstressed control of same day following three way ANOVA (repeated measure design).

Methylphenidate increased the entries in stressed group on 1st day (p<0.01) and 7th day (p<0.01) whereas the number of entries significantly decreased on 14th day (p<0.01) in stressed group with respect to their control unstressed group.

Effect of UCMS (figure 4) on 1st,7th and 14th day on time spent in male rats administered with methylphenidate (1.0 mg/kg/ml) as analyzed by three-way ANOVA(repeated measure designing) showed the effect of days (F=231.107,df=2,21;p<0.01), the effect of methylphenidate (F=7560.380,df=1,21;p<0.01),the effect of stress(F=4034.408,df=1,21;p<0.01) and interaction of stress, drug and days(F=307.300,df=2,21;p<0.01) were found significant. Individual comparison by Newman-Keuls Test showed that methylphenidate increased time spent in open arm of unstressed and stressed group animals as compared to their saline control. Time spent in open

© 2017 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

arm is significantly increased ($p<0.01$) on 1st,7th and 14th of administration in unstressed animals. Similarly in stressed group animal, time spent increased ($p<0.01$) on 1st, 7th and 14th day administration. Here in saline administrated animals, time spent first increased ($p<0.01$) on 1st day and then decreased ($p<0.01$) on 7thand 14th day as compared o their respective control. In comparison of stressed group with stressed group, Methylphenidate decreased ($p<0.01$) the time spent in open arm in stressed group on 1st, 7th and 14th day of administration.

4. DISCUSSION

The present study intended to investigate whether methylphenidate might be act as antidepressant to turn down the behavioral deficits induced in animal repeatedly exposed to chronic stress. Chronic mild stress rat models were used in this experiment and the work was designed to study of the effects of pre administration of methylphenidate at dose 1.0 mg/kg/day followed by the chronic mild stress for 14 days in laboratory rats. Results suggested the antidepressive effects of methylphenidate doses (1 hour before CMS) in chronic mild stress induced behavioral deficits. CMS exposed animals showed behavioral deficits, which recovered by the repeated administration of methylphenidate treatment.

Chronic stress has been considered the root cause of much psychologic illness including major depression. Depression which is 4th leading cause overloaded among all the diseases (Murray and lopez 1996). Unpredictable chronic mild stress can contribute to elevate the prevalence of depression by exaggerate the HPA axis. Hyperactivation of HPA axis further causes increased cortisol level which thereby produces many catabolic processes. Disruption in neurochemical and hormones may cause variations at psychological level and appear as psychiatric illnesses such as mood and anxiety disorder occurred. This psychological alteration is due to serotonergic dysfunctioning and has great impact on locomotive and explorative behavior (Willner 1991).

Psycho-stimulants like methylphenidate, affect the brain chemical particularly on dopamine which leads to hyperactivity and control of impulse and maintain the alertness, improving attention. Methylphenidate also increases the hyperphagic effects and growth rate. All of these effects combine to cause the antidepressive and anxiolytic effects.

Present study showed the recordings of antidepressive effects of methylphenidate. Figure 1 indicates the anxiolytic effects (increased time spent in light box) of methylphenidate in light dark box in stressed as well as unstressed rats. The activity of methylphenidate treating stressed rats is more significant on 14th day of administration as compared to saline treated stressed rat. Whereas the effect of methylphenidate is more prominent in unstressed rats as compared to stressed rats. Figure 2 also shows the monitoring of anxiolytic activity (higher number of entries in light box) of methylphenidate administration in light dark box and showed the opposite result with Figure 1 as the activity of methylphenidate treating stressed rats are more significant as compared to unstressed rats it can be the reason because unstressed rats spend more time in light box thereby reduced the entries. Anxiolytic profile of methylphenidate has also shown in Figure 3 and 4 and the effects is significant in stressed rats on 7th day (number of entries in open arm) and 14th day (time spent in open arm) respectively. Whereas, these effects are more prominent in unstressed rats as compared to methylphenidate treated stressed rats. It has been concluded by the results of present study that prior administration of methylphenidate have attenuated uncontrollable chronic mild stress induced depressive behavior in animal model of depression.

REFERENCE

1. Bourin M.; Hascoet, M (2003). The mouse light/ dark test. Eur. J. Pharmacol, 463: 55-65.
2. Chrousos GP, Torpy DJ, Gold PW (1998). Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. Ann Intern Med 129:229–240.
3. D'Aquila, P. S., Collu, M., Gessa, G. L., & Serra, G. (2000). The role of dopamine in the mechanism of action of antidepressant drugs. European Journal of Pharmacology, 405, 365-373.
4. Grippo, A. J., T. G. Beltz, et al. (2003). "Behavioral and cardiovascular changes in the chronic mild stress model of depression." Physiol Behav 78(4-5): 703-10.
5. Joca SR, Padovan CM and Guimaraes FS (2003). Activation of post synaotic 5-HT-1A receptors in the dorsal hippocampus prevents learned helpnessness development. Brain Res, 978: 177-184.
6. Katz R.J, Roth K.A, Carroll BJ (1981). Acute and chronic stress effects on open field activity in the rat: implications for a model of depression. Neurosci Biobehav Re, 5(2): 247-251.
7. Kronfeld-Schor N, Einat H (2012). Circadian rhythms and depression: Human psychopathology and animal models. Neuropharmacology. 62(1): 101-114.
8. Maes M and Meltzer H. (1995). The serotonin hypothesis of major depression, in Bloom F E and Kupfer DJ. (eds), Psychopharmacology: the fourth generation of progress, New York, Raven press: 933-44.
9. Muhammad Farhan, Ifaza Muneer, Safia Habib. Paroxetine: to meliorate the behavioral deficits-induced by repeated

© 2017 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS

exposure to chronic mild stress in rats. Medical Science, 2017, 21(87), 234-244.

10. Murray CJL, Lopez AD (1996). The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries, and risk factors in 1990 and projected to 2020. Cambridge: Harvard University Press.
11. Seeman P, Madras BK (1998) Anti-hyperactivity medication: methyl- phenidate and amphetamine. Mol Psychiatry 3:386 – 396.
12. Solanto M V (1998) Neuropsychopharmacological mechanisms of stimu- lant drug action in attention-deficit hyperactivity disorder: a review and integration. Behav Brain Res 94:127–152.
13. Song C, Wang H (2011). Cytokines mediated inflammation and decreased neurogenesis in animal models of depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 35(3): 760-768.
14. Volkow N D, Wang G-J, Fowler JS, Gatley JS, L ogan J, Ding Y-S, Hitzemann R, Pappas N (1998) Dopamine transporter occupancies in the human brain induced by therapeutic doses of oral methylphenidate. Am J Psychiatry 155:1325-1331.
15. Willner P, Klimek V, Golembiowska K, Muscat R (1991). Changes in mesolimbic dopamine may explain stress-induced anhedonia. Psychobiology. 19: 79-84.
16. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 93: 358–364.
17. Willner, P., Muscat, R., Papp, M. (1992). Chronic mild stress-induced anhedonia: a realistic animals model of depression. Neurosci. Biobehav. Rev. 16, 525–534.

© 2017 Discovery Publication. All Rights Reserved. www.discoveryjournals.com | OPEN ACCESS