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NEURO-PSYCHO-PHARMACOLOGICAL STUDIES ON INTERACTION OF STRESS AND METHYLPHENIDATE IN CHRONIC MILD STRESSMODEL OF DEPRESSION

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ABSTARCT

This paper describes neuro-psycho-pharmacological study on stress in rats. Male rats were divided into two groups; animals of stressed group were exposed to chronic mild stress. Animals of unstressed and stressed group were administrated with methylphenidate at dose 1.0 mg/kg/day for 14 days 1 h before the animals exposed to chronic mild stress. The purpose of the present study was to investigate whether methylphenidate might act as antidepressant to reduce the depressive like behavior in animal model of depression. Behavioral assessments were done by using different behavioral models.Repeated administration of methylphenidate attenuatedthe induced behavioral deficits. Animals which were exposed to chronic mild stress showed behavioral impairments which were potentially improved by the repeated administration of methylphenidate. Methylphenidate promotes hyperphagia. Unstressed-methylphenidate treating animals showeda higher growth rate than stress- methylphenidate treating animals. Behavioral sensitization monitored in familiar environment and exploratory activity in open field was higher in unstressed than stressed animals**.** The Results may help in understanding the pathophysiology of addiction and role of stress in the development of addiction.

Keywords: Behavioral Sensitization Chronic mild stress (CMS), Depression, Methylphenidate Locomotor Activity, Exploratory Activity.

INTRODUCTION

Stressis a nonspecific response of a body in a challenge which is often manifested as a threat for homeostasis, therefore, stress is consideredas disharmony that is accompanied by behavioral and physiological responses (Chrousos *et al.*, 1998). Chronic stress plays an important role in the progression of many psychological conditions like depression. Behavioral and neurochemical responses of drugs challenges have been studied in various animal models of depression (Kronfeld - Schorand Einat, 2012; Song and Wang, 2011). The first chronic stress model of depression was developed by Kaltz in 1981. In contrast to repeated exposure to similar stress, chronic exposure to unpredictable mild stress is more potentiate for developing depression, as it prevents adaptationto stress. A variety of stressors over a long period are required for the development of chronic mild stress protocol (Willner *et al*., 1987). If single or few stressors are used, it results in the habituation of behaviors (Muscat and Willner. 1992).Depression, which is 4th overloaded among all the diseases (Murray and Lopez, 1996). It results in behavioral deficits (Willner, 1991), 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 (Gessa *et al.*, 2000).

Repeated doses of psychostimulant develop a condition known as behavioral sensitization which enhances by the action of stressor and drug in somatodendritic region of dopamine neuron. Methylphenidate was introduced in 1944 as a psycho-stimulant. It enhances the release of neurotransmitters, particularly dopamine inthe brainwhich leads to hyperactivity and control of impulse. It also has an influenceon alertness and improving attention. Methylphenidate is approved by FDA for the treatment of Attention deficit hyperactivity disorder (ADHD). It is dopamine and nor epinephrine reuptake inhibitor (Sadile *et al.*, 2004; Solan, 1998). Significant effects of methylphenidate induced by therapeutic doses of 0.256-1mg/kg oral (Volkow *et al.*, 1998). Oral administration of methylphenidate elevates the level of extra cellular dopamine which inturnsactivates the dopamine auto receptor, increasing dopamine release in response to its activation (Seeman and Madareas 1998).

In previous studies, repeated restraint and chronic mild stress have been reported, respectively, to increase and to decrease the locomotor response to dopamine agonists (Cabib and Puglisi-Allegra, 1996; Papp *et al.,* 1993; Willner *et al.,* 1992). These observations prompted us to suggest that antidepressants influence the mesolimbic dopamine system sensitivity in a direction which is opposite to that induced by stress, at least as far as the neural circuits mediating locomotor activity are concerned. Stress induced change in the activity of the system is under the control of brain serotonergic system (Maes and Meltzer, 1995). Pharmacological studies using the serotonin precursor tryptophan, serotonin releasing drugs such as fenfluramine or drugs that act directly upon serotonin receptors have shown that increase in serotonin concentration may alter Hypothalamic pituitary adrenal (HPA) axis activation.

The aim of the presentwork was to highlight the interactions between stress and addiction. Depressive behavior is more often seen in addicts than normal individuals therefore, present work was designed to find out whether stress provokes addiction or addictive desires reinforce by irresistible stressful events.

METHODS AND MATERIALS

ANIMALS

Albino Wister adult male rats (weighing 150-200 gm) were bought from the Dow University of health and sciences Ojha Campus, Karachi. Experiments were performed in strict accordance and conducted in abalanced design with the guide for the care and use of laboratory animals (Institute of laboratory Animal resources on life sciences, US National Research Council, 1996) and the Institutional ethical Committee's guidelines for animal research.All animals were kept under 12-h light and dark conditions at 25 2^{o} and maintained on free access to standard rodent diet and tap water for familiarization for 3 days before the experimentation.

DRUG

Methylphenidate (Sigma, St. Louis, USA) was dissolved in saline (0.9% NaCl) and administrated orally through a stainless-steelfeeding tube at dose of 10 mg/kg /day to the respective group animals. The drugwas freshlyprepared before starting the experiment. Saline (0.9% NaCl solution; 1 ml/kg/day) was administrated to control animals.

EXPERIMENTAL PROTOCOL

Twenty-four male Albino Wistar rats were randomly divided into two groups: (i) Unstressed and (ii) Stressed groups. Animals of stressed groups were exposed to a schedule of chronic mild stress (Table 1) over a period of twoweeks while animals of unstressed groups remained in their home cages.The animals of each group were again subdivided into further groups each i.e., saline and methylphenidate injected. This resulted in a total of four groups (i) Unstressed-saline, (ii) Unstressed-methylphenidate, (iii) chronic mild stress-Saline and (iv) chronic mild stress -Methylphenidate injected animals.Animals were administrated accordingly with methylphenidate at dose 1.0 mg/kg/day or saline before of each stress. On the next day activities in a familiarand exploratory environment were monitored.

Table 1.Chronic Mild Stress.

S. No	***Chronic Mild Stress***	***Days***
1.	Restraint Tube Stress (3 h) and Without Sawdust (3h)	Day 01 and 14
2.	Tilted Cage (3 h) and Noise Stress (3h)	Day 02 and 13
3.	Cage Agitation (3h)and Cold Stress 4.c(3 h)	Day 03 and 12
4.	Water Stress (3 h) and Damp Sawdust (3h)	Day 04 and 11
5.	Repeated Light Dark Cycle (3 h) and Social Stress (3 h)	Day 05 and 10
6.	Crowding (3 h) and Coat State (3h)	Day 06 and 09
7.	Wet Cage (3 h) and12 h Water Deprivation	Day 07 and 08
8.	Noise Stress (3h) and Restrained Tube Stress (3 h)	Day 08 and 07
9.	Cold Stress (4 ·C) (3 h) and Cage Agitation (3 h)	Day 09 and 06
10.	Damp Sawdust (3 h) and Water Stress (3 h)	Day 10 and 05
11.	Social Stress (3 h) and Tilted Cage (3 h)	Day 11 and 04
12.	Coat State (3 h) and Wet Cage (3 h)	Day 12 and 03
13.	Without Sawdust (3 h) and Repeated Light Dark Cycle (3 h)	Day 13 and 02
14.	12 h Water Deprivation and Crowding (3h)	Day 14 and 01

Behavioral Assessment

1. Growth Rate

Body weight changes were monitored to find out the effect of treatments on growth of animals after 1st day of stress and then weekly. Growth rate changes were calculated as percentage of starting day (experiment day body weight/starting day body weight) x 100.

2. Food Intake

Daily and then weekly food intake was observed in order to determine the activity of the drug. In the Hooper of every cage, a weighed amount of rodent cubes were placed. Intake of food was monitored by weighing leftover food in the hooper.

3. Home cage activity

Home cage activity is used for determination of locomotors activity in familiar environment. Measurement of locomotors activity in rats is an easy and simple way to access behavior that reflects altered physiology of an animal. Using the home cage activity test, duration of monitoring is of 10 minutes for the study of stress or drug induced activity. Activity cage apparatus was a square Perspex cage (26x26x26 cm). All monitoring was done in balanced design. Observations were recorded simultaneously. The parameter observed was the number of crossings across the cage / 10 minutes.

4. Open Field Activity Test:

The open field activity test was performed to estimate exploratory activity.In this test, the open field apparatus is much larger than that of a home cage and is non-familiar to rodent. Rats were taken out from their cages and placed into an open field arena. The open apparatus comprises of opaque plastic walls of 42cm high and 76 x 76 cm square area.All rats were observed in a balanced design. The floor of the apparatusconsists of 25 equal squares. The rat was placed in the centre square of the field. The parameters observed were:

- The latency time (time which was taken by animal for exploring from the centre square of arena)
- Numbers of square crossed (the number of squares crossed by the animal with all four paws in 5 minutes)

Statistical Analysis

Values are presented as means ± SD. Data on drug administration of unstressed and stressed rats were analyzed by three-way ANOVA (repeated measures design). The softwareused 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.

RESULTS

Fig. 1 shows the effect of Unpredictable chronic mild stresson 1st,7th and 14th day on growth rate in male rats administrated with methylphenidate (1.0mg/ml/kg).Data analyzed by three-way ANOVA (repeated measure designing) the effect of days ($F=11.799,df=2,21$), the effect of Methylphenidate ($F=18.959,df=1,21$),the effect of stress ($F=12.432,df=1,21$) and the interaction of stress, drug and days ($F=18.924,df=2,21$) were found to be non-significant. Post hoc analysis by Newman-Keuls test showed that administration of methylphenidate increases the food intake in unstressed and stressed group animals. Significant increase in food intake was found on 7th day ($p<0.05$) and 14th day ($p<0.01$) of administration in stressed group. Similarly in unstressed group, food intake increased on 7th day ($p<0.05$) and 14th day ($p<0.01$) of administration of methylphenidate. The comparison of stressed group animal and unstressed group animal, saline treated animal decreased food intake significantly on 7th day ($p<0.05$) after being exposed to stress whereas methylphenidate treated animal stress decreased the food intake significantly on 14th day ($p<0.01$) of administration.

Fig.2 shows the effect of chronic mild stress on 1st, 7th and 14th day on growth rate in male rats administered with methylphenidate at the dose of 1.0 mg/ml/kg.Data analyzed by three-way ANOVA (repeated measure designing) the effect of days ($F=0.035,df=1,21$), the effect of methylphenidate ($F=6.182,df=1,21$), the effect of stress ($F=4.721,df=1,21$) and interaction of stress, drug and days($F=9.542,df=1,21$) were found non-significant . Post hoc analysis by Newman-Keuls Test showed that

exposure to chronic mild stress decreases the growth rate in saline treated animal significantly after 7th day ($p<0.01$) of stress as compared to similarly administered animal of unstressed group. Administration of methylphenidate increase the growth rate on 1st ($p<0.01$) and 7th day ($p<0.01$) of administration of stressed group. In unstressed group, growth rate of methylphenidatetreated animal increased on 7th day ($p<0.01$) and 14th day ($p<0.01$) of administration as compared to their saline group. In stressed group, the growth rate of methylphenidate animals showed to increase on 7th day ($p<0.01$) and 14thday ($p<0.01$) of administration.

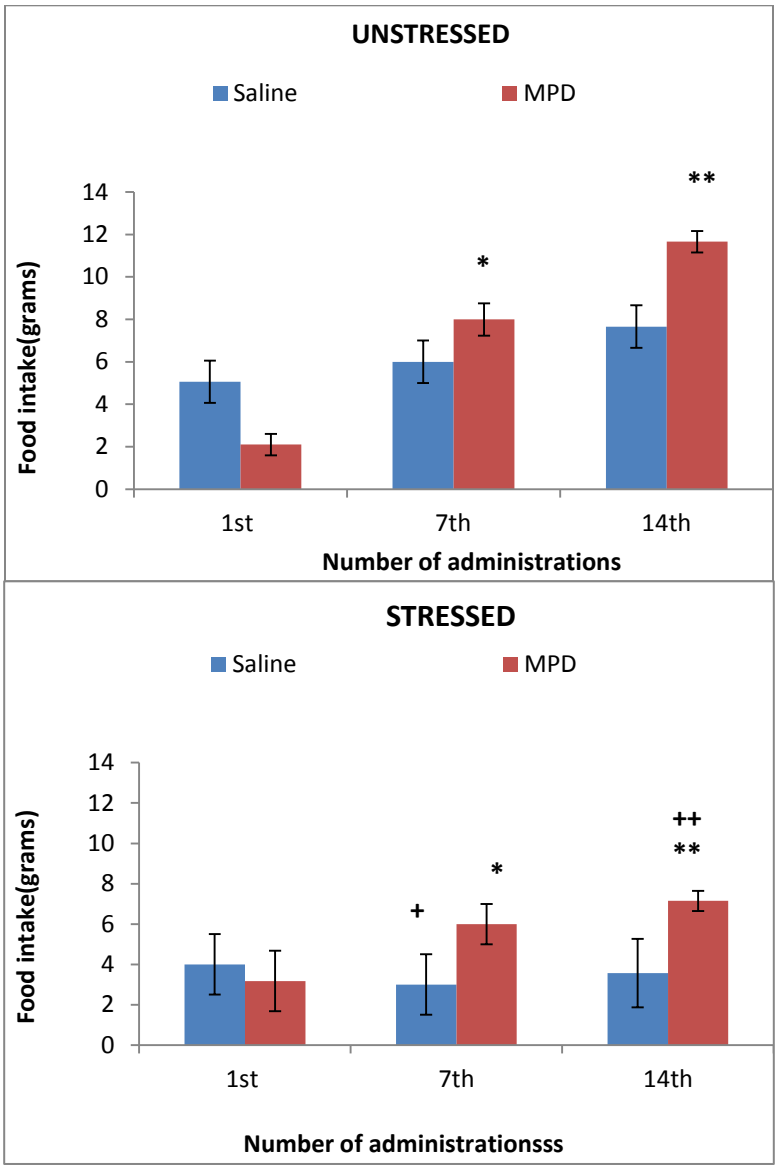

Fig. 1. Effect of methylphenidate administration on Food Intake of rats exposed to chronic mild stress. Values are means ± SD (n=6) as monitored on next day of 1st, 7th and 14th day of drug administration. Significant differences by Newman-Keuls test:*$p<0.05$, **$p<0.01$ from similarly treated saline control; +$p<0.05$, ++$p<0.01$ from respective Saline or drug administrated unstressed control following three-way ANOVA (repeated measure design).

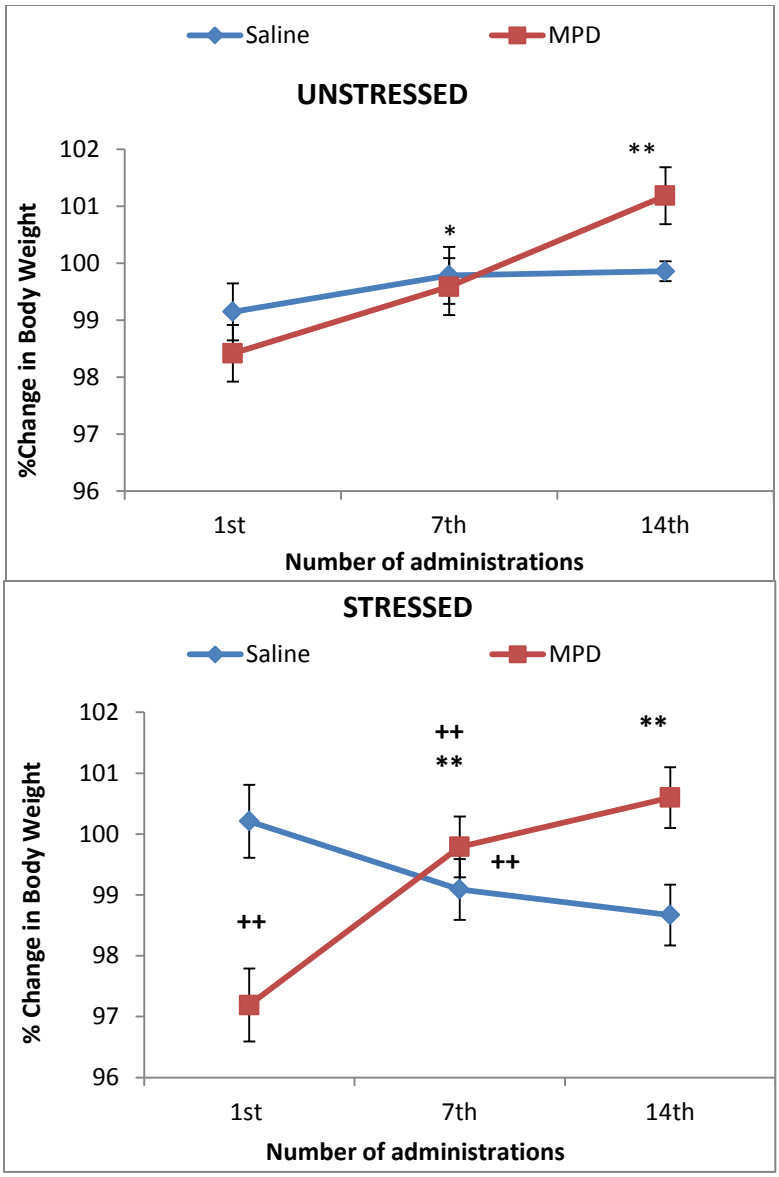

Fig. 2. Effect of methylphenidate administration on Growth rate of rats exposed to chronic mild stress Values are means ± SD (n=6) as monitored on 1st, 7th and 14th day of drug administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from similarly treated saline control; +p<0.05, ++p<0.01 from similarly administrated unstressed control of same day following three-way ANOVA (repeated measure design).

Fig. 3 shows the effect of chronic mild stress on 1st, 7th and 14th day on cage counts/10minutes in male rats administered with methylphenidate (1.0 mg/ml/kg/). Data as analyzed by three-way ANOVA (repeated measure designing) the effect of days (F=141.310,df=13,21;p<0.01), the effect of methylphenidate (F=384.267,df=1,21;p<0.01) and interaction of stress, drug and days (F=13.763,df=13,21;p<0.01) were found significant. Whereas the effect of stress (F=97.763, df=1, 21) was found non-significant. Post-hoc analysis by Newman-Keuls test showed that administration of methylphenidate increased the activity in unstressed group as well as chronic mild stress animals .In the stressed group, significant increase in activity was found after 10th, 11th, 12th, 13th and 14th day (p<0.01) of administration. Whereas in unstressed group activity was found to be increased after 8th-14th day (p<0.01) as compared to saline treated unstressed or chronic mild stress animals. In unstressed group, number of cage count decreases in saline

administration animal after 3rd day ($p<0.05$) as compared to unstressed methylphenidate animal. Repeated administration of methylphenidate increased the activity in both unstressed and stressed group.

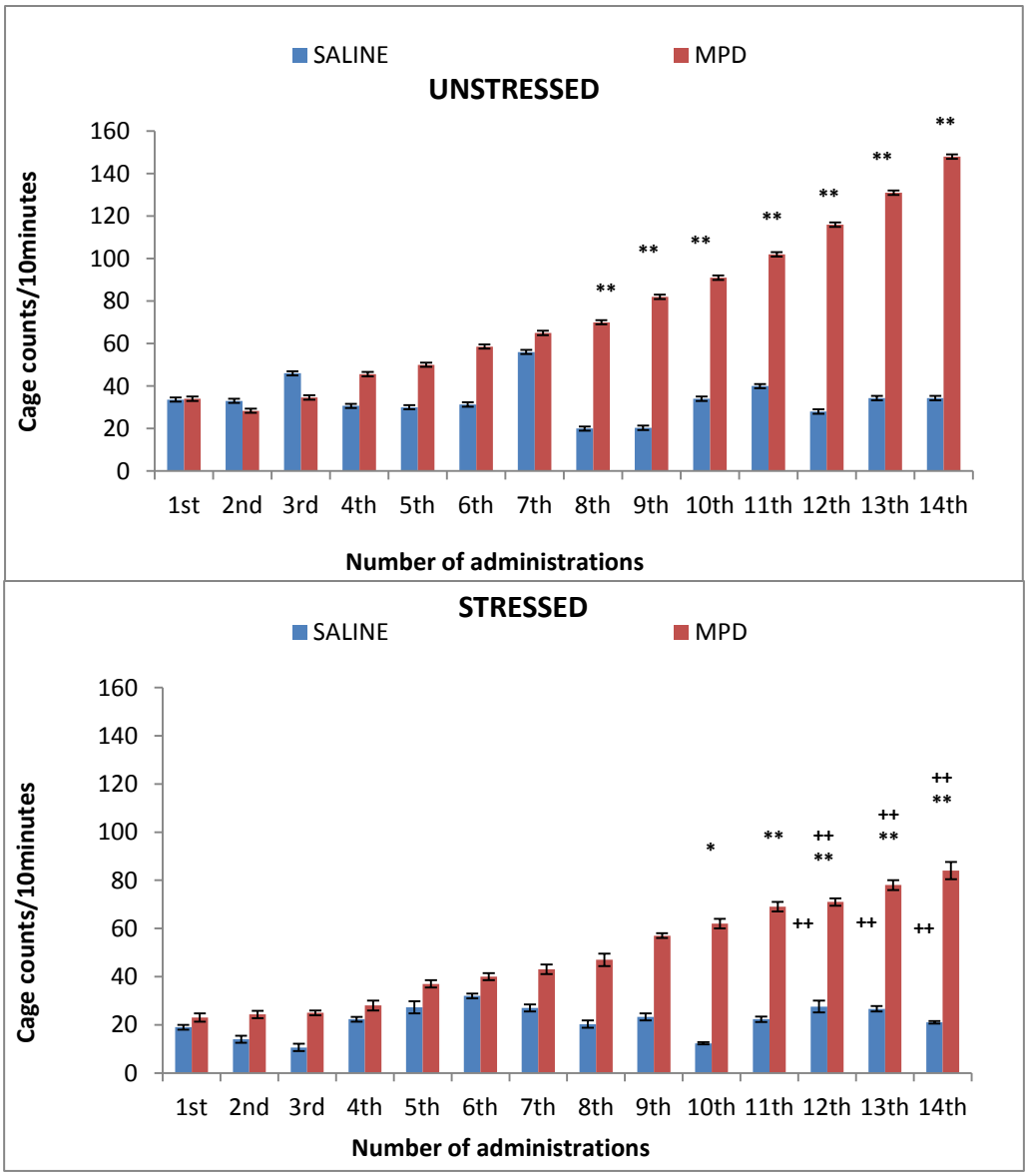

Fig.3. Effect of methylphenidate administration on activity in Home Cage of rats exposed to chronic mild stress. Values are means ± SD (n=6) as monitored on next day of 1st and then daily drug administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from similarly treated saline control; +p<0.05, ++p<0.01 from saline or drug administrated unstressed control following three-way ANOVA (repeated measure design).

Fig.4 shows the effect of chronic mild stress on 1st, 7th and 14th day on number of square crossed in male rats administered with methylphenidate (1.0 mg/ml/kg). Data analyzed by three-way ANOVA(repeated measure designing) the effect of days ($F=297.199, df=2,21; p<0.01$), the effect of methylphenidate ($F=615.659, df=1,21; p<0.01$), the effect of stress ($F=271.261, df=1,21; p<0.01$) and interaction of stress, drug and days($F=26.355, df=2,21; p<0.05$) were found significant. Post hoc analysis by Newman-Keuls test showed administration of methylphenidate increased the activity of rats in unstressed and stressed group animals. Significant increase ($p<0.01$) was found in unstressed group on 1st, 7th and 14th day of administration. Similarly in stressed animals methylphenidate significantly increased ($p<0.01$) the

number of square crossed on 1st, 7th and 14th day administration as compared to the saline control. In saline administrated animal, square crossed significantly decreased ($p<0.01$) after the exposure of stress on 1st, 7th and 14th day. In comparison of stressed animals and unstressed animals, methylphenidate administrated animals significantly decreased ($p<0.01$) number of square crossed on 1st, 7th and 14th day of administration.

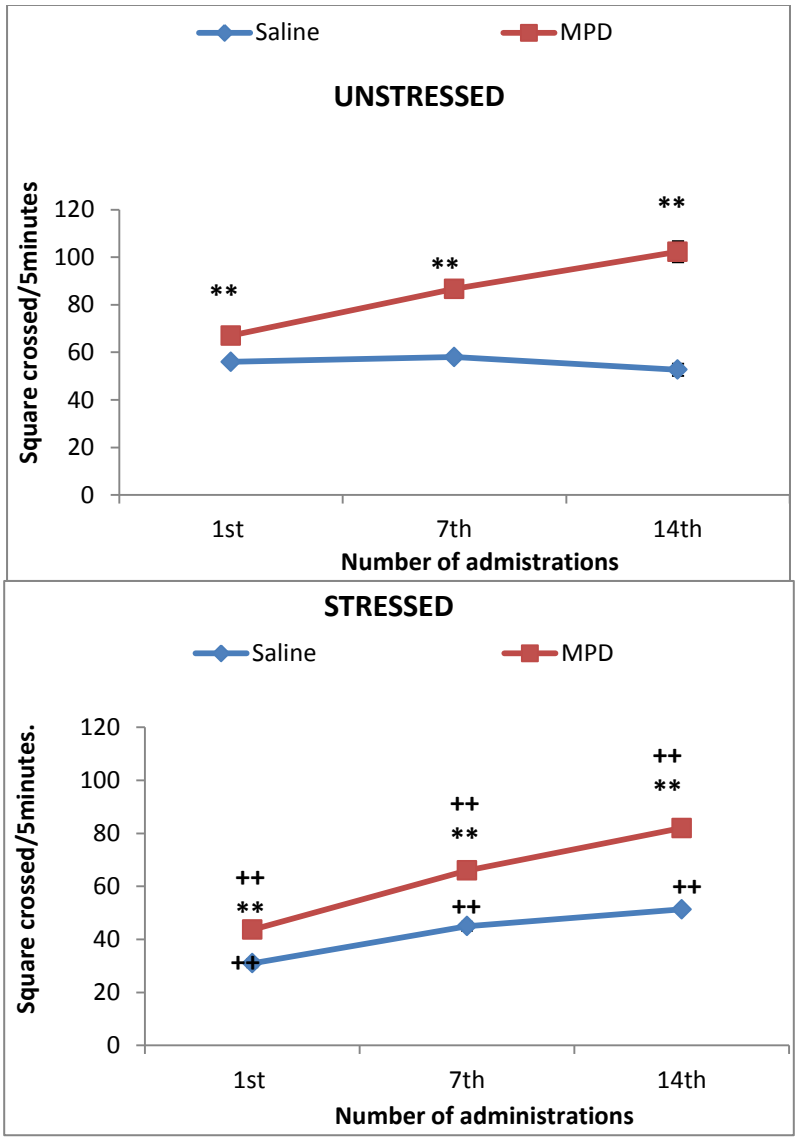

Fig. 4. Effect of methylphenidate administration on activity (Square crossed) in Open Field of rats exposed to chronic mild stress. Values are means ± SD (n=6) as monitored on 1st, 7th and 14th day of drug administration. Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 from similarly treated saline control; +p<0.05, ++p<0.01 from similarly administrated unstressed control of same day following three ways ANOVA (repeated measure design).

Fig. 5 shows the effect of chronic mild stress on 1st, 7th and 14th day on Latency time in male rats administered with methylphenidate at the dose of 1.0 mg/ml/kg. Data analyzed by three-way ANOVA(repeated measure design) the effect of days ($F=2.025, df=2,21$), the effect of methylphenidate ($F=3.576, df=1,21$), the effect of stress ($F=1.511, df=1,21$) and interaction of stress, drug and days($F=1.562, df=2,21$) were found non-significant. Post-hoc analysis by Newman-Keuls test showed that administration of methylphenidate decreased latency time as compare to saline administrated animals in

unstressed as well as chronic mild stress animals. Significant decreased was found after 1st administration in unstressed ($p<0.01$) and chronic mild stress ($p<0.05$). After two weeks administration, methylphenidate produced reverse effects as compared to saline administrated animals.

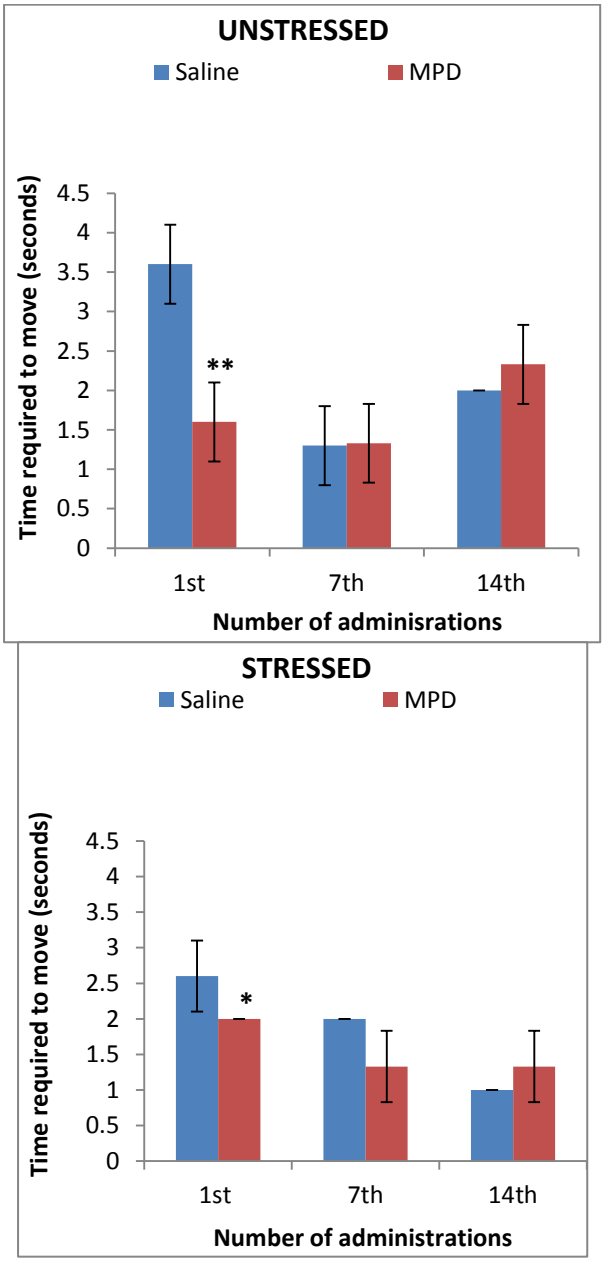

Fig. 5. Effect of methylphenidate administration on latency time in Open Field of rats exposed to chronic mild stress. Values are means ± SD (n=6) as monitored on 1st, 7th and 14th day of drug administration. Significant differences by Newman-Keuls test: *$p<0.05$, **$p<0.01$ from similarly treated saline control; following three-way ANOVA (repeated measure design).

DISCUSSION

The aim of the present study was to investigate whether repeated administration of methylphenidate at dose 1.0 mg/kg 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's reaction to stress and

beginning of depression. The present study provides the evidences 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.Methylphenidate is a psychomotor stimulant, which readily enters the brain to alter dopamine (DA) neurotransmission, used for the treatment of attention deficit hyperactivity disorder (Leddy *et al.,* 2009; Volkow *et al.,* 2012; Olfson *et al.,* 2007). Methylphenidate binds to DA transporters, therefore DA neurotransmitter remain in the synaptic cleft for longer time, produces an indirect DA agonist effect (Howell *et al.,* 2008). Administration of methylphenidate produced hyperphagia and increased in growth rate, which were higher in unstressed animals then stressed animals.Repeated administration of methylphenidate at dose 1.0 mg/kg/day induced behavioral sensitization and exploratory activity was greater in unstressed animals as compared to chronic mild stress group animals.Results from the present study showed that methylphenidate repeated administration increased food intake and growth rate (Figure 1& 2), more likely increase in the food intake and growth rate was found after 1st and 2nd week administration. Some previous studies demonstrated that methylphenidate produces temporary retardation in height and weight gain (Aronson, 2008). However, there is variance in the reported long-term ability of MPH to sustain weight loss ranging from three months to the duration of administration of a clinically effective dose (Leddy *et al.,* 2009; Barkley *et al.,* 1990). Stimulants are known to produce hypophagic (Goldfield *et al.,* 2011; Davis *et al.,* 2012; Dourish, 1995; Sugrue, 1987). The important observation of this study is that the side effect of stimulants such as anorexia is not observed.

Development of locomotor sensitization to psycho-stimulant drug is an important predictor of psycho-stimulant drug abuse in animal models (Robinson and Berridge., 1993). Present study revealed the expression of behavioral sensitization (Fig. 3) as observed greater in unstressed animals as compared to stressed animals gradually on repeated administration of methylphenidate in familiar and novel environment (Nausheen *et al.,* 2016). In open field, animals of stressed group take more time to start locomotion (latency time). On the other hand,unstressed animals latency time was short (Fig.5). Methylphenidate elevates the release of dopamine in neocortex (Berridge *et al.,* 2006) it also blocks the dopamine transporter (Ferris and Tang., 1979, Kollinset *al.,* 2001, Barrett*et al.,* 2005) whereas acute intake can be consequences of hyperactive behavior which exaggerates by repeated doses (Castellanos and Tannock. 2002, Rubia *et al.,* 2010, Schecklmann *et al.,* 2010). Dopamine system has been reported as a crucial part in the process of behavioral sensitization (Kalivas *et al.,* 1993a, 1993b).It has often seen that dopamine release is directly associated in response of psychoactive drugs, as they increase response of dopamine on repeated doses (Cox *et al.,* 2009). The activation of D2 receptors has significant influence on a variety of physiological function and there regulation, like locomotor activity controls (Picetti*et al.,* 1997). Dopamine transmission can be antagonized by serotonin in the region of mid brain and terminal region (Haleem, 2006).

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