Repeated treatment with reserpine as a progressive animal model of depression

Huma Ikram[1]* and Darakhshan J Haleem[1,2]
[1]Neurochemistry and Biochemical Neuropharmacology Research Unit, Department of Biochemistry, University of Karachi, Pakistan
[2]Neuroscience Research Laboratory, Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi, Pakistan

Abstract: Treatment-resistant depression is a major health problem worldwide. Restricted validity of the existing animal models of depression along with the need for the study of progressive development of resistance to antidepressants, demands the modeling of a progressive animal model of depression. Present study was designed to test the hypothesis that the repeated administration of reserpine could serve as a progressive animal model of depression. Animals were injected with reserpine (1.0mg/kg; once a day) for three weeks. Results from the present study showed impaired locomotive effects of reserpine in Skinner's box following second as well as third week. These hypolocomotive effects were more pronounced after third week than the second week. Reserpine-induced behavioral depression was evident in the animals after 2 weeks, as assessed by using forced swim test. Depletion of 5-HT, dopamine and metabolites was also observed in the brain samples. Results from the present study suggest that repeated administration of reserpine could be serve as a progressive model of depression and could be used as a convenient and economic animal model for the face validity of anxiolytic compounds. Findings have potential implications with reference to the understanding and the management of treatment-resistant depression.

Keywords: Reserpine, depression, open field, skinner's box, forced swim test, anxiety**,** serotonin**,** dopamine.

INTRODUCTION

Depression is the state of low mood and aversion to activity which can negatively influence person's thoughts and physical well-being. It is a major public health problem and affects individual's performance (Paykel, 2006). Stress might also serve as a predisposing and precipitating factor in the pathophysiology of depression (Haleem and Ikram, 2013; Haleem *et al*., 2013). Depression is also considered as primary target for treating some physical illnesses (Axford *et al*., 2008). This is a progressive process and the economic burden in terms of general medical health care expenditures continues to increase with the degree of treatment-resistant depression (Olchanski *et al*., 2013). Widely used animal models of depression include "despair" paradigms such as tail suspension tests, Porsolt's forced swim test and learned helplessness (Chourbaji *et al*., 2005; Cryan *et al*., 2005; Druss *et al*., 2000; Fautino *et al*., 2000; Farhan *et al*., 2014). The same purpose could also be achieved by olfactory bulbectomy, maternal/ social deprivation and others (Green *et al*., 2009; Hellweg *et al*., 2007; Mourlon *et al*., 2010). A major concern in this regard is, that these animal models may not work all the times, have restricted validity and may result in the false conclusions due to mishandlings or any other such reasons (Fernandes *et al*., 2012; Ikram *et al*., 2011) A need of establishing new animal models of depression therefore arises. Apart from the face validity of new drugs, new animal models are also needed for the better understanding of the pathophysiology and factors contributing to the development of depression.

Another problem associated with the treatment of depression is, that up to 30% of patients with clinical depression, do no not respond to classic antidepressant therapies (Al-Harbi, 2012; Shelton *et al*., 2010) due to various underlying reasons (Thase, 2008). Further research in this area and the development of new therapeutic agents and their face validation requires new animal models to study pathophysiological mechanisms involved during the progression of depression. This may further be extended to study and understand the 'progressive' resistance to the treatment regimens available nowadays.

Reserpine is an indole alkaloid used lately as an antihypertensive drug (Shamon and Perez, 2009). However, it's no more in practice because of the associated side-effects. Reserpine acts mainly by depleting catecholamines. It inhibits the uptake of catecholamines by acting as an irreversible inhibitor of vesicular amine pump and ultimately results in the depletion of catecholamine stores (Liu and Gershenfeld, 2003). Repeated administration of reserpine can present an animal model with the progressive development of the symptoms of depression. Present study was therefore designed to develop an animal model to monitor the progressive development of depressive symptoms. Apart from studying the antidepressant effects of certain novel agents, it would be beneficial for studying the progressive development of depression.

**Corresponding author:* e-mail: huma_biochemist@yahoo.com

MATERIALS AND METHODS

Animals

Locally bred male Albino Wistar rats (weighing 180-200 g) were purchased from HEJ Research Institute of Chemistry, Karachi and were housed individually under 12 hr light and dark cycles (lights on at 06:00 hr) and controlled room temperature (24±2°C) with free access to tap water and cubes of standard rodent diet at least 7 days before starting the experiment so that they could become familiar to the environment. Animals were tested in light phase. Before starting the experiment, rats were accustomed to various handling procedures in order to nullify the psychological affliction of environment. All protocols for experimentation were approved by the Institutional Animal Ethics Committee (IAEC).

Drugs and doses

Reserpine (Sigma, St. Louis, USA) was dissolved in saline and injected subcutaneously. Drug was freshly prepared before each experiment. Control animals were injected with saline (1.0 mg/kg).

Experimental protocol

Twenty-four male Albino Wistar rats (weighing 180-220 g) were randomly assigned to two groups each containing twelve animals each: (i) saline (1.0ml/kg) and (ii) reserpine (1.0mg/kg) injected groups. Animals were injected with the saline or reserpine respectively for a period of three weeks (single injection/ day). Food intake, growth rates and activities in Skinner's box were monitored on weekly basis. Three weeks later, at the end of treatment, forced swim test was performed and activities in light dark activity box were monitored as well. On day 22, animals were sacrificed and brain samples were collected for neurochemical analysis by HPLC-EC.

Behavioral assessment

Activity in Skinner's Box

Transparent Perspex cages (26×26×26 cm) with sawdust covered floor were used to monitor activity in familiar environment. Rats were placed individually in these cages to get familiar with the environment. 15 min later the animals were injected with drug or saline. Numbers of cage crossings were counted 5 min post-injection for 10 min (Ikram *et al*., 2007; Ikram and Haleem, 2010; Ikram and Haleem, 2011)

Forced swim test

Weekly assessment of depressive symptoms was performed by forced swim test. Test was performed as described elsewhere (Ikram *et al*., 2014). Rats were placed individually in a tank (53x19x28 cm). Water was filled in the tank up to the level of 18cm and animal was supposed to swim. Animal was introduced in the container for 5 minutes and behavioral scoring was performed by noting struggle time. After testing session, rats were dried with towel and kept back in their home cages.

Decapitation of rat brain

Dissection procedure was same as described earlier (Bano *et al*., 2014; Mirza *et al*., 2013). Animals were killed 1hr post injections, on day 22. The skull plates were cut and membrane covering the brain was removed with the help of fine forceps. Using spatula, brain was taken out and washed with ice-cold saline. The collected brains were immediately stored at -70°C for the estimation of biogenic amines and metabolites using High performance liquid chromatography with electrochemical detection (HPLC-EC).

HPLC-EC Analysis of Biogenic amines and metabolites

Extraction of biogenic amines and metabolites was same as described previously (Ikram *et al*., 2012). Extraction was performed using 70% perchloric acid. 5 times volume of the extraction medium was added to the brain tissues. Samples were homogenized by using electrical homogenizer and subjected to ultracentrifugation at 6000rpm for 20min at 4°C. Supernatant was separated and injected to HPLC-EC for neurochemical assay. A 5µ Shimpack ODS separation column of 4.0mm internal diameter and 150mm length was used. 0.1M phosphate buffer (PH 2.9) containing EDTA (0.0035%), methanol (14%) and octyl sodium sulfate (0.023%) was used at an operating potential of 2000-3000 psi on Schimadzu HPLC pump. Electrochemical detection (using Schimadzu LEC 6A detector) was done at an operating potential of +0.8V.

STATISTICAL ANALYSIS

Results are represented as means ± SD. Statistical analyses were performed by two-way analysis of variance (ANOVA) or Student's t-test (whatever applicable). Post hoc individual comparisons of groups were performed by Newman-Keuls test, following ANOVA. Values of $p<0.05$ were considered significant.

RESULTS

Fig. 1 shows effects of repeated administration (once a day for 3 weeks) of reserpine (1.0 mg/kg) on weekly food intake. Data analyzed by two-way ANOVA showed significant effects of drug ($df= 1,66$; $F= 162.45$; $p<0.01$), weekly monitoring ($df= 2,66$; $F= 5.67$; $p<0.05$) as well as interaction between the two ($df= 1,66$; $F= 21.74$; $p<0.01$). Post hoc analysis by Newman–Keuls test showed a significant increase in food intake by reserpine following second (0.05) and third week (0.01saline) as compared to respective saline injected controls.

Fig. 2 shows effects of repeated administration (once a day for 3 weeks) of reserpine (1.0 mg/kg) on weekly

growth rates. Data analyzed by two-way ANOVA showed significant effects of drug (df= 1,66; F= 36.99; p<0.01), weekly monitoring (df= 2,66; F= 5.17; p<0.01) as well as and interaction between the two (df= 1,66; F= 22.09; p<0.05). Post hoc analysis by Newman–Keuls test showed a significant increase in growth rates by reserpine following third week (0.01saline) as compared to respective saline injected controls.

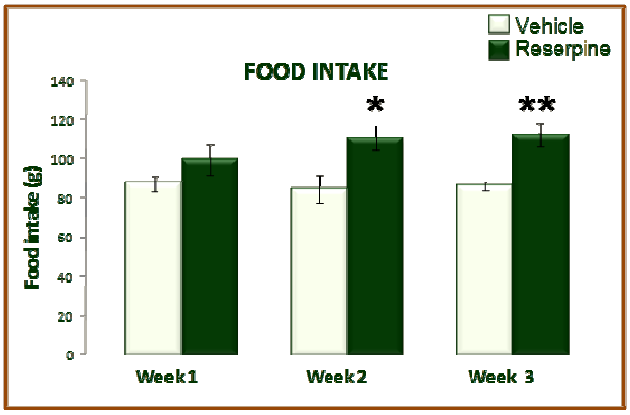

Fig. 1: Effect of repeated administration (once a day for 3 weeks) of reserpine (1.0 mg/kg) on weekly food intake. Values are means ±S.D. (n=12). Significant differences by Newman-Keuls test: *p<0.05, **p<0.01 in repeated reserpine injected rats from their respective repeatedly saline injected controls following two-way ANOVA.

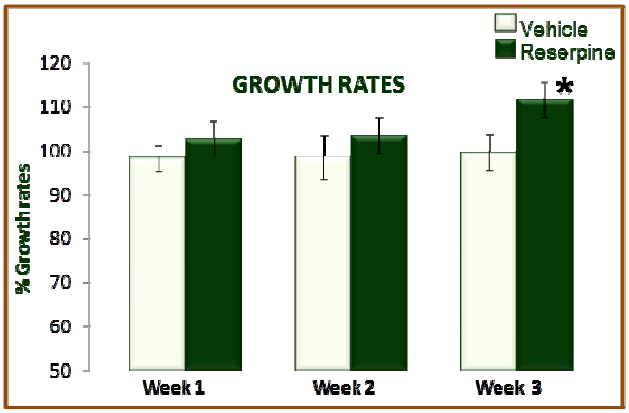

Fig. 2: Effect of repeated administration (once a day for 3 weeks) of reserpine (1.0 mg/kg) on weekly growth rates. Values are means ±S.D. (n=12). Significant differences by Newman-Keuls test: *p<0.01 in repeated reserpine injected rats from their respective repeatedly saline injected controls following two-way ANOVA.

Fig. 3 shows effects of repeated administration (once a day for 3 weeks) of reserpine (1.0mg/kg) on Skinner's box activity. Data analyzed by two-way ANOVA showed significant effect of drug (df= 1,66; F= 92.14; p<0.01), weekly monitoring (df= 2,66; F= 76.57; p<0.01) and interaction between the two (df= 1,66; F= 69.42; p<0.01). Post hoc analysis by Newman–Keuls test showed a decrease in locomotive activities of reserpine injected animals after second (p<0.05) as well as third (p<0.01) week, as compared to their respective saline injected controls. These decreased locomotive activities of reserpine injected animals following second week and afterwards, were also comparable (p<0.01) with the activities of the same animals after first week.

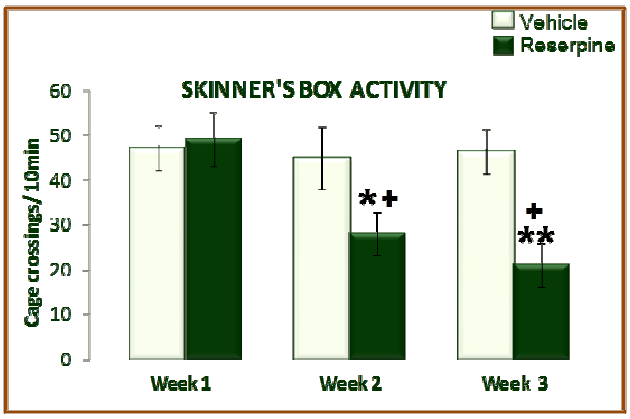

Fig. 3: Effect of repeated administration (once a day for 3 weeks) of reserpine (1.0 mg/kg) on Skinner's box activity as monitored on weekly basis. Values are means ±S.D. (n=12). Significant differences by Newman-Keuls test: *p <0.05, **p<0.01 in repeated reserpine injected rats from their respective repeatedly saline injected controls; +p<0.01 from repeated reserpine injected rats (week 1), following two-way ANOVA.

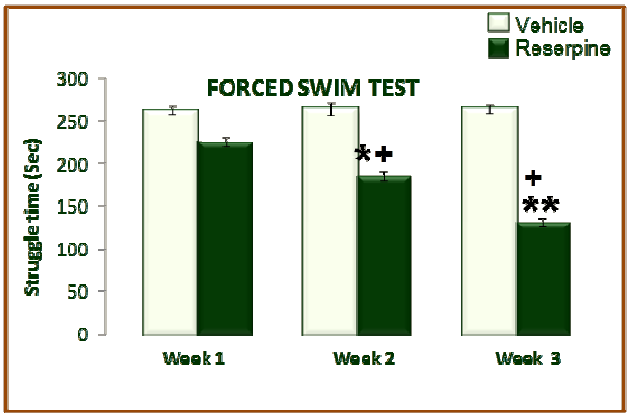

Fig. 4: Effect of repeated administration (once a day for 3 weeks) of reserpine (1.0 mg/kg) on forced swim test, as monitored 30min post last injection (weekly basis). Values are means ±S.D. (n=12). Significant differences by Newman-Keuls test: *p <0.05, **p<0.01 in repeated reserpine injected rats from their respective repeatedly saline injected controls; +p<0.01 from repeated reserpine injected rats (week 1), following two-way ANOVA.

Fig. 4 shows effects of repeated administration (once a day for 3 weeks) of reserpine (1.0 mg/kg) on forced swim test. Data analyzed by two-way ANOVA showed significant effect of drug (df= 1,66; F= 115.45; p<0.01), weekly monitoring (df= 2,66; F= 831.58; p<0.01) and interaction between the two (df= 1,66; F= 243.39;

p<0.01). Post hoc analysis by Newman–Keuls test showed a decrease in the struggle time of reserpine injected animals after second (p<0.05) as well as third (p<0.01) week, as compared to their respective saline injected controls. These decreased locomotive activities of reserpine injected animals following second week and afterwards, were also comparable (p<0.01) with the activities of the same animals after first week.

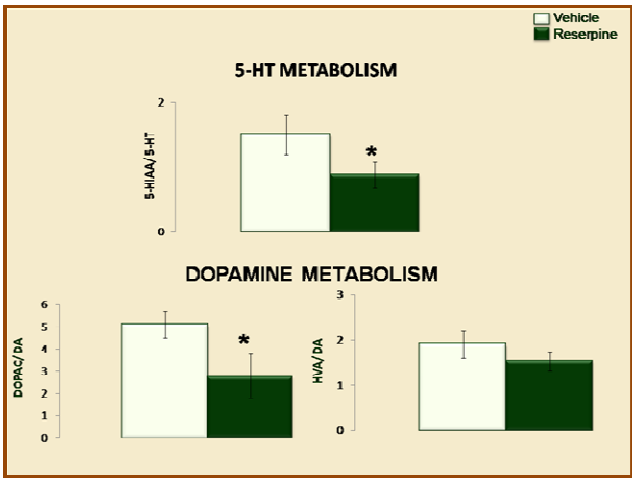

Fig. 5: Effect of repeated administration (once a day for 3 weeks) of reserpine (1.0 mg/kg) on levels of biogenic amines and metabolites. Values are means ±S.D. (n=12). *p <0.01 in repeated reserpine injected rats from their respective repeatedly saline injected controls following Student's t-test.

Fig. 5 shows effects of repeated administration (once a day for 3 weeks) of reserpine (1.0 mg/kg) on the levels of biogenic amines in brain. Data analyzed by student's t-test showed significantly decreased dopamine (p<0.01), HVA (p<0.01), and 5-HIAA (p<0.01) levels.

DISCUSSION

In the present study, we investigated the effects of repeated reserpine administration at the dose of 1.0 mg/kg. We observed motor impairment as well as the depressive effects of the drug, as monitored on weekly basis. Results suggest that repeated administration of reserpine could be used as a progressive animal model of depression. Although, behavioral depression develops as a result of reserpine administration and Skalisz and coworkers (2002) have suggested use of reserpine-injected animal models of depression for studying Parkinson's-related depression. However, they had monitored effects of single reserpine injection (1.0 mg/kg and 2.0 mg/kg) on sucrose preference test, 24hr post injection. They have reported reserpine-induced depression, as well as hypolocomotive symptoms of depression–Parkinson's association. We therefore have studies the effects of repeated administration of reserpine at the dose of 1.0 mg/kg for a period of three weeks, so as to monitor the depressive effects.

Results from the present study, showed that food intake and growth rates were increased progressively in the reserpine-injected animals (fig. 1 & 2). Geiger and coworkers (2009) have suggested that deficits of mesolimbic dopamine neurotransmission in rats would lead to dietary obesity. Since reserpine also induces depletion of dopamine, reserpine-induced hyperphagia (fig. 1) could be explained in terms of decreased dopaminergic neurotransmission. However, this reserpine-induced hyperphagia became evident after week 2, while reserpine-induced increases in growth rates of the animals (fig. 2) were observed after week 3.

In the present study, reserpine-induced progressive motor impairment was observed in animals. Fernandes and coworkers (2012) have reported gradual potentiation of the hypolocomotive effects of reserpine, as observed following repeated administration of reserpine at a low (1.0 mg/kg). This progressive motor impairment (fig. 3) was apparent after week 2 and further potentiated after week 3. It is also been suggested that repeated administration of reserpine (2.0 mg/kg) in the animals, could be used as an animal model to study the depression prevalent in Parkinsonian patients (Skalisz *et al*., 2002). However, authors have monitored the effects of single reserpine injection. We do hereby report progressive hypolocomotion in the reserpine-induced animal model of depression, besides the progression of behavioral depression. Forced swim test also exhibited depressive effects of reserpine (fig. 4). Forced swim test is a well-established animal model for studying rodent behavioral despair and monitoring the anti-depressant affinity of novel compounds (Fautino *et al*., 2004; Estanislau *et al*., 2011). We used forced swim test to monitor the depressant effects of reserpine. Forced swim test is used for the face validation of depression in animal models. Results have shown progressive expression of depression in reserpine treated animals after week 2 but not earlier. The behavioral deficits induced by the repeated administration of reserpine were accompanied by the monoamine depletion in the brain (fig. 5). Results are in accordance with the dominated hypothesis for regarding the pathophysiology of depression i.e., monoamine hypothesis of depression (Delgado, 2000).

CONCLUSION

In conclusion, results from the present study suggest a progressive animal model of depression following repeated administration of reserpine at a low dose (1.0 mg/kg). These depressive symptoms were accompanied by the depletion of monoamines in the brain; the commonly suggested pathophysiological mechanism in depression. The treatment protocol applied, also produced locomotive deficits, which suggests that this animal model of depression could also be used as the 'progressive' animal model for the understanding and face

validity of depressive symptoms in Parkinsonian patients. Results could be implicated for the better understanding and treatment of depression and related disorders.

ACKNOWLEDGMENT

The authors would like to thank Higher Education Commission of Pakistan for the research grant.

REFERENCES

Al-Harbi KS (2012). Treatment-resistant depression: therapeutic trends, challenges and future directions. *Patient Prefer. Adher.*, **6**: 369-388.

Axford J, Heron C, Ross F and Victor CR (2008). Management of knee osteoarthritis in primary care: pain and depression are the major obstacles. *J. Psychosom. Res.*, **64**: 461-467.

Bano F, Ikram H and Akhtar N (2014). Neurochemical and behavioral effects of Cinnamon (Cinnamomi cassiae) bark aqueous extract in obese rats. *Pak. J. Pharm. Sci.*, **27**(3): 559-563.

Chourbaji S, Zacher C, Sanchis-Segura C, Dormann C, Vollmayr B and Gass P (2005). Learned helplessness: validity and reliability of depressive-like states in mice. *Brain Res. Protoc.*, **16**: 70-78.

Cryan JF, Valentino RJ and Lucki I (2005). Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neurosci. Biobehav. Rev.*, **29**: 547-569.

Delgado PL (2000). Depression: the case for a monoamine deficiency. *J. Clin. Psychiatry*, **61**(6): 7-11.

Druss BG, Rosenheck RA and Sledge WH (2000). Health and disability costs of depressive illness in a major U.S. corporation. *Am. J. Psychiatr.*, **157**: 1274-1278.

Estanislau C, Ramos AC, Ferraresi PD, Costa NF, de Carvalho HMCP and Batistela S (2011). Individual differences in the elevated plus-maze and the forced swim test. *Behav. Proc.*, **86**: 46-51.

Farhan M, Ikram H, Kanwal S and Haleem DJ (2014). Unpredictable Chronic Mild Stress induced Behavioral Deficits: A Comparative Study in Male and Female Rats. *Pak. J. Pharm. Sci.*, **27**(4): 879-884.

Fautino LR, Joseph K and Russel EP (2004). Total sleep deprivation decreases immobility in forced swim test. *Neuropsychopharmacology*, **29**: 1105-1111.

Fernandes VS, Santos JR, Leão AHFF, Medeiros AM, Melo TG, Izídio GS, Cabral A, Ribeiro RA, Abílio VC, Ribeiro AM and Silva RH (2012). Repeated treatment with a low dose of reserpine as a progressive model of Parkinson's disease. *Behav. Brain Res.*, **231**: 154-163.

Geiger BM, Haburcak M, Avena NM, Moyer MC, Hoebel BG and Pothos EN (2009). Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. *Neuroscience*, **159**: 1193-1199.

Green AD, Barr AM and Galea LAM (2009). Role of estradiol withdrawal in 'anhedonic' sucrose consumption: A model of postpartum depression. *Physiol. Behav.*, **97**: 259-265.

Haleem DJ and Ikram H (2013). Immobilization-induced behavioral deficits are attenuated but coping with repeated stress impaired in apomorphine injected rats. *Current Psychopharmacology*. **2**: 254-259.

Haleem DJ, Ikram H, Haider S, Parveen T and Haleem MA (2013). Enhancement and inhibition of apomorphine-induced sensitization in rats exposed to immobilization stress: Relationship with adaptation to stress. *Pharmaco. Biochem. Behav.*, **112**(1): 22-28.

Hellweg R, Zueger M, Fink K, Hörtnagl H and Gass P (2007). Olfactory bulbectomy in mice leads to increased BDNF levels and decreased serotonin turnover in depression-related brain areas. *Neurobiol Dis.*, **25**(1): 1-7.

Ikram H, Ahmed S and Haleem DJ (2011). Effects of Apomorphine on Locomotor Activity and Monoamine Metabolism: A Dose Related Study. *Pak. J. Pharm. Sci.*, **24**(3): 315-321.

Ikram H, Choudhary AM and Haleem DJ (2012). Regional Neurochemical Profile following Development of Apomorphine-induced Reinforcement. *Pak. J. Pharm. Sci.*, **25**(3): 513-519.

Ikram H and Haleem DJ (2010). Haloperidol-induced Tardive Dyskinesia: Role of 5HT2C Receptors. *Pak. J. Sci. Ind. Res.*, **53**(3): 136-145.

Ikram H, Haleem DJ (2011). Attenuation of Apomorphine-Induced Sensitization by Buspirone. *Pharmacol. Biochem. Behav.*, **99**(3): 444-450.

Ikram H, Mushtaq F and Haleem DJ (2014). Dose-Dependent Effects of Tryptophan on Learning and Memory. *Pak. J. Pharm. Sci.*, **In press**

Ikram H, Samad N and Haleem DJ (2007). Neurochemical and Behavioral Effects of m-CPP in a Rat Model of Tardive Dyskinesia. *Pak. J. Pharm. Sci.*, **20**(3): 188-195.

Liu X and Gershenfeld HK (2003). An exploratory factor analysis of the tail suspension test in 12 inbred strains of mice and an F2 intercross. *Brain Res. Bull.*, **60**: 223-231.

Mirza B, Ikram H, Bilgrami S, Haleem DJ and Haleem MA (2013). Neurochemical and behavioral effects of green tea (camellia sinensis): A model study. *Pak. J. Pharm. Sci.*, **26**(3): 511-516.

Mourlon V, Baudin A, Blanc O, Lauber A, Giros B, Naudon L and Daugé V (2010). Maternal deprivation induces depressive-like behaviours only in female rats. *Behav. Brain Res.*, **213**(2): 278-287.

Olchanski N, McInnis Myers M, Halseth M, Cyr PL, Bockstedt L, Goss TF and Howland RH (2013). The economic burden of treatment-resistant depression. *Clin. Ther.*, **35**(4): 512-522.

Paykel ES (2006). Depression: Major problem for public health. *Epidemiol. Psichiatr. Soc.*, **15**(1): 4-10.

Shamon SD and Perez MI (2009). Blood pressure lowering efficacy of reserpine for primary

hypertension. *Cochrane Database Syst. Rev.* **7**(4): CD007655.

Shelton RC, Osuntokun O, Heinloth AN and Corya SA (2010). Therapeutic options for treatment-resistant depression. *CNS Drugs,* **24**(2): 131-161.

Skalisz LL, Beijamini V, Joca SL, Vital MA, Da Cunha C and Andreatini R (2002). Evaluation of the face validity of reserpine administration as an animal model of depression-Parkinson's disease association. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **26**(5): 879-883.

Thase ME (2008). Management of patients with treatment-resistant depression. *J. Clin. Psychiatry,* **69**(3): e8.