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Effect of Anti-Stress Activity of Buspirone on Restrained Stress Induced Male Albino Rats

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Abstract

Experiments were conducted with the aim of evaluating the protective potential of Buspirone, an anxiolytic drug, on alteration in gravimetry and hematological parameters in male albino rats. 28 adult Wistar albino rats weighing about 175 to 225g were taken for the study and were divided at random into four groups of six animals each. Group I (control), kept undisturbed in the metabolic cage throughout the 42 days experimental period. Group II (stress) rats were kept in a wire mesh restrainer for 6 hr/day for 42 days. Group III (stress+ withdrawal) rats were stressed for 21 days and withdrawal of stress for remaining 21 days (total 42 days). Group IV (stress + Buspirone) rats were only stressed for 21 days and treated with drug Buspirone (12mg/kg body weight, IP) in continuation with stress for remaining 21 days (total period is 42 days). At the end of the study period, all the experimental rats were sacrificed and gravimetrical, hematological parameters were evaluated. Results revealed after 42 days, there was a significant ($P \leq 0.05$) decrease final body weight, Hb, RBC, MCV, MCH, total WBC and Platelets, while increase in neutrophils, lymphocytes, eosinophils and monocytes in Group II of restraint stress rats when compared to control group I. Group III and Group IV shown significantly ($P \leq 0.05$) improvement in gravimetry and hematological parameters in restraint stress compared to only group II. Results indicate a possible antioxidant effect of Buspirone on restrained stress induced alteration of gravimetrical and hematological profiles in male albino rats.

Key words: Stress, Buspirone, Gravimetry, Hematology, Rats.

INTRODUCTION

Stress can be explained as any stimulus that creates an imbalance in the homeostasis processes [1]. Stress has been postulated to be involved in the etiopathogenesis of a variety of disease state and host of other diseases. The resultant disturbances may vary depending upon type, intensity, and the duration of a particular stressor and the strain\sex differentiation of the subjects [2].

Different animal models for stress have been used to evaluate the anti-stress activity of compounds of both natural and synthetic origin and research associated with focusing on identification, quantification, and characterization of the injured tissue for evaluation of different therapeutic modalities and understanding the mechanism of stress response [3]. The blood is one of the major homeostatic systems of the body supporting normal viability, integrity and adaptive responses. The functional state of the blood systems changes dynamically according to the nature, strength and duration of exposure to external factors. Many studies performed on humans and animals have provided evidence for significant shifts in physiological and biochemical processes during the modeling of emotional tension. The nervous and blood systems are the quickest to react to emotional stimuli. Stress and emotional reactions affect the immune system is by complete blood counts, including the hematopoietic system, leukocyte profile or biochemical markers of stress.

Anti-stress drugs are widely used for the treatment of stress. Selective serotonin reuptake inhibitors (SSRIs) are the major and dominant class of antidepressants used over the last decade whereas ancient groups of most widely used antidepressants were Tricyclic antidepressants (TCA) and monoamine oxidase inhibitors. BuSpar (Buspirone) is an antianxiety medication indicated for management of selected anxiety disorders or for short-term relief of anxiety symptoms [4]. Studies reported that administration of Buspirone has been shown to exhibit antidepressant-like activity [5-7] and often suggested that therapeutic effect of Buspirone are not due to its immediate action on the 5-HT system, but rather adaptive changes that occur after prolonged treatment [8]. However, the underlying mechanisms of its therapeutic efficacy remain unclear. Therefore, this study was planned to investigate the effect of drug Buspirone on restrained stress on gravimetrical and hematological parameters in male albino rats.

MATERIAL AND METHODS

Animals

Three months old colony bred healthy adult male albino rats of Wistar strain, weighing approximately 175 to 225g, were acclimatized for 7 days to the laboratory conditions at 22-24°C, relative humidity (55%) and a 12h light: dark (circadian) cycle under hygienic condition. The animals were utilized from Central Animal Facility, Indian Institute of Science, Bengaluru for the experiments. Wister rats fed with laboratory stock diet (Hindustan lever, Mumbai, India) and water *ad libitum*. All the animals were sacrificed at the end of the last dose after an overnight fast. The experimental procedures followed were performed in accordance with the approval of the Institutional Animal Ethics Committee (IAEC/477/2016) and animals were cared in accordance with the guidelines by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for the experimental studies.

Experimental study

The acclimatized animals divided into four groups of six animals each and three animals were kept in each metabolic wire cage (60cmX30cmX20cm). Group I (untreated

control) rats were healthy controls, kept undisturbed in the metabolic cage throughout the experimental period for 42 days. Group II (stress induced) rats were stressed in wire mesh restrainer for 6h/day for 42 days [9]. Group III (stress + withdrawal) rats were stressed for 21 days by keeping them inside mesh restrainer and withdrawal of stress for remaining 21 days by keeping animals in normal cages (total 42 days). Group IV (stress + Buspirone, 12mg/kg body weight, IP) rats were stressed for 21 days and treated with drug Buspirone [10, 11] for remaining 21 days in continuation with stress i.e., total 42 days.

Stress Procedure

Rats were subjected to restrained stress in a wire mesh restrainer for 6 hours per day for 21 days. The wire mesh restrainer had a wooden base and stainless steel wire mesh restrainer hinged to the base. The restrainer having the dimensions of 8 cm (L) x 4 cm (B) x 4 cm (H) was used for the experiments. A pad lock and latch helped to secure the rat in the restrainer [9].

Gravimetry

The body weight of all the rats was recorded on the day 1 of treatment, alternate 10^{th} day and the day of sacrifice (i.e. 42^{nd} day). Percent body weight gain was determined in experimental groups of the rats with a ratio of final body weight to the initial body weight. Organ somatic index was determined by the ratio of organ weight to body weight of rat before sacrifice (final body weight).

Hematological parameters

The blood collected from retro orbital method. Blood was collected in centrifuge tubes, kept at room temperature for about 2h and centrifuged at 1500×*g* for 15min to collect serum. Hematological parameters such as PCV, Hb, RBC, MCV, MCHC, MCH, TWBC, Platelets, Neutrophils, Lymphocytes, Eosinophils and Monocytes were analyzed by using SYS MAX-35 automated cell counter machine.

Statistical analysis

Data were expressed as mean ± standard deviation of the mean. Statistical comparisons were performed by one-way ANOVA, followed by post-hoc t-test and $P \leq 0.05$ is considered to indicate a significant difference between experimental and controls.

RESULTS AND DISCUSSION

Gravimetry

In the present study, no adverse reactions were observed in any of the experimental groups and remained active and healthy with normal feeding behavior. Rats in the control group had a normal weight accumulation, whereas the weight gain in the restrained stress group (chronic moderate stress) was significantly lower than the control group. Stress induced rats (Group II) were found to be lethargic and their body weights decreased remarkably which is indicated by the mean percentage of body weight gain of 14.07%, as compared to their mean initial body weight gain (25.06%). Group IV (stress + Buspirone) rats exhibited no significant change in mean percentage of body weight gain (23.86%) from the mean initial percentage of body weight gain. However, Group III (stress withdrawal) rats had a non-significant increase in mean initial percentage of body weight gain (23.86%) as compared to untreated controls. The decrease in body weight may be due to low food consumption, hormonal imbalance and protein metabolism in the rats under the influence of stress. Several studies have demonstrated that chronic exposure to restraint stress reduces the body weight and food intake of rodents [12-15]. However, the mechanisms underlying these restraint-induced changes in body weight and food intake remain to be elucidated. Evidences shown the decrease in body weight could be due to the direct effect of stress on the food intake behavior of the rats [16], metabolic processes are mediated by glucocorticoids [17], increase in metabolic demands, reduced digestion, and increased adrenal steroid secretion [18] and stress might have increased the protein catabolism and hampered the utilization of food consumed during the stress period, thereby causing decrease in body weight [19]. The treatment with drug Buspirone had cut down chronic stress has been related to changes in body weight and physiology of different organs of group IV rats.

Hematological parameters

Stress is thought to impair immune function [20,21] through emotional or behavioral manifestations such as anxiety, fear, tension, cognition, anger, and sadness and physiological changes of heart rate, blood pressure, glucose metabolism, and sweating. Stress induced changes in hematological parameters clearly showed anemia, and it may be due to release of immature RBCs in circulation. However, in our study, the decrease in hemoglobin concentration, RBC count, total WBC count and MCV may be due to non-regenerative anemia arising from stress induced disorder of hematopoietic stem cells resulting in decreased erythrocyte, leukocyte and platelet count. Similar to this study, other experimental studies have been shown by the significant reduction in hematological parameters when exposed to immobilization stress [22, 23] and restrained stress animals [24, 25]. Decreased in the level of hematological parameters in the stressed group, when compared with control, at the same time treated with drug Buspirone for remaining 21 days in continuation with stress were shown near normal values as control and stress with-drawal rats might be due to the antioxidant, anti-lipidperoxidation, immunostimulation and anticonvulsant effects of Buspirone.

Buspirone also has some evidence as augmentation strategy with selective serotonin reuptake inhibitors for treatment of major depressive disorder [26]. Experimental study shown that after an acute stress event, eosinophil levels were significantly lower in aggressive animals compared to slightly aggressive ones [27]. However, there are contradictory findings concerning the effect of chronic stress on eosinophil counts [28-30]. The blood eosinophil level is a hormone-dependent parameter, and taken together with neutrophil/lymphocyte ratios, it may help distinguish between leukocyte responses to stress. Both emotional [31] and physiological stress, such as long-lasting cold [32] or immobilization [29] have been proved to reduce the eosinophil level.

Table 1: Effect of drug Buspirone on restrained stress induced alteration of gravimetry in male albino rats				
Gravimetrical parameters	**Group I (Untreated control)**	**Group II (Stress induced)**	**Group III (Stress Withdrawal)**	**Group IV (Stress + Buspirone)**
Initial Body Weight (g)	192.50±45.96[a]	197.50±10.61[a]	220.50±4.95[a]	193.67±12.11[a]
10 Days after (g)	203.00±46.67[a]	198.00±7.07[a]	238.00±9.90[b]	199.67±10.37[a]
20 Days after (g)	227.00±46.67[a]	208.00±8.49[a]	256.50±10.61[b]	218.00±11.87[a]
30 Days after (g)	231.50±44.55[a]	218.50±12.02[a]	262.50±7.78[b]	228.17±25.52[a]
Final Body Weight (g)	242.50±47.38[a]	224.00±4.24[b]	278.50±4.95[c]	241.67±15.82[a]
Percentage Body Weight Gain (%)	25.06±5.51[a]	14.07±3.27[b]	26.35±2.98 [a]	23.86±1.95[a]

Table 2: Effect of drug Buspirone on restrained stress induced alteration of hematological parameters in male albino rats				
Hematological parameters	**Group I (Untreated control)**	**Group II (Stress induced)**	**Group III (Stress Withdrawal)**	**Group IV (Stress + Buspirone)**
PCV (%)	43.83±1.25[a]	42.03±1.43[a]	42.93±1.51[a]	42.86 ± 1.57 [a]
Hb (g/dL)	13.91±0.37[a]	10.15±0.44[b]	12.84±0.38[a]	13.62 ± 0.40 [a]
RBC (x 10^6/µL)	7.48 ±0.35[a]	5.77±0.34[b]	7.10±0.37[a]	6.28 ± 0.41 [a]
MCV (FL)	59.05±1.50[a]	49.33±1.25[b]	57.36±0.92[a]	56.82 ±1.35 [a]
MCHC (g/dL)	34.51 ± 1.21[a]	39.58 ± 0.92[a]	35.11 ± 1.35[a]	33.06 ± 0.92 [a]
MCH (pg)	19.73 ± 0.78[a]	16.85 ± 1.23[b]	18.40 ± 0.75[a]	17.03 ± 1.24 [a]
Total WBC (x 10^3/µL)	11.30 ± 0.62[a]	9.70 ± 0.83[b]	11.04 ± 0.37[a]	11.56 ± 0.78 [a]
Platelets (x 105/µL)	1.12 ± 0.05[a]	0.85 ± 0.08[b]	1.10 ± 0.07[a]	1.32 ± 0.05 [a]
Neutrophils (%)	29.03 ± 0.77[a]	39.50 ± 0.79[b]	31.20 ± 1.23[a]	26.99 ± 0.75 [a]
Lymphocytes (%)	79.13 ± 7.44[a]	105.7 ± 7.80[b]	93.88 ± 5.51[b]	82.28 ± 7.54 [a]
Eosinophils (%)	2.20 ± 0.30[a]	3.60 ± 0.24[b]	2.58 ± 0.17[a]	2.90 ± 0.30 [a]
Monocytes (%)	2.03 ± 0.35[a]	3.57 ± 0.34[b]	2.53 ± 0.28[a]	1.90 ± 0.17 [a]

Each value is Mean ± SD of six observations in each group. In each row, values with different superscripts (a, b, c) were significantly different from each other (P<0.05). *Post-hoc t*-test analysis was used to test for differences among the means when ANOVA indicated a significant P<0.05.

The present study indicated that restrained stress induced significant adverse effect on hematological parameters in rats 21 days after treatment with Buspirone had successfully ameliorated the hematological disturbances and also showed a protective role in anemia and leucopenia (Table 2).

A protective effect of Buspirone on leucocytes from oxidative stress was observed and results revealed that exposure to restraint stress induced peripheral oxidative stress, which is defined by an increase in the generation of reactive oxygen species (ROS) in peripheral blood lymphocytes, granulocytes and monocytes. These adverse effects were partially reversed by Buspirone indicating that it is capable of alleviating oxidative damage induced by psychological stress on the peripheral immune system. Our results are in concurrence with other studies showing that the production of ROS by immune cells might be influenced by psychological stress. However, the available results in reference to the influence of psychological stress on the production of ROS are contradictory [19, 33], while others have shown a decreased ROS production [34, 35]. This discrepancy may have been the result of a number of research design problems, including age, sex, intensity and type of stressor, plasma concentration of catecholamine and lack of adequate non-stressed controls, which are very important since a circadian rhythm in the generation of these compounds has been described [36] Many of effects mediated by stress-induced neurochemical and hormonal abnormalities that are often associated with oxidative stress [37, 38]. Taking into account the available evidences of main pathways of ROS generation in the course of depression have been described, at present, we believe that the potentially favorable antioxidant effect of the Buspirone could be mediated by activation of immune and inflammatory response systems [39].

CONCLUSION

The study therefore concluded that the treatment with Buspirone has a significant effect to counteract restrained stress induced foregoing alterations. Buspirone prevented the stress-induced abnormalities in hematological parameters indicating the protective effect against stress. Further our particular interest experimentation needs to be done with this anxiolytic drug to explore responsible for such activity and to elucidate the possible biochemical mechanism and at the level of ultrastructural of cerebellum particularly on the neuronal cells of cerebellar cortex which is one of the central regions in which ordered organizational patterns are most obvious.

Each value is Mean ± SD of six observations in each group. In each row, values with different superscripts (a, b, c) were significantly different from each other (P<0.05). *Post-hoc t*-test analysis was used to test for differences among the means when ANOVA indicated a significant P<0.05.

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CONFLICTS OF INTEREST

Authors declared there is no Conflict of interest.

REFERENCES

[1] Oxington, K.V., *Psychology of Stress.* New York, USA, Nova Science Publishers Inc 2005, pp. 54-55.

[2] Kioukia-Fougia, N., Antoniou, K., Bekris, S., Liapi, C., Christofidis, I., Papadopoulou-Diafoti, Z., The effects of stress exposure on hypothalamic-pituitary-adrenal axis, thymus, thyroid hormones and glucose levels. *Prog. Neuropharmacol. Biol. Psychiatry.* 2002, *26,* 823-830.

[3] Bhatia, N, Maiti, P.P., Choudhary, A., Tuli, A., Masih, D., Khan, M.M.U., Ara, T., Jaggi, A.S., Animal models in the study of stress: A review. *NSHM J Pharmacy and Healthcare Management.* 2011, *2,* 42-50.

[4] Howland, R.H., Buspirone: Back to the Future. *J Psychosoc Nurs Ment Health Serv*. 2015, *53,* 21–24.

[5] Schreiber. R., De Vry, J., 5-HT 1A receptor ligands in animal models of anxiety, impulsivity and depression: multiple mechanisms of action? *Prog Neuropsychopharmacol. Biol. Psychiatry.* 1993, *17,* 87-104.

[6] Kinney, G.G., Grifftith, J.C., Hudzik, T.J., Antidepressant-like effects of 5-hydroxytryptamine-1A receptor agonists on operant responding under a response duration differentiation schedule. *Behav.Pharmacol*. 1998, *9,* 309-318.

[7] Blier, P., Ward, N.M., Is there a role for 5-HT (1A) agonists in the treatment of depression? *Biol. Psychiatry.* 2003, *53,* 193-203.

[8] Okazawa, H., Yamane, F., Blier, P., Diksic, M., Effect of acute and chronic administration of the serotonin-1A agonist buspirone on serotonin synthesis in rat brain. *J. Neurochem.* 1999, *72,* 2022-2031.

[9] Kumar, R.S., Rao, M.S., Nayak, S., Sareesh, N.N., Effect of *Ocimum sanctum* (Linn) extract on restraint stress induced behavioural defects in male Wister rats. *Pharmacologyonline*.2007, *3,* 394-404.

[10] Kai, S., Kohmura, H., Ishikawa, K., Ohta, S., Kuroyanagi, K., Kawano, S., Kadota, T., Chikazawa, H., Kondo, H., Takahashi, N., Reproductive and developmental toxicity studies of buspirone hydrochloride (I)--Oral administration to rats during the period of fetal organogenesis. *J Toxicol Sci. Suppl.* 1990a, *1,* 31-60.

[11] Kai, S., Kohmura, H., Ishikawa, K., Ohta, S., Kuroyanagi, K., Kawano, S., Kadota, T., Chikazawa, H., Kondo, H., Takahashi, N., Reproductive and developmental toxicity studies of buspirone hydrochloride (II)--Oral administration to rats during perinatal and lactation periods. *J Toxicol Sci. Suppl.* 1990b, *1,* 61-84.

[12] Marti, O., Marti, J., Armario, A., Effects of chronic stress on food intake in rats: influence of stressor intensity and duration of daily exposure. *Physiol Behav*. 1994, *55,*747-753.

[13] Harris, R.B., Zhou, J., Youngblood, B.D., Rybkin, I.I., Smagin, G.N., Ryan, D.H., Effect of repeated stress on body weight and body composition of rats fed low-and high-fat diets. *Am J Physiol.* 1998, *275,* 1928-1938.

[14] Gamaro, G.D., Manoli, L.P., Torres, I.L., Silveira, R., Dalmaz, C., Effects of chronic variate stress on feeding behavior and on monoamine levels in different rat brain structures. *Neurochem Int.* 2003, *42,* 107-114.

[15] Jeong, J.Y., Lee,D.H., Kang, S.S., Effects of Chronic Restraint Stress on Body Weight, Food Intake, and Hypothalamic Gene Expressions in Mice. *Endocrinol Metab.* 2013, *28,* 288-296.

[16] First, M., Gil-Ad, I., Taler, M., Tarasenko, I., Novak, N., Weizman, A., The effects of fluoxetine treatment in a chronic mild stress rat model on depression-related behavior, brain neurotrophins and ERK expression. *J Mol Neurosci.* 2011, *45,* 246-255.

[17] Bhatnagar, S., Vining, C., Iyer, V., Kinni, V., Changes in hypothalamic- pituitary-adrenal function, body temperature, body weight and food intake with repeated social stress exposure in rats. *J Neuroendocrinol,* 2006, *18,* 13-24.

[18] Nayanatara, A.K, Tripathi, Y., Nagaraja, H. S., Jeganth, P.S., Ramaswamy, C., Ganaraja, B., Effect of chronic immobilization stress on some selected physiological, biochemical and lipid parameters in Wistar Albino Rats. *Res J Pharmaceutical Biol Chem Sci,* 2012, *1,* 34–42.

[19] Ng. F., Berk, M., Dean, O., Bush, A.I., Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. *Int J Neuropsychopharmacol.* 2008,*11,* 851-876.

[20] Zorrilla, E.P., Luborsky,L., McKay, J.R., Rosenthal. R., Houldin, A., Tax, A., McCorkle, R., Seligman, D.A., Schmidt, K., The relationship of depression and stressors to immunological assays: a metaanalytic review. *Brain Behav Immun.* 2001, *15,* 199–226.

[21] Herbert, T.B., Cohen,S., Stress and immunity in humans: a meta-analytic review. *Psychosomatic Medicine,* 1993, *55,* 364–379.

[22] Caporossi, L.S., da Silva, A.R., Semenoff, T.A.,Pedro, F.M.,Borges, A.H., Semenoff-Segundo, A.S., Effect of two models of stress associated with ligature-induced periodontitis on hematological parameters in rats. *J Dental Sci*, 2010, *25,* 371–375.

[23] Sarumathi, A., Saravanan, N., A study on the haematological parameters and brain acetylcholine esterase activity in immobilization induced stress and co-treatment with *Centella asiatica* leaves extract to Wistar rats. *Int J Nutrition Pharmacol Neurological Diseases.* 2013, *3,* 102–107.

[24] Kori, R.S., Aladkatti, R.H., Desai, S.D., Das, K.K., Effect of Anti-stress Activity of Fluoxetine on Restrained Stress Induced Male Albino Rats in Hematological Parameters and Whole Brain Histopathology. *J Young Pharm,* 2017, *9,* 246-250.

[25] Kori, R.S., Aladkatti, R.H., Desai, S.D., Das, K.K., Effect of Drug Alprazolam on Restrained Stress Induced Alteration of Serum Cortisol and Antioxidant Vitamins (Vitamin C and E) in Male Albino Rats. *J Clin Diag Res.* 2016, *10,* 07-09.

[26] Appelberg, B.G., Syvälahti, E.K., Koskinen, T.E., Mehtonen, O.P., Muhonen, T.T., Naukkarinen, H.H., Patients with severe depression may benefit from buspirone augmentation of selective serotonin reuptake inhibitors: results from a placebo-controlled, randomized, double-blind, placebo wash-in study. *J Clin Psychiatry*.2001, *62,* 448–452.

[27] Hansen, S.W., Damgaard, B.M., Behavioral and adrenocortical coping strategies and the effect on eosinophil leukocyte level and heterophil/lymphocyte-ratio in beech marten (*Martes foina*). *Appl. Anim. Behav. Sci.* 1993. *35,* 369-388.

[28] Jeppesen, L.L., Pedersen, V., Effect of whole year nest-boxes on cortisol, circulating leukocytes, exploration and agonistic behavior in silver foxes. *Behav. Processes,* 1991, *25,* 171-177.

[29] Heller, K.E., Jeppesen, L.L., Behavioral and eosinophil leukocyte responses to single and repeated immobility stress in mink. *Scientifur.* 1985, *9,* 174-178.

[30] Hansen, S.W., Damgaard, B.M., Effect of environmental stress and immobilization on stress physiological variables in farmed mink. *Behav. Processes.* 1991, *25,* 191-201.

[31] Kerr, A.C., The effect of mental stress on the eosinophil leukocyte count inman. *Q. J. Exp. Physiol. Cogn. Med. Sci.* 1956, *41,* 18-24.

[32] Denison, M.E., Zarrow, M.X., Eosinophils of blood during prolonged exposure to cold and chronic administration of cortisone acetate. *Proc.Soc.Exp.Biol.Med.*1954, *85,*433-437.

[33] Patki, G., Solanki, N., Atrooz, F., Allam, F., Salim, S., Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. *Brain Research.* 2013, *1539,* 73-86.

[34] Atanackovic, D., Schulze, J., Krcger, H., Brunner-Weinzierl, M.C., Deter, H.C., Acute psychological stress induces a prolonged suppression of the production of reactive oxygen species by phagocytes. *J Neuroimmunol.* 2003, *142,* 159-165.

[35] McCarthy, D.O., Ouimet, M.E., Daun, J.M., The effects of noise stress on leukocyte function in rats. *Res Nurs Health.* 1992, *15,* 131-137.

[36] Atanackovic, D., Brunner-Weinzierl, M.C., Krcger, H., Serke, S., Deter, H.C., Acute psychological stress simultaneously alters hormone levels, recruitment of lymphocyte subsets, and production of reactive oxygen species. *Immunol Invest.* 2002, *31,* 73-91.

[37] Shaw, P.J., Bergmann, B.M., Rechtschaffen, A., Effects of paradoxical sleep deprivation on thermoregulation in the rat. *Sleep.* 1998, *21,* 7-17.

[38] Knutson, K.L., Spiegel, K., Penev, P., Van Cauter, E., The metabolic consequences of sleep deprivation. *Sleep Med Rev.* 2007, *11,*163-178.

[39] Galecki. P., Szemraj, J., Bien´kiewicz, M., Florkowski, A., Galecka, E., Lipid peroxidation and antioxidant protection in patients during acute depressive episodes and in remission after fluoxetine treatment. *Pharmacol Rep.* 2009, *61,*436-447.