Caloric Vestibular Stimulation Alleviates Cold Water Swimming Stress Induced Changes in BDNF and Haematological Parameters

Archana R[1], Rama Kranthi Tumu[2]

[1]*Department of Physiology, Annaii Medical College and Hospital, Rajalakshmi Health City, Chennai, Tamilnadu, India.*
[2]*Department of Physiology, Government Medical College, Wanaparthy, Telangana, India*

KEYWORDS
stress; cold water swimming stress; corticosterone; brain derived neurotrophic factor, wet weight of organs.

ABSTRACT
Introduction: Stress is inevitable in daily life. Stress in long term has been implicated in the development of physical and neuronal disorders. Various studies have described the levels and role of BDNF in the brain following stress. Studies representing the changes in serum BDNF and wet weight of organs in stress are limited and controversial. Aim: The aim of the present study is to find the effect of stress on serum BDNF concentration, wet weight of selected organs, haematological parameters and to evaluate the effectiveness of caloric vestibular stimulation on stress-induced changes in these parameters. Methods and material: Male Wistar rats were exposed to cold water swimming stress for 14 days for a duration of 30 minutes per day. Serum corticosterone and serum BDNF were measured using the ELISA method. Weight of liver, spleen, thymus and lymph nodes were measured and their relation to body weight was calculated. Total RBC count, total WBC count, platelet count and haemoglobin concentration were analysed. Results: A statistically significant increase in serum corticosterone and BDNF was observed following stress. Liver weight, thymus weight and lymph nodes weight did not show any significant changes but spleen weight has reduced in the normal recovery group following stress. Total RBC count, total WBC count, platelet count and haemoglobin levels were significantly higher in the stress group. Caloric vestibular stimulation was effective in managing stress-induced changes in serum corticosterone, BDNF and spleen weight. Conclusion: Caloric vestibular stimulation is effective in restoring the stress-induced changes.

1. Introduction

Stress and illness have complex relationship and in long term aggravate each other. Chronic illness is often associated with stress which leads to anxiety and depression [1]. Brain derived neurotrophic factor (BDNF) along with nerve growth factor is a member of the neurotrophin family. It is widely distributed in various regions of the brain and peripherally in the lungs, heart, gastrointestinal tract, liver. It is expressed in vascular smooth muscle cells, fibroblasts and thymic stroma, platelets [2,3]. In the CNS especially the hippocampus chronic stress cause downregulation of BDNF mRNA and protein [4]. BDNF has a role in the proliferation and differentiation of embryonic and adult neural stem or progenitor cells whereas stress hormones have a negative impact on the same cells and are related to the pathophysiology of the brain diseases like autism spectrum disorder and depression [5]. Altered levels of BDNF levels have been reported in many conditions like major depressive disorder [3,6]. Studies are available describing the role of stress on BDNF in different areas of the brain but studies representing the plasma BDNF levels in stress are limited and controversial. The present study is taken up to see the effect of cold water swimming stress on serum BDNF in Wistar rats and to evaluate the efficacy of caloric vestibular stimulation on stress induced changes in serum BDNF.

Haematological tests are performed as part of routine clinical examination. During stress, red blood cells are essential for supplying oxygen to meet the demands of tissues which play an active role in combating the stressor. White blood cells are essential and are redistributed according to the changes brought in the body by the stressor. Platelets alter as per the stressor. When the stressor is in the form of physical trauma and the compensatory mechanisms demand altered coagulation platelets activity alter accordingly. The weight of the organs helps in the determination of the state of the organ if it's normal or pathological. Any deviation from normal weight indicates pathology or compensatory mechanism in response to the stress applied to an organ (7). The objectives of the present study also include the assessment of stress induced changes in the wet weight of selected organs, haematological parameters and to evaluate the effect of caloric vestibular stimulation on stress induced changes on these parameters.

2. Methods and Materials:

Animals:

Male, Wistar rats of 3 -6 months of age weighing 180 to 280 grams were included in the study. Pellet diet was provided with water ad libitum. Animals were maintained as per the guidelines of the Committee for Control and Supervision of Experiments on Animals. Four animals were housed in a polypropylene cage. The present study was carried out after obtaining Institutional animal ethical committee clearance (1/PIMS/2017). Following experiment blood samples were obtained from retro-orbital site under the influence of isoflurane and serum was obtained after centrifugation. Animals were sacrificed as per the standard guidelines.

Experimental design:

Twenty-eight rats were randomly selected and grouped into six. Four rats were included in each group. Six rats were included in caloric vestibular stimulation groups in view of side effects and mortality.

Group I (n=4) – Control

Group II (n=4) – Stress for 14 days

Group III (n=4) – Stress for 14 days + normal recovery for 15 days (stress+NR-15).

Group IV (n=4) – Stress for 14 days + normal recovery for 30 days (stress+NR-30).

Group V (n=6) – Stress for 14 days + CVS for 15 days (stress+CVS-15).

Group VI (n=6) – Stress for 14 days + CVS for 30 days (stress+CVS-30).

Coldwater swimming stress: Stress was induced in rats by making them swim in cold water maintained at 100C for 30 min a day between 9.00 AM to 12.00 PM. Plastic containers of 40 cm diameter, 60 cm height, were used and the water level was maintained at 30 cm [8].

Caloric vestibular stimulation: Caloric vestibular stimulation was induced in rats bilaterally by irrigating external auditory meatus for a duration of 2 minutes using 2mL water maintained at 410C using a polyethylene tube for 15 and 30 days (9) in respective groups.

Determination of corticosterone: Serum corticosterone was analysed using solid phase enzyme-linked immunosorbent assay (ELISA) method. Procedure in brief, diluted wash solution by 10X. Taken 10 µL of calibrator and sample in separate wells and then added 100 µL of incubation buffer to each well followed by 50 µL by enzyme conjugate and incubated for 2 hours. Washed wells 4 times with 300 µL of wash solution. Added 200 µL of substrate solution in to each well and incubated for 30 min in dark room at room temperature. Later added 50 µL of stop solution and determined the absorbance of each well at 450 nm wavelength within 15 minutes.

Determination of BDNF: Serum BDNF was analysed using enzyme-linked immunosorbent assay (ELISA) method. Procedure in brief, taken 50 µL of standard and 40 µL of serum and added 10 µL of special diluent to samples. 50 µL of HRP was added to each well, incubated for 60 minutes at 370C. Removed excess, dried wells and washed with was buffer. 50 µL of chromogen A and B were added and incubated at 37oC for 15 minutes in dark. 50 µL of stop solution was added and optical density was measured at 450nm.

Determination of wet weight of organs and haematological parameters: Liver, spleen, thymus and inguinal lymph nodes were isolated immediately after sacrificing the animal. Organs were cleaned and blotted dry before weighing. Organ weight in relation to body weight was calculated (10). Whole blood samples were used for determination haematological parameters. Total RBC count, total WBC count, platelet count and haemoglobin were estimated using automated cell counter.

Statistical Analysis: Data was analysed using SPSS 20. One-way analysis of variance followed by Tukey's post hoc test was used for multiple comparisons and expressed as mean ±S.E.M. p value < 0.05 was considered to be statistically significant.

3. Results:

Serum corticosterone: In comparison to control, serum corticosterone has increased in stress group, stress+NR-15 and stress+NR-30 groups ($p<0.001$). In comparison to stress group, corticosterone has significantly reduced

in both stress+CVS-15 (p-0.03) and stress+CVS-30 groups (p<0.001). This proves caloric vestibular stimulation is effective in reducing stress induced increase in serum corticosterone [Table 1].

Table 1: Effect of caloric vestibular stimulation on cold water swimming stress induced changes in serum BDNF and wet weight of organs

Groups	Cortico-sterone (ng/mL)	BDNF (pg/mL)	Liver weight (g/100g)	Spleen weight (g/100g)	Thymus weight (g/100g)	Lymph nodes weight (g/100g)
Control	11.7 ± 0.5	318.5 ± 11.4	4.6 ± 0.45	0.48 ± 0.04	0.19 ± 0.04	0.06 ± 0.01
CVS-15	20.4 ±3.3	359.1 ± 26.4	3.7 ± 0.26	0.40 ± 0.03	0.24 ± 0.06	0.07 ± 0.00
CVS-30	18.7 ±2.2	309.3 ± 36.4	3.3 ± 0.28	0.47 ± 0.06	0.19 ± 0.03	0.07 ± 0.00
Stress	181.9± 36.3[a]	536.7± 22.6[a]	3.6 ± 0.38	0.26 ± 0.00	0.14 ± 0.02	0.08 ± 0.02
Stress+NR-15	171.4± 35.8[a]	449.3± 16.9[a]	4.2 ± 0.96	0.22 ± 0.02[a]	0.17 ± 0.02	0.09 ± 0.01
Stress+NR-30	111.9± 15.7[a]	435.3± 24.0[a]	5.0 ± 0.70	0.43 ± 0.04	0.17 ± 0.01	0.11 ± 0.01
Stress+CVS-15	73.6 ± 8.8[b]	278.0± 14.1[b]	3.9 ± 0.22	0.29 ± 0.03	0.13 ± 0.01	0.07 ± 0.01
Stress+CVS-30	54.8 ± 9.5[b]	263.8± 11.2[b]	3.7 ± 0.44	0.39 ± 0.05	0.15 ± 0.03	0.07 ± 0.00

Values are mean + SE (n = 4 each; stress+CVS-15 and stress+CVS-30 groups, 6 each).

CVS = caloric vestibular stimulation; NR = natural recovery.

15 and 30 are, 15 days and 30 days caloric vestibular stimulation.

a Significantly different from control group. b Significantly different from stress group.

BDNF: In comparison to control group, stress (p<0.001), stress+NR-15 (p-0.005) and stress+NR-30 (p-0.01) groups have shown an elevation in serum BDNF levels. Rats which received CVS intervention, stress+CVS-15 and stress+CVS-30 (p<0.001) showed a significant decline in serum BDNF as compared to stress group. The result proves the effectiveness of caloric vestibular stimulation in reducing the stress induced elevation of serum BDNF [Table 1].

Organ weights: There was no significant difference (p>0.05) in weight of the liver, thymus and lymph nodes among the groups. However, spleen weight in the stress+NR-15 group (p-0.02) has reduced significantly in comparison to control group whereas stress+CVS-15 group showed no such decline in weight. This finding proves the protective role of caloric vestibular stimulation in stress [Table 1].

Haematological parameters: As compared to the control group, the stress group have showed a significant elevation (p<0.001) in total RBC count, total WBC count, platelet count and haemoglobin levels. Both normal recovery groups and caloric vestibular stimulation groups showed significant decline (p<0.05) in haematological parameters in comparison to stress group [Table 2].

Table 2: Effect of caloric vestibular stimulation on cold water swimming stress induced changes in Haematological parameters

Groups	Total RBC count (millions/dl)	Total WBC count (1000/dl)	Platelet count (x1000 cells/dl)	Haemoglobin (gm%)
Control	7.7 ± 0.3	9095.0 ± 684.4	901.5 ± 21.6	13.1 ± 0.7
CVS-15	6.4 ± 0.4	7250.0 ± 196.7	766.5 ± 46.0	12.8 ± 0.4
CVS-30	6.7 ± 0.4	7487.5 ± 499.3	775.0 ± 24.7	13.5 ± 0.4
Stress	13.1 ± 0.6[a]	12575.0 ± 205.6[a]	1403.3 ± 39.4[a]	16.6 ± 0.3[a]
Stress+NR-15	6.6 ± 0.3[b]	10625.0 ± 501.3[b]	1129.5 ± 31.0[b]	13.0 ± 0.2[b]
Stress+NR-30	7.3 ± 0.9[b]	10475.0 ± 497.7[b]	1097.8 ± 20.7[b]	14.0 ± 0.5[b]
Stress+CVS-15	6.6 ± 0.2[b]	7608.3 ± 312.7[b]	840.2 ± 43.3[b]	13.2 ± 0.4[b]
Stress+CVS-30	7.0 ± 0.6[b]	7377.3 ± 531.2[b]	787.0 ± 44.8[b]	13.2 ± 0.2[b]

Values are mean + SE (n = 4 each; stress+CVS-15 and stress+CVS-30 groups, 6 each).

CVS = caloric vestibular stimulation; NR = natural recovery.

15 and 30 are, 15 days and 30 days caloric vestibular stimulation.

a Significantly different from control group. b Significantly different from stress group.

4. Discussion and Conclusion:

Stressors, physical, perceived or chemical all evoke adaptive responses to maintain homeostasis. If the stressor

is severe or a longer duration, these responses become maladaptive and threaten homeostasis. Response to stress is mainly regulated by the hypothalamic-pituitary-adrenocortical (HPA) and sympathoadrenal medullary (SAM) axis which causes increased levels of glucocorticoids and hyperactivation of the sympathetic nervous system. Most of the stress-induced changes in the body are mediated either by glucocorticoids, catecholamines and neurotrophic factors (11).

The vestibular apparatus senses the position of head and three-dimensional movement in space (12). Although it was used as a diagnostic tool for evaluation of neurological disorders, research has proven the beneficial effect of vestibular stimulation in various conditions like Parkinson's, chronic stress , helping in alleviation of motor dysfunction and inhibits neuronal degeneration[14,15]. Vestibular stimulation can influence the activity of the hypothalamic-pituitary-adrenal axis (HPA) by direct and indirect pathways. Vestibular system has direct connections with the hypothalamus and the hippocampus. Indirectly by stimulating the hippocampal formation and increasing the release of the gamma-amino butyric acid (GABA) vestibular system can inhibit the HPA axis. Hypothalamus and vagus mediates the modulation of aldosterone by vestibular stimulation. Any stimuli which influence the sympathetic nervous system can alter the production of adrenal medullary hormones. Vestibular influence on sympathetic activity is mediated by otolith organs. The vestibular system has projections to diencephalon which can alter the sympathetic activity (16).

In the present study, a statistically significant increase in serum corticosterone levels was observed in stress and normal recovery groups. Rats that received CVS following stress have shown a decline in serum corticosterone which proves caloric vestibular stimulation is effective in reducing serum corticosterone, which is a marker of stress. this finding is in accordance with the findings of previous studies that reported a decline in glucocorticoids following vestibular stimulation in stress (17). Previous studies have shown a negative correlation between stress and serum levels of BDNF (18,19,20). In the present study, an increase in serum BDNF has been observed following stress and this finding is in accordance with other studies which has shown post-stress increase in serum BDNF [21,22]. Animals that received caloric vestibular stimulation as intervention following stress showed lower BDNF levels in comparison to the stress group and recovery groups. The mechanisms that interplay in the role of cortisol mediated BDNF elevation are unsolved. It has also been proposed that Glucocorticoids regulate translation, processing, and secretion of BDNF [5]. CVS by reducing glucocorticoids may contribute to the reduction in stress-induced elevation of serum BDNF.

The liver plays a crucial role in metabolism and energy homeostasis, where hepatocytes harmonize these metabolic mechanisms through gene expression in response to various humoral signals. GCs adjust systemic metabolic activities through GC receptors located on hepatocytes [23]. Thymus and lymph nodes play important role in enabling an individual to immunological adjustments. Spleen is one of the most abundant immune organs that plays a vital role in the modulation of the immune system, differentiation of T and B cells, production of antibodies, and clears circulating apoptotic cells. Stress alters myeloid cells, dendritic cells, granulocytes, and NK cells in the spleen [24,25]. In the current study, we have not found a significant alteration in liver weight, thymus weight and lymph nodes weight whereas spleen weight has decreased. Our study is in accordance with the earlier studies which have witnessed stress-induced decrease in weight of the spleen [26,27]. A significant reduction in spleen weight was observed both in stress and recovery groups that did not receive caloric vestibular stimulation following stress. CVS groups did not show a difference from the control group which proves CVS is helpful in restoring stress-induced changes in spleen weight. The weight reduction in the spleen could be due to stress-induced vasoconstriction in splanchnic circulation which shifts the splenic blood into general circulation, mediated through sympathetic-adrenal co-activation [28]. CVS by reducing stress mediators can help in protecting the stress-induced decline of the spleen weight.

Glucocorticoids cause the redistribution of blood cells, which might be facilitated by alterations of cell adhesion molecules. Glucocorticoids amend functions and trafficking of peripheral immune cells. Glucocorticoids lower circulating lymphocytes, monocytes, macrophages, basophils, eosinophils, and proliferate neutrophils. Peripheral circulation of leukocytes is necessary for effective immune function. The distribution and number of these immune cells represent comprehensive vigilance of the immune system [29]. Glucocorticoids enhance the oxygen-carrying capacity and enrich the rate of erythropoiesis to meet the demands of stress, by acting on the bone marrow. Previous studies have reported that stress increases total RBC count, WBC count, platelets as well as haemoglobin concentration [30,10]. Our present study is in accordance with previous studies and showed a significant increase in total RBC, WBC, platelet counts and haemoglobin level in the stress group. Stress increases blood cells in circulation through glucocorticoids and vestibular stimulation improves cold water stress

induced immunological alterations [31,32].

Conclusion: The present study proves stress elevates serum BDNF, total RBC count, total WBC count, platelet count, haemoglobin level and reduces spleen weight. Caloric vestibular stimulation is effective in preventing the stress-induced changes in serum corticosterone, BDNF, and spleen weight.

Study Impact: Caloric vestibular stimulation may prove to be a simple, cost effective, non invasive intervention for stress and stress related disorders.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

References

[1] Kaur, G., Guat, H. T., Suthahar, A., Ambigga, S. K., Karuthan C. Depression, anxiety and stress symptoms among diabetics in Malaysia: a cross sectional study in an urban primary care setting. BMC Family Practice. 2013; 14: 69.

[2] Bathina, S., Das, U. Brain-derived neurotrophic factor and its clinical implications. Arch Med Sci. 2015; Dec 10; 11(6): 1164–1178.

[3] Fujimura, H., Anthony, A. C., Chen, R., Nakamura, T., Takeshi, N. T., Kambayashi, J., Sun, B., Tandon, N. Brain-derived Neurotrophic Factor Is Stored in Human Platelets and Released by Agonist Stimulation. Thromb Haemost. 2002; 87(4):728-34.

[4] Zaletel, I., Filipović, D., Puškaš, N. Hippocampal BDNF in Physiological Conditions and Social Isolation. Rev Neurosci . 2017; 28(6):675-692.

[5] Numakawa, T., Odaka, H., Adachi, N. Actions of Brain-Derived Neurotrophic Factor and Glucocorticoid Stress in Neurogenesis. Int J Mol Sci .2017; 18(11):2312.

[6] Phillips, C. Brain-Derived Neurotrophic Factor, Depression, and Physical Activity: Making the Neuroplastic Connection. Neural Plast. 2017: 7260130.

[7] Mubbunu. L., Bowa, K., Petrenko, V., Silitongo, M. Correlation of Internal Organ Weights with Body Weight and Body Height in Normal Adult Zambians: A Case Study of Ndola Teaching Hospital. Anat Res Int.2018: 4687538.

[8] Nagaraja, H. S., Jeganathan, P. S. Forced swimming stress-induced changes in the physiological and biochemical parameters in albino rats. Indian Journal of Physiology and Pharmacology. 1999; 221 43(1):53–59.

[9] Nishiike, S., Nakamura, S., Arakawa, S., Takeda, N., Kubo, T. GABAergic inhibitory response of locus coeruleus neurons to caloric vestibular stimulation in rats. Brain Res. 1996; 712(1):84-94.

[10] Purushothaman, D., Kumar, S. S., Archana, R., Mukkadan, J. K. Neuroimmuno modulation by vestibular stimulation in cold water swimming Stress induced wistar albino rats. Asian J Pharm Clin Res. 2015; 8(4):117-120.

[11] Barton, B. A. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integr Comp Biol. 2002; 42(3):517-525.

[12] Pfeiffer, C., Serino, A., Blanke, O. The vestibular system: a spatial reference for bodily self-consciousness. Frontiers in Integrative Neuroscience. 2014; 31.

[13] Sherly Deborah George, Archana Rajagopalan, Subramani Parasuraman . Caloric Vestibular Stimulation Induced Enhancement of Behavior and Neurotrophic Factors in Chronic Mild Stress Induced Rats. Frontiers in Pharmacology, No: 834292, 2022 (13):1-9

[14] Harini Narayanam, Archana Rajagopalan , Subramani Parasuraman , Parayil Varghese Christapher , Suresh V Chinni Vestibular stimulation alleviates the motor dysfunctions in a mouse model of Parkinson's disease. Int. J. Pharm. Investigation, 2022; 12(4) : 479-482

[15] Thanalakshmi J, Archana R, Senthilkumar S, Shakila R, Pazhanivel N, Subhashini S. Role of caloric vestibular stimulation in improvement of motor symptoms and inhibition of neuronal degeneration in rotenone model of Parkinson's disease–An experimental study. Physiology International. 2020 Oct 17;107(3):390-405.

[16] Ray, C. A., Monahan, K. D. The Vestibulosympathetic Reflex in Humans: Neural Interactions Between Cardiovascular Reflexes. Clin Exp Pharmacol Physiol. 2002; 29(1-2):98-102.

[17] White-Traut, R. C., Schwertz, D., McFarlin, B., Kogan, J. Salivary cortisol and behavioral state responses of healthy newborn infants to tactile-only and multisensory interventions. J Obstet Gynecol Neonatal Nurs. 2009; 38(1):22-34.

[18] Klein, A. B., Williamson, R. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. International journal of neuropsychopharmacology. 2011; 14(3): 347-353.

[19] Mitoma, M., Yoshimura, R., Sugita, A., Umene, W., Hori, H., Nakano, H., Ueda, N., Nakamura, J. Stress at work alters serum brain-derived neurotrophic factor (BDNF) levels and plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels

in healthy volunteers: BDNF and MHPG as possible biological markers of mental stress?; Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2008; 32(3):679-685.

[20] Giese, M., Unternaehrer, E., Brand, S., Calabrese, P., Holsboer-Trachsler, E., Eckert, A. The Interplay of Stress and Sleep Impacts BDNF Level. PLoS One. 2013; 8(10): e76050.

[21] Linz, R., Puhlmann L. M. C., Apostolakou, F., Mantzou, E., Papassotiriou, I., Chrousos, G. P., Engert, V., Singer, T. Acute Psychosocial Stress Increases Serum BDNF Levels: An Antagonistic Relation to Cortisol but No Group Differences After Mental Training. Neuropsychopharmacology. 2019; 44(10):1797-1804.

[22] Buselli, R., Veltri, A., Baldanzi, S., Marino, R., Bonotti, A., Chiumiento, M., Girardi, M., Pellegrini, L., Guglielmi, G., Dell'Osso, L., Cristaudo, A. Plasma Brain-Derived Neurotrophic Factor (BDNF) and serum cortisol levels in a sample of workers exposed to occupational stress and suffering from Adjustment Disorders. Brain Behav. 2019; 9(7):e01298.

[23] Mueller, K. M., Themanns, M., Friedbichler, K., Kornfeld, J. W., Esterbauer, H., Tuckermann, J. P., Moriggl, R. Hepatic growth hormone and glucocorticoid receptor signaling in body growth, steatosis and metabolic liver cancer development. Mol Cell Endocrinol. 2012; 361(1-2): 1–11.

[24] Jiang, W., Li, Y., Sun, J., Li. L., Li, J. W., Zhang, C., Huang, C., Yang, J., Kong, G. Y., Li, Z. F. Spleen contributes to restraint stress induced changes in blood leukocytes distribution. Sci Rep. 2012; 7: 6501.

[25] Tarantino, G., Scalera, A., Finelli, C. Liver-spleen axis: Intersection between immunity, infections and metabolism. World J Gastroenterol. 2013; 19(23): 3534–3542

[26] Sardessai, S. R., Abraham, M. E., Mascarenhas, J. F. Effect of stress on organ weight I rats. Indian J Physiol Pharmacol.1993; 37(2):104-8.

[27] Hara, C., Manabe, K., Ogawa, N. Influence of activity-stress on thymus, spleen and adrenal weights of rats: Possibility for an immunodeficiency model. Physiology & Behavior. 1981; 27(2):243-248.

[28] Sembulingam, K., Jeevanandam, T. E., Sembulingam, P., Parveen, S. Effect of noise stress on adrenal glands, liver, spleen and thymus of albino rats. Biomedicine. 2000; 20: 246 - 254.

[29] Sapolsky, R. M., Romero, L. M., Munck, A. U. How Do Glucocorticoids Influence Stress Responses? Integrating Permissive, Suppressive, Stimulatory, and Preparative Actions. Endocrine Reviews. 2000; 21 (1): 55–89.

[30] Lombardi, G., Ricci, C., Banfi, G. Effect of winter swimming on haematological parameters. Biochem Med (Zagreb). 2011;21(1):71-8.

[31] Krishnan, J., Kumar, S. S., Archana, R., Mukkadan, J. K. Effect of Forced Freshwater and Cold Water Swimming Stress Induced Changes in Selected Physiological and Biochemical Parameters in Wistar Albino Rats. Research Journal of Pharmaceutical, Biological and Chemical sciences. 2015: 6(3):105.

[32] T. Rama Kranthi, R. Archana, S. Senthilkumar. Vestibular Stimulation as An Interventional Approach for Cold Water Stress Induced Immunological and Histopathological Changes in Rats .Indian Journal of Animal Research, Volume 56 Issue 7: 830-836 ,2022