ORIGINAL ARTICLE

HARNESSING THE THERAPEUTIC POTENTIAL OF SYNBIOTIC CONSUMPTION: MITIGATING STRESS AND ENHANCING NEURAL PLASTICITY IN MALE SPRAGUE DAWLEY RATS

Aysha Mushtaq, Shazia Ali*, Tooba Jamal*, Shagufta Feroz, Ayesha Zafar***, Mehvish Ashfaq†**

Department of Physiology, HBS Medical and Dental College, Islamabad, *Islamic International Medical College, Riphah International University, Rawalpindi, **Riphah Institute of Lifestyle Medicine, Riphah International University, Islamabad, ***Dental College, HITEC-IMS, Taxila, †HITEC-IMS Taxila, Pakistan

Background: The hypothalamic-pituitary-adrenal (HPA) axis regulates homeostasis, and chronic stress triggers cortisol release, contributing to metabolic, cardiovascular, and neuropsychiatric disorders. Brain Derived Neurotrophic Factor (BDNF) plays a vital role in neural plasticity and stress adaptation. Objective was to determine and correlate serum cortisol and BDNF levels in rats consuming standard, probiotic, and synbiotic diets under stressors of immobilization and fasting. **Methods:** A randomized controlled trial was conducted at the National Institute of Health, Islamabad. Fifty male Sprague Dawley rats were divided into Control (Group A) and Interventional (Group B; subgroups B1–B4). Group A received a standard lab diet, whereas subgroups B1–B4 were exposed to immobilization and fasting stress with varying dietary interventions: standard diet (B1, B2), probiotic yogurt (B3), and synbiotic yogurt+legumes (B4). Serum cortisol and BDNF were measured at baseline, 4, and 10 weeks using ELISA. Data were analysed with SPSS-21 and $p<0.05$ was considered significant. **Results:** After 10 weeks, Group B3 and B4 showed significantly decreased cortisol ($p<0.001$) and increased BDNF ($p<0.001$) compared to Group B2. A strong negative correlation was found between cortisol and BDNF ($r= -0.738$, $p<0.001$). **Conclusion:** Synbiotic supplementation mitigated stress-induced neuroendocrine changes by lowering cortisol and elevating BDNF levels, suggesting that dietary modulation with probiotics and synbiotics may improve stress resilience and neural health.

Keywords: BDNF, Cortisol, HPA axis, Stress, Synbiotics

Pak J Physiol 2025;25(4):22–5, **DOI:** https://doi.org/10.69656/pjp.v21i4.1857

INTRODUCTION

Stress is an inevitable element of modern life and a major contributor to disease progression. Chronic exposure to stress triggers maladaptive physiological responses, increasing the risk of cardiovascular, inflammatory, metabolic, and neuropsychiatric disorders.[1] The body perceives stress as a threat to homeostasis, engaging the hypothalamic-pituitary-adrenal (HPA) axis, which mediates the secretion of cortisol.[2] Persistent activation leads to metabolic dysregulation, inflammation, and impaired neural function.[3]

Decades of research have shown that stress has a role in gastrointestinal disease pathogenesis. Gut Dysbiosis associated with stress, alters the secretory activity, motility, and visceral sensitivity of the GI tract, as well as the permeability of the gut barrier.[4] This goal is achieved through the auto-regulatory neural and hormonal systems, which work closely with the central and peripheral clocks to achieve this goal. A key regulatory pathway in the maintenance of these homeostatic processes is the HPA Axis.[5]

Circadian rhythms are mental, physical, and behavioural changes that follow a daily cycle, which respond primarily to light and darkness in an organism's environment. Timing of food abstinence and intake may affect circadian rhythms.[6] Dysregulation of the circadian cortisol rhythm is one marker that appears to coincide with the deleterious effects of chronic stress.[7] Chronic and severe psychological and physical stress leads to HPA hyperactivity and cortisol hypersecretion.[8]

The scaffolding of the Gut-Brain axis consists of CNS, ENS, autonomic nerve systems, neuroendocrine and neuro-immune systems, and gut microbiota that has recently emerged as a critical regulator of gut-brain axis, particularly under times of stress. The gut micro biome has been associated to anxiety, depression, and irritable bowel syndrome.[9] The introduction of Probiotics, Prebiotics or Synbiotic into human diet is favorable for the intestinal microbiota.[10]

There is a pulsatile pattern in which cortisol is secreted. The hypothalamic-pituitary-adrenal (HPA) pathway is involved in the rise of cortisol in response to physical and psychological stress. The hypothalamus stimulates corticotropin-releasing factor (CRF), which boosts adrenocorticotropin hormone (ACTH) production in the pituitary.[11] ACTH causes cortisol secretion from the adrenal glands. Long-term stressors induce on going cortisol production and so malfunction. The negative feedback mechanism causes neuronal degeneration, inflammation, hippocampal volume loss, anxiety and depression-like behaviours, increased susceptibility to infections, metabolic syndrome, obesity, mental health disorders, cancer, cardiovascular diseases, insomnia, severe mood swings, depression, and severe hypotension.[12]

BDNF (Abrineurin) is a protein essential for synaptic transmission, neuroplasticity, and cognitive development. Low BDNF levels are linked to cognitive decline, while high levels boost cognitive function.[13] Lifestyle factors like poor diet and physical inactivity can negatively impact mental and cognitive health. Dietary changes, such as consuming probiotics and prebiotics, may help increase BDNF levels and preserve cognitive and metabolic health.[14] Probiotic and prebiotic supplements have been developed to manage intestinal micro ecological environment.[15]

Probiotics have been shown to prevent obesity, inflammatory diseases, cancer, and degenerative diseases. They positively influence innate immunity, exert antiviral properties, and impact mood, cognition, and gut microbiome. Probiotics also enhance gut barrier functions, stimulating B cells and influencing cytokine production. Consuming probiotic-rich yogurt and prebiotic legumes has been linked to cardio-metabolic benefits, reduced obesity and diabetes, and improved biomarkers of chronic diseases, including oxidative stress and inflammation.

In 1995, Gibson and Roberfroid introduced the term 'Synbiotic', defining it as a mixture of probiotics and prebiotics that benefits the host by improving the survival and introduction of live microbial dietary supplements. This concept has led to the development of therapeutic approaches targeting intestinal microflora to treat stress-related diseases. However, these treatments often have adverse effects and are unaffordable for many. Fortunately, we can now modify health through dietary regimes and lifestyle modifications, measuring effects through microbial or metabolic changes.[15]

Research on probiotics and prebiotics as well as the creation of synbiotics is the need of hour. There is a possibility that future research will find that certain components have beneficial impacts on human health. This research project serves the purpose by combining synbiotics (yogurt and legumes) and synchronizing their consuming time with circadian rhythm. Limited data is available in which this combination is used as a shield against stress. It is unclear whether probiotics might affect cognitive performance independent or dependent of the detrimental effects of stress.[16]

There is needed to understand the link between gut microbiota, stress, and neuropsychiatric disorders. This study investigates cortisol and BDNF levels in response to stressful lifestyles and co-morbidities. Large-scale clinical trials are necessary to determine the efficacy of synbiotic-based interventions in stress-related disorders, which could shed light on the impact of microbiota perturbations on chronic stress and cognitive-related disorders.

MATERIAL AND METHODS

This randomized controlled animal study was conducted in collaboration with the Animal House, National Institute of Health (NIH), Islamabad, after approval from the Institutional Ethics Review Committees (Riphah/IIMC/IRC/21/65, NIH No. F.1-5/ERC/2021).

Fifty male Sprague Dawley rats weighing 250–300 g were randomly allocated into Control (A) and Interventional (B) groups. Group B was subdivided into 4 subgroups of 10 each. All rats were acclimatized for 7 days at 22±0.5 °C under a 12-hour light/dark cycle and fed a standard lab diet.

Stressors:
Immobilization (2 h/day, 5 days/week) and overnight fasting (10 PM–10 AM) for 10 weeks.

Dietary interventions:
B1: Prolonged fasting+immobilization (standard diet)
B2: Fasting+immobilization (standard diet, control for intervention)
B3: Standard diet+probiotic yogurt (8 g/day)
B4: Standard diet+synbiotic (yogurt 8 g/day+legumes 75 g/day)

Yogurt contained Lactobacillus acidophilus and Streptococcus thermophilus, verified at NARC Islamabad. Legumes (Phaseolus vulgaris, Cicer arietinum, Lens culinaris, Vigna radiata) were authenticated and cooked before mixing with the standard diet.

Blood samples were collected at baseline, 4, and 10 weeks. Serum cortisol and BDNF levels were measured using ELISA. Data were expressed as Mean±SD and analysed using ANOVA and Pearson correlation in SPSS-21, and *p*≤0.05 was considered significant.

RESULTS

The Mean±SD of serum levels of cortisol (ng/mL) of all the groups on day 0, 4th and 10th week are shown in Table-1. The Mean±SD of serum levels of BDNF (ng/mL) of all the groups on day 0, 4th and 10th week are shown in Table-2.

A strong negative correlation was also found between serum Cortisol and BDNF levels at week 10 (Pearson Correlation Coefficient= -0.738, *p*≤0.001)

Table-1: Serum levels of cortisol in male Sprague Dawley rats exposed to stressors of immobilization and overnight fasting at day 0, 4th and 10th week

Cortisol (ng/mL)	Group A (Control)	Group B (Interventional)			
		Group B1	Group B2	Group B3	Group B4
0 week	21.02±1.97	15.58±3.16	19.82±3.48	16.64±5.09	13.83±3.30
4th week	21.69±1.74	92.29±8.31	73.20±9.67	69.30±11.62	70.60±9.47
10th week	20.72±3.42	99.39±12.99	71.69±5.94	45.78±5.79	28.82±6.76

Table-2: Serum levels of BDNF in male Sprague Dawley rats exposed to stressors of immobilization and overnight fasting at day 0, 4th and 10th week

		Group B (Interventional)			
BDNF (ng/mL)	Group A (Control)	Group B1	Group B2	Group B3	Group B4
0 week	26.01±0.94	26.96±1.35	26.20±0.93	26.28±1.45	26.57±0.98
4th week	26.05±0.94	16.09±1.34	19.17±0.95	16.96±1.28	17.38±1.22
10th week	26.32±0.96	14.37±1.82	18.41±1.89	28.99±2.87	34.26±2.14

DISCUSSION

Stress is how a person reacts to external or internal issues and challenges. The organism employs various strategies to coordinate adaptive responses at the systemic and cellular levels.[17] Chronic stress disrupts biological rhythms contributing to cerebrovascular illness, obesity, diabetes, poor neuronal plasticity, inflammation and uncontrollable eating disorders.[18]

The present research offers a variety of modalities for modifying one's lifestyle that may be practiced in the privacy of the individual's home and at a reduced cost to mitigate the negative consequences of chronic stress.[19] Findings of current study showed that, serum cortisol of interventional Group-B after exposure to stresses at 1st experimental level were significantly raised at 4 weeks as compared to Day 0 and also compared with Control Group-A. This is in agreement with Sharma *et al*[20]. Findings of this study showed that chronic stress increased serum cortisol levels when exposed to stresses of immobilization and fasting. The finding is consistent with the findings of a Buynitsky T *et al*[21] and Noushad S *et al*[22] that rise in serum cortisol was significant after restraining the rats as compared to other stressors.

The cortisol levels of Group B2 also remained significantly raised at 10 weeks but values were less than B1 indicating that prolonged stress and skipping morning meal could keep the cortisol levels highly raised. These findings are similar to study by Bonnet JP *et al*[23] that hyper activation of HPA axis due to chronic stress results from impaired negative feedback control, thus the system remains elevated for a long time once triggered resulting in increased levels of cortisol.

The mean cortisol levels of Group B3 and B4 were significantly decreased at 10 weeks as compared to that at 4 weeks due to synbiotic supplementation in regulating the serum cortisol levels close to baselines and preventing its increase.[24] These findings are also consistent with study conducted by Tulk HM *et al*[25].

Work by Fumagalli *et al*[26] found that restraint stress leads to a significant decrease in BDNF protein levels in the prefrontal cortex and striatum of males when they reach adulthood. Our study is in agreement with that study.

Current study is contradictory to work by Zuena *et al*[27] who found that restraint of the pregnant rat led to no change in BDNF expression in the female but an increase in BDNF expression in male rat. In current study BDNF levels were markedly lowered after chronic stress. This indicates that early life stress can have long lasting effect on gene expression associated with neural development and ongoing neural plasticity.

In this study, serum levels of BDNF of Group B1 and of Group B2 remained significantly decreased at 10 weeks as compared to other groups due to stresses. The serum BDNF levels of Group B3 and B4 were significantly raised at 10 weeks with synbiotic treatment. This is due to the fact that gut bacteria affect BDNF levels via the creation of neurotransmitters, neuroactive short-chain fatty acids (SCFAs), secondary bile acids, and other physiologically active small molecules. By activating G protein-coupled receptors, SCFAs may regulate several brain areas (GPCRs).

According to Nazanin Heidarzadeh-Rad *et al*[28] post-intervention serum BDNF levels in the probiotics group were considerably higher than in the prebiotic placebo groups. That study investigating the impact of probiotics on BDNF levels in animal models is consistent with our findings. Yong SJ *et al*[29] discovered that injection of L. helveticus NS8 to rats with chronic stress increased Hippocampal BDNF mRNA, suggesting putative antidepressant-like effects.

Eight-week probiotic supplementation (composed of L. acidophilus, Lactobacillus casei, and B. bifidum) resulted in a larger reduction. In the present investigation, in conjunction with the findings of Mohammad Morshedi *et al*[30], probiotics/synbiotics treatment significantly increased BDNF levels. It may be argued that combining multiple strains into a single product could be more effective. By altering the intestinal microbiota, prebiotics can affect the brain and behaviour via microbe-derived molecules such as SCFAs and tryptophan metabolites, neuroactive molecules such as neurotransmitters, and neuronal signalling via stimulation of the vagus nerve, all of which play a role in reducing the inflammatory state and increasing the serum levels of BDNF.[30]

CONCLUSION

Synbiotic supplementation significantly modulated stress-related biomarkers in rats exposed to chronic stress. A daily intake of probiotic yogurt and legumes normalized cortisol and elevated BDNF, promoting neuroplasticity and stress resilience.

RECOMMENDATIONS

Human-based, large-scale studies are required to confirm these findings. Molecular and microbiome analyses should be incorporated to elucidate mechanisms. Time-sequence stool collection scan be performed on individually housed animals, providing opportunity to assess changes in colonic microbiota over time.

REFERENCES

1. Selye H (Ed). The Stress of Life. New York: McGraw-Hill;1956.
2. McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med 1998;338(3):171–9.
3. Chrousos GP. Stress and disorders of the stress system. Nat Rev Endocrinol 2009;5(7):374–81.
4. Karl PJ, Hatch AM, Arcidiacono SM, Pearce SC, Pantoja-Feliciano IG, Doherty LA, *et al.* Effects of psychological, environmental and physical stressors on the gut microbiota. Front Microbiol 2018;9:1–32.
5. Dinan TG, Cryan JF. The impact of gut microbiota on brain and behavior: Implications for psychiatry. Curr Opin Clin Nutr Metab Care 2017;20(6):460–6.
6. Mayer EA. Gut feelings: The emerging biology of gut-brain communication. Nat Rev Neurosci 2011;12(8):453–66.
7. Schneiderman N, Ironson G, Siegel SD. Stress and health: Psychological, behavioral, and biological determinants. Annu Rev Clin Psychol 2005;1:607–28.
8. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. Nat Rev Neurosci 2009;10(6):434–45.
9. Cryan JF, O'Mahony SM. The microbiome–gut–brain axis: From bowel to behaviour. Neurogastroenterol Motil 2011;23(3):187–92.
10. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J Nutr 1995;125(6):1401–12.
11. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, *et al.* Expert consensus on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014;11(8):506–14.
12. Russell G, Lightman S. The human stress response. Nature Rev Endocrinol 2019;15(9):525–34.
13. Notaras M, van den Buuse M. Brain-derived neurotrophic factor (BDNF): novel insights into regulation and genetic variation. Neuroscientist 2019;25(5):434–54.
14. Sapolsky RM. Why stress is bad for your brain. Sci 1996;273(5276):749–50.
15. Mohammadi AA, Jazayeri S, Khosravi-Darani K, Solati Z, Mohammadpour N, Asemi Z, *et al.* (Retracted Article): The effects of probiotics on mental health and hypothalamic-pituitary-adrenal axis: A randomized, double-blind, placebo-controlled trial in petrochemical workers. Nutri Neurosci 2016;19(9):387–95.
16. Li C, Niu Z, Zou M, Liu S, Wang M, Gu X, *et al.* Probiotics, prebiotics, and synbiotics regulate the intestinal microbiota differentially and restore the relative abundance of specific gut microorganisms. J Dairy Sci [Internet] 2020;103(7):5816–29. Available from: http://dx.doi.org/10.3168/jds.2019-18003
17. McEwen BS. Brain on stress: How the social environment gets under the skin. Proc Natl Acad Sci 2012;109(Suppl 2):17180.
18. Maqsood R, Stone TW. The Gut-Brain Axis, BDNF, NMDA and CNS Disorders. Neurochem Res [Internet]. 2016;41(11):2819–35. Available from: http://dx.doi.org/10.1007/s11064-016-2039-1
19. Valdes AM, Walter J, Segal E, Spector TD. Role of the gut microbiota in nutrition and health. BMJ 2018;361:36–44.
20. Sharma R, Gupta D, Mehrotra R, Mago P. Psychobiotics: the next-generation probiotics for the brain. Curr Microbiol 2021;78(2):449–63.
21. Buynitsky T, Mostofsky DI. Restraint stress in biobehavioral research: recent developments. Neurosci Biobehav Rev 2009;33(7):1089–98.
22. Noushad S, Ahmed S, Ansari B, Mustafa UH, Saleem Y, Hazrat H. Physiological biomarkers of chronic stress: A systematic review. Int J Health Sci 2021;15(5):46.
23. Bonnet JP, Cardel MI, Cellini J, Hu FB, Guasch-Ferré M. Breakfast skipping, body composition, and cardiometabolic risk: A systematic review and meta-analysis of randomized trials. Obesity 2020;28(6):1098–109.
24. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, *et al*. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Nat Rev Gastroenterol Hepatol [Internet]. 2020;17(11):687–701. Available from: http://dx.doi.org/10.1038/s41575-020-0344-2
25. Tulk HM, Blonski DC, Murch LA, Duncan AM, Wright AJ. Daily consumption of a synbiotic yogurt decreases energy intake but does not improve gastrointestinal transit time: a double-blind, randomized, crossover study in healthy adults. Nut J 2013;12(1):87.
26. Fumagalli F, Bedogni F, Perez J, Racagni G, Riva MA. Corticostriatal brain-derived neurotrophic factor dysregulation in adult rats following prenatal stress. Eur J Neurosci 2004;20(5):1348–54.
27. Zuena AR, Mairesse J, Casolini P, Cinque C, Alemà GS, Morley-Fletcher S, *et al.* Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. PloS One 2008;3(5):e2170.
28. Heidarzadeh-Rad N, Gökmen-Özel H, Kazemi A, Almasi N, Djafarian K. Effects of a psychobiotic supplement on serum brain-derived neurotrophic factor levels in depressive patients: a post hoc analysis of a randomized clinical trial. J Neurogastroenterol Motil 2020;26(4):486.
29. Yong SJ, Tong T, Chew J, Lim WL. Antidepressive mechanisms of probiotics and their therapeutic potential. Front Neurosci 2020;13:1361.
30. Morshedi M, Saghafi-Asl M, Hosseinifard ES. The potential therapeutic effects of the gut microbiome manipulation by synbiotic containing-Lactobacillus plantarum on neuropsychological performance of diabetic rats. J Translat Med 2020;18(1):18.

Address for Correspondence:

Dr Ayesha Zafar, Department of Physiology, HITEC-Institute of Medical Sciences, Taxila, Pakistan. **Cell:** +92-336-5601133
Email: ayeshazafar_30@hotmail.com

Received: 20 Jul 2025 **Reviewed:** 17 Oct 2025 **Accepted:** 23 Dec 2025

Contribution of Authors:

AM: Methodology, Data analysis, interpretation, critical review, revision of manuscript, Final approval, final proof to be published
SA: Conceptualization, study design, methodology, data analysis, and Writing Draft
TJ: Conceptualization, Study Design, Methodology, data analysis, interpretation and Writing Draft
SF: Methodology, Data analysis and interpretation
AZ: Study Design, writing draft
MA: Methodology, Data analysis and interpretation and writing draft

Conflict of interest: None to declare **Funding disclosure:** No funds received