Original Article

Impact of synbiotics on stress-induced alterations in cortisol levels and lipid profile in male Sprague Dawley Rats

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Objective: To compare and correlate serum cortisol and lipid profile among different groups of Male Sprague Dawley rats exposed to the stressors of immobilization and fasting while consuming standard lab diet, probiotics and synbiotics.

Methodology: This randomized control trial was carried out at Islamic International Medical College from September 2021 to September 2022, on 50 male Sprague Dawley rats. These were divided into Control-A (10 rats) and Experimental groups B1, B2, B3, and B4 (10 rats each), which were subjected to stressors of immobilizations (2 hours/5 days/10 weeks) and fasting 10 hours/5 days/10 weeks. Group B1 was given an extra 2 hours of fasting. Standard lab food for B1 and B2 while B3 received a conventional lab diet supplemented with probiotics. B4 received a standard lab diet supplemented with synbiotics. Serum cortisol and lipid profile was measured at 0, 4, and 10 weeks.

Results: Group B4 shows significant reduction ($p \leq 0.001$) in cortisol and lipid profile except HDL on comparison with other groups after 10 weeks. There was positive correlation between serum cortisol and cholesterol (0.840), triglyceride (0.741) and LDL (0.879) and negative correlation between serum cortisol and HDL -0.799.

Conclusion: Synbiotics ameliorate raised levels of cortisol and lipid profile due to stress exposure.

Keywords: Stress, hypothalamic-pituitary axis, cortisol, lipid profile, synbiotics.

INTRODUCTION

Stress affects many biochemical regulatory systems in the body. Acute stress is short-term while chronic stress is long-term.[1] The changes in gut microbiota are one of the biological effects that are produced due to day-to-day stress. In turn, the gut microbiota affects the stress response of the host therefore, establishing the gut microbiota as a critical mediator of host health. This interaction influences a variety of important processes, like host's metabolism, immunology, and the operation of the intestinal barrier.[2]

Circadian clock is accountable for ensuring that the internal time is in synchronization with the time of the external world throughout the day. One of the important factors of circadian rhythm is taking meal accordingly, especially breakfast.[3] Fasting has been associated with increased negative emotions, such as irritability negative mood and stress due to the decreased sugar levels and increased laver of cortisol.[4] Stress entrains the circadian rhythm via glucocorticoids, sympathetic nerves, oxidative stress, hypoxia, pH, cytokines, and temperature.

Chronic stress response, is primarily manifested by the adrenal glands' secretion of glucocorticoids into the systemic circulation leading to metabolic syndrome, insulin resistance, and high blood pressure.[5] During stress, liver alter lipoproteins including very low-density lipoprotein, low density lipoprotein, and high-density lipoprotein.[6]

Synbiotics are combination of probiotics, which are live micro-organism in the gut and prebiotics which are mainly carbohydrates in nature and they improve the survival of gut microbiomes.[7] Therefore, they regulate the production of neurotransmitters, various hormones, that affect host's metabolism.[8] Limited studies are available on synbiotics and their effects on cortisol and lipid profile. Therefore, in current study it is hypothesized that effects of synbiotics will alter the levels of cortisol, and lipid profile on exposure to chronic stress.

METHODOLOGY

This randomized control trial conducted in the Department of Physiology, Islamic International Medical College Rawalpindi in collaboration with National Institute of Health Islamabad. Institutional review committee of Islamic International Medical College (Appl. # Riphah/IRC/21/66) and Ethics Committee for Animal Care and Experimentation (IECACE) of NIH (NO.F.1-5/ERC/2021) approved the study.

Animals: Seven days prior to experiment acclimatization to the environmental conditions of humidity of 50-70% and a room temperature of 24 ± 2°C, with a 12-hour light/dark cycle was done. The animals were then separated and placed in their respective enclosures. They were given a standard diet in pellet form.

Stressors: For **immobilization,** rats were kept in a wooden container, measuring 57×19×.5cm. Each container comprised of 10 small spaces measuring 17×5.5×5 cm for individual rats, these were covered by metallic sheet from inside to avoid biting. On the surface a mesh was placed for ventilation and a hole on one side for rat tails was made. The rats were restrained in these containers for 2 hrs/5day/week.

Fasting: Rats of experimental groups B2, B3 and B4 underwent fasting (overnight fasting). They were given the last meal at 10 pm and then food was removed from their cages. Morning meal was given to rats at 8 am ensuring10 hours fast. Rats of group B1 were on prolonged fast from 10 pm to 10 am (12 hours fast) and they were given morning meal at 10 am.[9]

Synbiotics: Synbiotic comprised of probiotic and prebiotic components. For probiotics yogurt 8g/day was used and for prebiotics legumes of 75g/day were daily consumed, which were verified by the National Agriculture Research Center, Islamabad.

Sampling: Day-0, 2ml blood was collected through rat tail vein, while intracardiac puncture was used to collect 3ml of blood at four and ten weeks of experimentation for measuring serum cortisol and lipid profile. Animals were euthanized after 10 weeks of experimentation.

Statistical Analysis: Data analysis was done through SPSS version 23. ANOVA and Post hoc-Tukey tests used for comparison between groups. Pearson’s correlation was done between serum levels of cortisol and lipid profile. p≤0.05 was considered significant.

RESULTS

The rats were randomly allocated to the following groups (Table 1). Mean±SD of serum cortisol levels in B1 (94.67±13.37) at 10 weeks was significantly increased (p<0.05) compared to Mean±SD serum cortisol levels in B2 (87.17±13.24). Serum cortisol levels in B3 (54.15±7.72 ng/ml) and B4 (42.96±6.31), were significantly decreased (p<0.05) compared cortisol levels in Group B2 (94.67±13.37) (Table 2).

Table 1: Group distribution of rats.

Groups	Experimental conditions	No. of Rats
Group A	Control group	10
Group B1	Immobilization and prolonged fasting with standard lab diet	10
Group B2	Immobilization and fasting with standard lab diet	10
Group B3	Immobilization and fasting with probiotic diet	10
Group B4	Immobilization and fasting with synbiotic diet	10

B1 shows a significant difference with B3(p<0.002) and B4(p<0.001), while B2 also exhibits a significant difference with B3(p<0.002) and B4(p<0.001). Multiple comparisons of Mean±SD indicate significant differences between A and B1(p<0.001), B2(p<0.001), and B3(p<0.03). GroupB1 exhibits significant

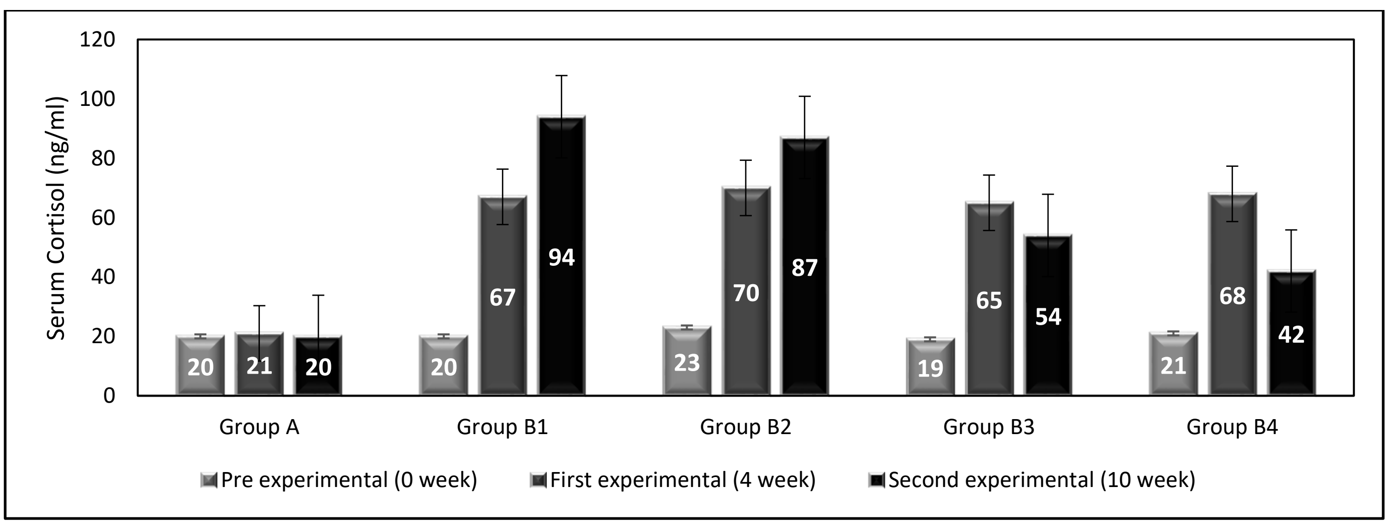

Fig. 1: Comparisons of Mean ± SD of serum levels of cortisol.

differences with B2(p<0.04), B3(p<0.02), and B4(p<0.007). B2 also demonstrates significant differences with B3(p<0.04) and B4(p<0.01).

Multiple comparisons of LDL revealed significant differences between A and B1(p<0.001), B2(p<0.001), and B3(p<0.03). Significant difference was observed between B1 and B3(p<0.02), as well as between B1 and B4(p<0.001). Significant difference was found between group B2 and B3(p<0.03), and between B2 and B4(p<0.001). Furthermore, a significant difference was identified between group B3 and B4(p<0.05) (Table 3).

Multiple comparisons triglycerides of Mean±SD significant difference between A and B1(p<0.001), B2(p<0.001), B3(p<0.001), and B4(p<0.001). B1 exhibits a significant difference with B2(p<0.03), B3(p<0.001), and B4(p<0.001). Similarly, B2 demonstrates a significant difference with B3(p<0.004) and B4(p<0.001). Moreover, a significant difference is observed between B3 and B4(p<0.02).

Cortisol has a strong positive correlation with serum cholesterol, LDL and triglyceride as Pearson correlation coefficient is 0.840, 0.741 and 0.879, respectively. Cortisol shows negative correlation with HDL as Pearson correlation coefficient is -0.799 at 10 weeks (Fig. 1).

Table 2: Comparison between the Mean±SD of serum cortisol done by Post-hoc Tucky test.

Groups		Serum cortisol mean difference	p value
A Control	B1	39.77	0.001***
	B2	38.44	0.001***
	B3	25.68	0.001***
	B4	23.30	0.001***
B1	B2	1.32	0.1
	B3	14.09	0.002**
	B4	16.64	0.001***
B2	B3	12.76	0.002**
	B4	15.14	0.001***
B3	B4	2.37	0.08

Tables 3: Mean±SD of lipid profile among different groups at 0, 4th and 10th week.

Lipid profile	Sampling time	Group A	Group B1	Group B2	Group B3	Group B4
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Cholesterol mg/dl	0 week	45.29 ± 4.88	44.49 ± 6.13	45.55 ± 2.84	40.07 ± 4.46	43.86 ± 2.86
	4thweek	45.49 ± 3.96	61.78 ± 7.27	67.42 ± 5.99	73.09 ± 5.08	67.10 ± 6.51
	10thweek	44.16 ± 4.04	74.67 ± 6.84	71.28 ± 5.72	62.73 ± 4.64	54.02 ± 5.98
HDL levels mg/dl	0 week	40.63 ± 2.90	38.09 ± 3.18	37.92 ± 4.98	42.21 ± 5.96	40.69 ± 3.03
	4thweek	42.15 ± 3.28	36.09 ± 3.18	36.63 ± 5.06	36.93 ± 2.64	34.13 ± 2.67
	10thweek	42.17 ± 4.15	28.96 ± 2.04	30.81 ± 3.41	37.53 ± 2.67	39.24 ± 2.98
LDL levels mg/dl	0 week	15.35 ± 3.15	15.59 ± 1.81	17.26 ± 2.28	16.48 ± 2.43	14.81 ± 2.73
	4^{t} week	15.53 ± 1.83	22.82 ± 3.09	21.10 ± 2.42	18.95 ± 1.53	19.45 ± 2.73
	10thweek	14.83 ± 2.60	24.90 ± 2.47	22.27 ± 2.49	17.06 ± 1.64	15.83 ± 1.97
Triglyceride levels- mg/dl	0 week	50.33 ± 10.12	45.44 ± 6.47	51.52 ± 4.59	46.27 ± 8.78	45.33 ± 6.20
	4thweek	44.74 ± 7.39	138.81 ± 42.50	139.26 ± 11.67	131.49 ± 11.67	136.27 ± 13.62
	10thweek	45.85 ± 5.82	161.42 ± 9.39	145.60 ± 9.07	104.64 ± 11.72	93.73 ± 35.29

DISCUSSION

Stress causes an increase in cortisol levels in the body which can be reduced by utilizing synbiotics in the diet. It is found that synbiotics can reduce cortisol levels, similar to our study, where there is a significant decrease in cortisol levels after using the synbiotic diet.[10] However, the results of another study differed from ours, as usage of a synbiotic diet had no significant influence on cortisol levels.[11]

In our study, rats who skipped their morning meals and fasted over night for an extended period of time had significantly higher cortisol levels than other groups.

These findings are contrary to another study, in which individuals who skipped breakfast had lower cortisol levels this difference can be due to short duration of fasting that was less than 12 hours.[12]

In the current study, continuous stress exposure lead to raised lipid levels. Previous research showed that continuous stress caused a raised lipid profile and obesity via visceral fat deposition.[13] Current study proved that intake of synbiotics reduced lipid levels when compared to other groups. Another prior study found that daily use of synbiotics for 8 weeks improved lipid profiles significantly, while in current study, beneficial effects of synbiotics on lipid profile were observed after 6 weeks.[14] Altered lipid profile was observed in the group of current study, who skipped their morning meal as it provides macro- and micronutrients that influence eating behavior throughout the day, leading to a healthy diet. These findings are like another study in which skipping breakfast causes an increase in morning hunger, leading to weight gain.[15]

CONCLUSION

Synergistic effect of probiotics and prebiotics as synbiotic had greater potential in modulating gut microbiota, which reduce the cortisol secretion during stress through alteration in the

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Conception and design: Tooba Jamal, Shazia Ali, Aysha Mushtaq, Shagufta Feroz.
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Drafting of the article: Tooba Jamal, Shazia Ali.
Critical revision of article for important intellectual content: Tooba Jamal, Shazia Ali.
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