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Efficiency of Probiotics in Immobilized Rats through Involvement of the Antioxidant and Anti-Inflammatory Systems

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ABSTRACT

Aims Immobilization is known as a type of stress that influences antioxidant status and inflammation factors. On the other hand, probiotics could significantly improve antioxidant status and inflammatory response. This study was thus conducted to evaluate the effects of probiotics in immobilized rats by involving antioxidant and anti-inflammatory systems.
Materials & Methods A total number of 72 Albino Wistar rats were divided into 3 groups, including a normal control group without stress (Control), 2) Immobilized rats without additive (Immobilized), and 3) Immobilized rats given with 104 CFU probiotics.day-1(Probiotic). Inflammatory and pro-inflammatory cytokines, corticosterone, and serum antioxidants were evaluated on days 7 and 14.
Findings Results showed that immobilization could significantly increase the serum concentrations of TNF-α, TGF-β corticosterone, MDA, and NO and also decrease SOD, GPx, and IL-10 in days 7 and 14, as immobilized rats were compared with control rats ($p<0.05$). However, administration of probiotics could significantly improve the above-mentioned parameters ($p<0.05$).
Conclusion The use of probiotics could alleviate the adverse effects of stress. It could be advised to use probiotics in the form of dairy and other supplements to overcome stress.

Keywords Antioxidant Status; Immobilization Stress; Inflammatory Responses

CITATION LINKS

[1] Effect of Bacopa monniera on stress-induced changes in plasma corticosterone ... [2] Effects of Salvadora persica extract on the hematological and biochemical alterations ... [3] Depression, anxiety-like behavior, and memory impairment are associated with increased oxidative stress and inflammation in ... [4] Stress-induced enhancement of leukocyte trafficking into sites ... [5] Exposure to a social stressor alters the structure of the intestinal microbiota ... [6] Free radicals, antioxidants, and human ... [7] Inducible nitric oxide synthase expression in the brain cortex after acute restraint stress is regulated by ... [8] Suppression of stress immobilization-induced phosphorylation of ERK 1/2 by biting in the rat ... [9] Single and repeated immobilization stress differentially triggers induction and phosphorylation of several transcription factors and mitogen-activated ... [10] Restraint stress-induced central monoaminergic and oxidative changes in rats and their prevention by ... [11] The microbiome in infectious ... [12] Effects of specific lactic acid bacteria on the intestinal permeability to ... [13] Lactobacillus rhamnosus GG attenuates interferon-{gamma} and tumor necrosis factor-a-induced ... [14] Anti-inflammatory effects of the genus Bifidobacterium on macrophages by modification of phosphor ... [15] Probiotic attributes, antioxidant, anti-inflammatory and neuromodulatory effects of Enterococcus faecium CFR ... [16] Effects of Salvia sclarea on chronic immobilization stress ... [17] Immunoglobulin E levels in ... [18] Preventive effect of safranal against oxidative ... [19] Comparison of growth performance and immune responses of broiler chicks reared under heat ... [20] Heat stress impairs performance and induces intestinal inflammation in broiler ... [21] Oxidative stress induced by corticosterone administration in broiler chickens (Gallus ... [22] Mycobacterium mucogenicum infection ... [23] Induction of oxidative stress by restraint stress ... [24] Enhance the antioxidant capacity ofsoymilk ... [25] Effect of fermentation of pomegranate juice by lactobacillus plantarum and lactobacillus acidophilus on the antioxidant activity and metabolism ... [26] Functional Properties of Microorganisms ... [27] Heat stress decreases expression of the cytokines, avian β-defensins 4 and 6, and Toll-like receptor 2 in broiler ... [28] Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory ... [29] Lactobacillus fermentumBR11 and fructooligosaccharide partially reduce jejunal inflammation in ... [30] Hemeoxygenase-1 signals are involved in preferential inhibiting pro-inflammatory cytokine release by surfactin in cells ... [31] The development of probiotics for controlling multiple bacterial diseases of rainbow ...

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Introduction

Stress is known as a factor that causes negative effects on general physiology and leads to psychology [1] and heart and causes diabetes and loss of weight [2]. It also causes faulted working memory, anxiety, and depression [3]. Studies have accepted the role of stress in suppression of immunity [4] and intestinal microflora [5]. It also causes the production of free radicals and oxidative damage to macromolecules [6]. Stress also increases the production of pro-inflammatory cytokines such as transcription factor NF-κB-mediated pathways and other inflammatory mediators [7].

In addition, stress is related to increased oxidative stress that stimulates phosphorylation of mitogen-activated protein kinases in animal models [8, 9]. Immobilization is a type of physical stress that limits mobilization and increases aggression in the animal model [2]. It is also used to study stress-induced changes [10].

Experimental studies that have shown probiotics' possible effects on the host's psychological status are rare. The gastrointestinal tract is an active metabolic organ containing various microbial species. It has been accepted that decreased healthy microflora provides an opportunity for pathogenic bacteria and increases inflammation [11]. It is shown that specific probiotic strains reduce the production of pro-inflammatory cytokines by decreasing the integrity of the tight junctional complexes between epithelial cells [12, 13].

Probiotic lactobacilli and bifidobacteria's profitable role in inflammatory conditions has been reported [14]. A study has also shown that probiotics significantly decrease the basal levels of some oxidative stress markers and increase the power of antioxidant enzymes, showing the antioxidant properties of probiotics [15]. Immobilization is the type of stress that influences inflammation and oxidative stress. On the other hand, probiotics can positively affect oxidative stress and inflammation. So far, no study has been conducted to evaluate the effects of probiotics on immobility stress. This study was thus conducted to evaluate the effects of probiotics in immobilized rats through the involvement of antioxidant and anti-inflammatory systems.

Materials and Methods

Materials

Commercial kits of interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), nitric oxide (NO), corticosterone, transforming growth factor-beta (TGF-β), malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were purchased from Sigma Aldrich Company. Commercial strains of Lactobacillus plantarum and Bifidobacterium B94 were selected to test.

Animals

72 Albino Wistar rats (6 weeks-age, 170±10g) were adapted for one week before trial and kept based on the animal welfare laws. All the animals were maintained at an optimal temperature (25±1°C), and humidity (55±5%) and illumination period (12 h light and 12 h dark) were kept in the experimental period. Animals received ad libitum water and feed. Animals were exposed to stress to induce immobilization stress, as reported by others [16]. Animals were exposed to immobilization for 2h/d and two weeks by a restraining chamber [17]. On days 7 and 14 of the trial, animals were anesthetized, blood samples were collected, and sera were collected.

Groups

Animals were divided into three groups (n=24), each divided into four sub-groups. Groups were included: 1) Normal control group without stress (Control), 2) Immobilized rats without additive (Immobilized), 3) Immobilized rats given 104 CFU probiotics.

Inflammatory cytokines and antioxidant status

Inflammatory and pro-inflammatory cytokines, including IL-10, TNF-α, TGF-β, NO, and corticosterone, were measured on days 7 and 14. Commercial kits evaluated the MDA, SOD, and GPx concentrations in serum according to the manufacturers' instructions on days 7 and 14.

Statistical Analysis

The obtained data were analyzed using Graph Pad Prism 7.0 (GraphPad Software, Inc., 7825 Fay Avenue, Suite 230, La Jolla, CA, USA) and reported as mean±standard deviation. Groups were compared by the Tukey test.

Findings

Corticostrone, MDA and NO concentrations

Immobilization could significantly increase corticosterone, MDA, and NO serum concentrations on days 7 and 14, as immobilized rats were compared with control rats ($p<0.05$). However, the administration of probiotics could significantly decrease corticosterone, MDA, and NO ($p<0.05$; Table 1).

Table 1. Effects of experimental treatments on serum concentrations of corticosterone, MDA, and NO in immobilized rats

Parameter	Control	Immobilized	Probiotic
Day 7			
Corticostrone (ng/mL)	32.10±1.10^{c}	62.31±1.20^{a}	42.10±3.12^{b}
MDA (μmol/L)	41.2±2.10^{c}	73.21±2.10^{a}	62.10±3.12^{b}
NO (μmol/L)	21.30±1.12^{c}	52.10±3.10^{a}	41.2±2.10^{b}
Day 14			
Corticostrone (ng/mL)	32.40±3.10^{c}	96.81±2.10^{a}	63.51±3.12^{b}
MDA (μmol/L)	41.70±1.13^{c}	84.35±3.35^{a}	63.10±2.12^{b}
NO (μmol/L)	21.15±2.32^{c}	65.21±3.42^{a}	45.1±3.20^{b}

Superscripts (a-c) show significant differences among groups in the same row.

Inflammatory factors

Our findings for inflammatory responses are presented in Figure 1. Results showed that exposure

to immobilization increased levels of TNF-α and TGF-β and decreased levels of IL-10 in days 7 and 14 ($p<0.05$). Treatment with probiotics could significantly decrease TNF-α and TGF-β and increase levels of IL-10 in days 7 and 14 ($p<0.05$).

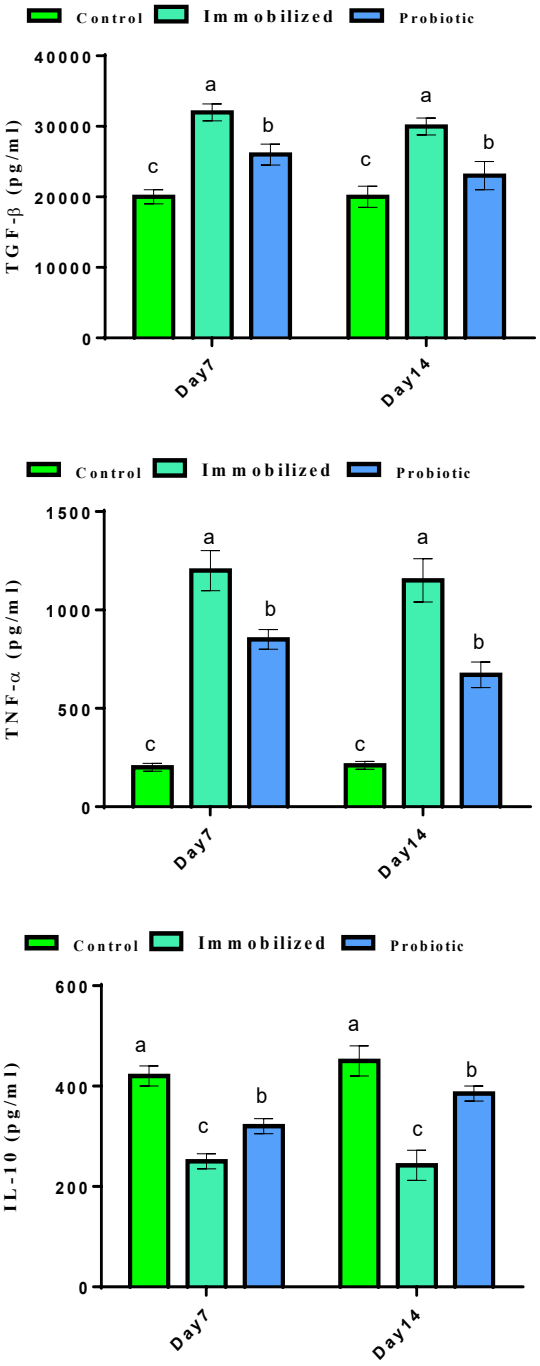

Figure 1. Effects of experimental treatments on inflammatory responses in immobilized rats. Superscripts (a-c) show significant differences among groups on the same day.

Antioxidant enzymes

Our findings for antioxidant status are shown in Figure 2. Results showed that the serum concentrations of SOD and GPx were decreased in immobilized rats, but treatment with probiotics could significantly improve antioxidant status in days 7 and 14 ($p<0.05$).

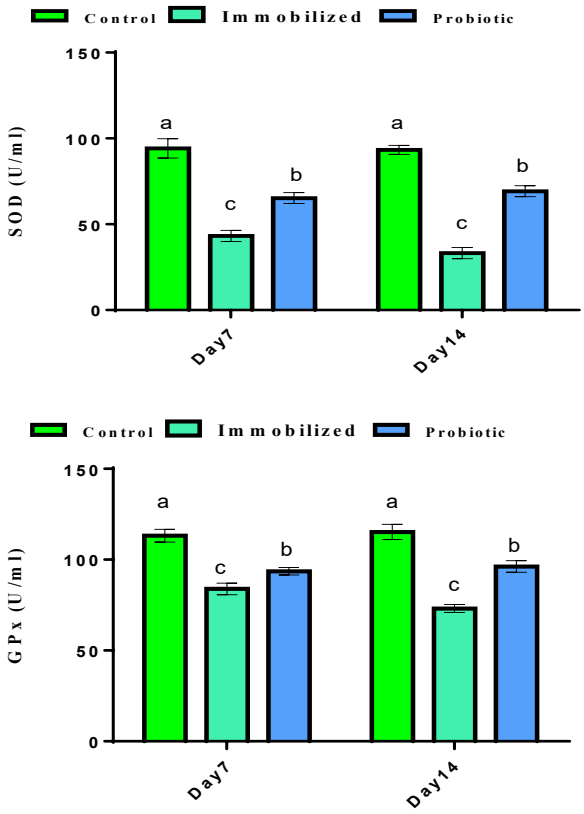

Figure 2. Effects of experimental treatments on antioxidant status in immobilized rats. Superscripts (a-c) show significant differences among groups on the same day.

Discussion

Immobility stress increased levels of MDA, NO, and corticosterone. It has been accepted that stress increases the production of reactive oxygen species by biological system ability [18] and increases MDA production as a marker for lipid peroxidation. MDA shows increased oxidative damage and reduced antioxidants. It has been reported that there is a relationship between stress, increased corticosterone, and intestinal injuries [19]. Stress changes the neuroendocrine system by activating the hypothalamic-pituitary-adrenal axis and increasing the production of corticosterone [20]. Stress increases corticostrone production and changes structural proteins [21]. It has been reported that MDA and NO are inflammatory biomarkers [22]. On the other hand, oxidative stress plays a major role in the pathogenesis of immobilized stress [23]. Improved MDA and NO were observed in probiotic groups. Improved MDA and NO could be attributed to antioxidant and anti-inflammatory responses, which will be discussed later. It has been reported that probiotic strains of *L. rhamnosus* [24], *Enterococcus faecium* [15], and *L. acidophilus* [25] improve antioxidant capacity, which could be

attributed to microbial metabolic activity during fermentation [26]. Increased inflammatory responses were observed in rats exposed to stress. It has been reported that stress increases corticostrone, and increased corticostrone decreases mRNA expression of anti-inflammatory cytokines, i.e., IL-10 [27], as observed in the current study. IL-10 is known as one of the most important anti-inflammatory cytokines, which prevent the production of pro-inflammatory cytokines such as TNF-α [28]. IL-10 has been reported to have anti-inflammatory properties that are formed by both T-cells and monocytes/macrophages. The TNF-α has been known as an inflammatory cytokine that initiates inflammation. It means that immobilization stress increases inflammation, but probiotics decrease inflammation. Stress provides an opportunity for pathogens that increase inflammation. Previous studies have reported that *L. fermentum* could significantly decrease jejunal inflammation of the upper small intestine after 5-FU administration in rats [29]. Improved anti-inflammatory properties could be attributed to reduced pro-inflammatory cytokines, increased immature leukocyte production, and interferon production [30, 31]. Probiotics also decrease pathogen-induced inflammation that is caused to improve the intestinal ecosystem. It could be stated that probiotics improve inflammation conditions by the mentioned mechanisms. Stress also decreased levels of antioxidant enzymes. Stress stimulates and increases the metabolic rate, which causes high free radicals and oxidative damage [6]. Stress decreases antioxidant enzymes, but probiotics improve them. Antioxidant properties of probiotics have previously been reported [15], but the mechanism is unknown.

Conclusion

Similar to other stresses, immobilization increased inflammation and oxidation. Probiotics could improve inflammatory responses and have antioxidant properties. It could be recommended to use probiotics as a daily supplement in patients with stress-related conditions, such as depression.

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