

# Original Article: Efficiency of Probiotic in Immobilized Rats Through Involvement of the Antioxidant and Anti-Inflammatory Systems



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## ABSTRACT

**Background:** Immobilization is known as 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 through involvement of the antioxidant and anti-inflammatory systems.

**Materials and Methods:** A total number of 72 Albino Wistar rats were divided into 3 groups, including: 1. Normal control group without stress (Control); 2. Immobilized rats without additive (Immobilized); 3. Immobilized rats given with 104 CFU probiotic.day-1(Probiotic). Inflammatory and pro-inflammatory cytokines, corticosterone and serum antioxidants were evaluated in days 7 and 14.

**Results:** showed that immobilization could significantly increase the serum concentrations of TNF- $\alpha$ , TGF- $\beta$  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 probiotic could significantly improve the above mentioned parameters ( $P < 0.05$ ).

**Conclusion:** It could be concluded that the use of probiotics could alleviate adverse effects of stress. It could be advised to use of probiotics in form of dairy and other supplements to overcome on stress.

## Introduction

**S**tress is known as a factor that causes negative effects on general physiology and leads psychology [1], heart and causes diabetes

and loss weight [2]. It also causes faulted working memory, anxious and depression [3]. Studies have accepted the role of stress in suppression of immunity [4] and intestinal microflora [5]. It also causes to produce the free radicals, oxidative damages on macromolecules [6].

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Stress also increases production of pro-inflammatory cytokines such as transcription factor NF- $\kappa$ B-mediated pathways and other inflammatory mediators [7]. In addition, stress is related with increased oxidative stress that stimulates phosphorylation of mitogen-activated protein kinases in animal models [8, 9]. Immobilization is type of physical stress that limits mobilization and increases aggression in the animal model [2] and is also used to study the stress-induced changes [10].

Experimental studies that have shown the possible effects of probiotics in the psychological status of the host are rare. Gastrointestinal tract is known as metabolic active organ which contains various microbial species. It has been accepted that decreased healthy microflora provides opportunity for pathogenic bacteria and increases inflammation [11]. It is shown that specific probiotic strains reduce production of pro-inflammatory cytokine through decreasing integrity of the tight junctional complexes between epithelial cells [12, 13]. The profitable role of probiotic lactobacilli and bifido bacteria for inflammatory condition has been reported [14].

A study has also been shown that probiotics significantly decrease the basal levels of some oxidative stress markers and increase the powerful of antioxidant enzymes; showing antioxidant properties of probiotics [15]. Immobilization is type of stress that influences inflammation and oxidative stress. On the other hand, probiotics can have positive effects on 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 involvement of the antioxidant and anti-inflammatory systems.

## Materials and methods

### Materials

Commercial kits of Interlukin-10 (IL-10), Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), Nitric Oxide (NO), corticosterone, Transforming Growth Factor-beta (TGF- $\beta$ ), 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

A total number of 72 Albino Wistar rats (6 weeks-age, 170 $\pm$ 10 g) were adapted for one week before of trial and kept on basis the animal welfare laws. All the animals

were maintained in an optimal temperature (25 $\pm$ 1 $^{\circ}$ C), and humidity (55 $\pm$ 5%) and illumination period (12 h light and 12 h dark) were kept in the experimental period. Animals received ad libitum water and feed. To induce the immobilization stress, animals were exposed to stress as reported by others [16]. Animals were exposed to immobilization for 2 h/d and for 2 weeks by a restraining chamber [17]. In days 7 and 14 of conduction of trial, animals were anesthetized; blood samples were collected and sera were collected.

### Groups

Animals were divided into 3 groups (n=24) and each group was divided into 4 sub-groups. Groups were included: 1. Normal control group without stress (Control), 2. Immobilized rats without additive (Immobilized), 3. Immobilized rats given with 104 CFU probiotic. day-1 (Probiotic).

### Inflammatory cytokines and antioxidant status

Inflammatory and pro-inflammatory cytokines including IL-10, TNF- $\alpha$ , TGF- $\beta$  and also NO and corticosterone were measured in days 7 and 14. The MDA, SOD and GPx concentrations in serum were evaluated by commercial kits according to the manufacturers' instructions in days 7 and 14.

### Statistical analysis

The obtained data were analyzed by using Graph Pad Prism 7.0 (GraphPad Software, Inc., 7825 Fay Avenue, Suite 230, La Jolla, CA, USA) and reported as Mean $\pm$ SD. Groups were compared by Tukey test.

## Results

### Corticosterone, MDA and NO concentrations

Our results for serum concentrations of corticosterone, MDA and NO in immobilized rats are presented in Table 1. As results shows, immobilization could significantly increase the serum concentrations of corticosterone, MDA and NO in days 7 and 14, as immobilized rats were compared with control rats (P<0.05). However, administration of probiotic could significantly decrease the levels of corticosterone, MDA and NO (P<0.05).

### Inflammatory factors

Our findings for inflammatory responses are presented in Figure 1. Results showed that exposing to immobilization increased levels of TNF- $\alpha$  and TGF- $\beta$  and de-

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

Days	Parameters	Control	Immobilized	Probiotic	P*
7	Corticosterone (ng/mL)	32.10±1.10 <sup>c</sup>	62.31±1.20 <sup>a</sup>	42.10±3.12 <sup>b</sup>	*
	MDA (µmol/L)	41.2±2.10 <sup>c</sup>	73.21±2.10 <sup>a</sup>	62.10±3.12 <sup>b</sup>	*
	NO (µmol/L)	21.30±1.12 <sup>c</sup>	52.10±3.10 <sup>a</sup>	41.2±2.10 <sup>b</sup>	*
14	Corticosterone (ng/mL)	32.40±3.10 <sup>c</sup>	96.81±2.10 <sup>a</sup>	63.51±3.12 <sup>b</sup>	*
	MDA (µmol/L)	41.70±1.13 <sup>c</sup>	84.35±3.35 <sup>a</sup>	63.10±2.12 <sup>b</sup>	*
	NO (µmol/L)	21.15±2.32 <sup>c</sup>	65.21±3.42 <sup>a</sup>	45.1±3.20 <sup>b</sup>	*

Superscripts (a-c) show significant differences among groups in same row. \*: P<0.05.



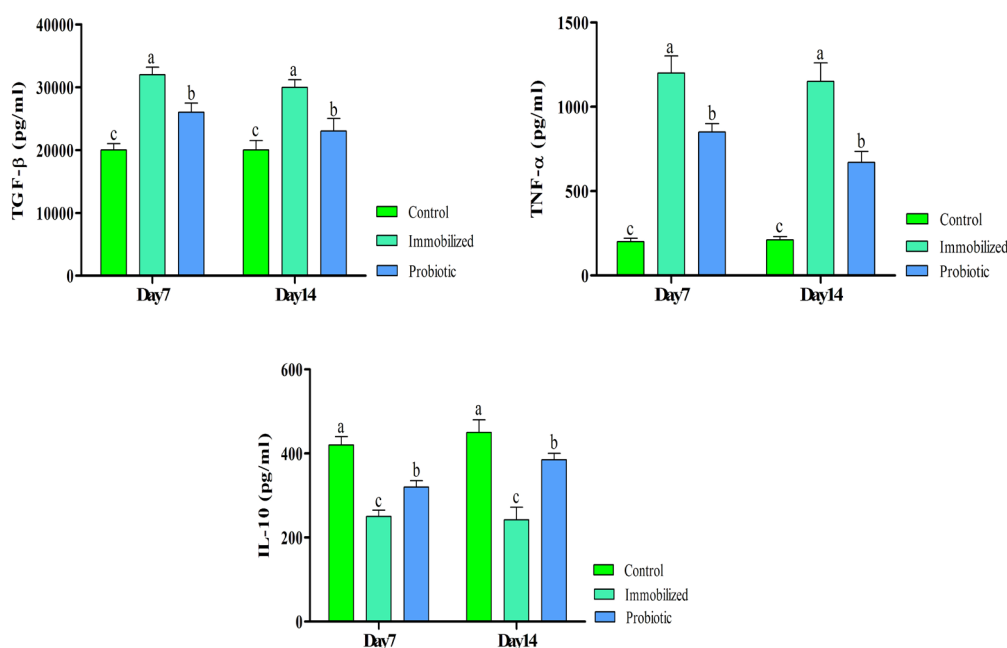
creased levels of IL-10 in days 7 and 14 (P<0.05). Treatment with probiotic could significantly decrease TNF-α and TGF-β and increase levels of IL-10 in days 7 and 14 (P<0.05).

**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 probiotic could significantly improve antioxidant status in days 7 and 14 (P<0.05).

**Discussion**

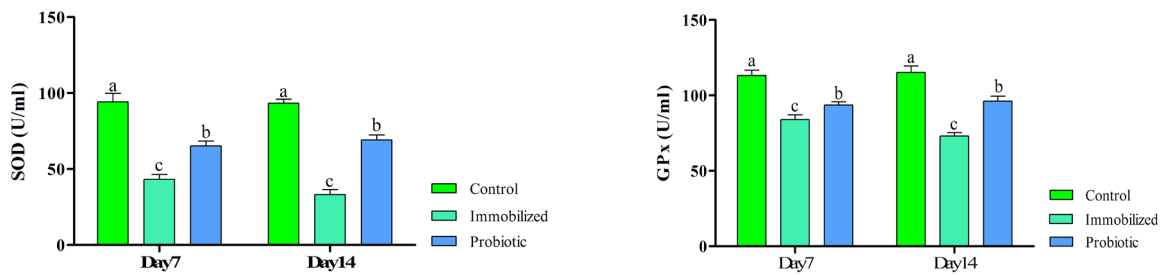
Immobility stress increased levels of MDA, NO and corticosterone. It has been accepted that stress increases production of reactive oxygen species by biological system ability and increases MDA production as marker for lipid per-oxidation [18]. MDA shows increased oxidative damage and reduced antioxidants. It has been reported relation between stress, increased corticosterone and intestinal injuries [19]. Stress changes neuroendocrine system through activation of the hypothalamic-pituitary-adrenal axis and increases production of corticosterone [20]. Stress increases corticosterone production and changes structural proteins [21]. It has been reported



**Figure 1** Effects of experimental treatments on inflammatory responses in immobilized rats

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





**Figure 2.** Effects of experimental treatments on antioxidant status in immobilized rats

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

that MDA and NO as inflammatory biomarkers [22]. On the other hand, oxidative stress plays major role in 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 that would be discussed. It has been reported that probiotic strains of *L. rhamnosus* [24], *Enterococcus faecium* [25] and *L. acidophilus* [26] improve antioxidant capacity which could be attributed to microbial metabolic activity during fermentation [27].

Increased inflammatory responses were observed in rats exposed to stress. It has been reported that stress increases corticosterone and increased corticosterone decreases mRNA expression of anti-inflammatory cytokines, i.e. IL-10 [28], as observed in the current study. IL-10 is known as one of the most important anti-inflammatory cytokines which prevents production of pro-inflammatory cytokines such as TNF- $\alpha$  [29]. IL-10 has been reported to have anti-inflammatory properties that is formed by both T-cells and monocytes/macrophages. The TNF- $\alpha$  has been known as an inflammatory cytokine that initiates inflammation. It means that immobilization stress increases inflammation but probiotic decreased inflammation.

Stress provides opportunity for pathogens that increases inflammation. Previous studies have reported that *L. fermentum* could significantly decrease jejunal inflammation of the upper small intestine after 5-FU administration in rats [30]. Improved anti-inflammatory properties could be attributed to reduced pro-inflammatory cytokines, increased immature leukocyte production and interferon production [31, 32]. Probiotics also decrease pathogen-induced inflammation that is caused to improve the intestinal ecosystem. It could be stated that probiotics improve inflammation condition by mentioned mechanisms. Stress also decreased levels of antioxidant enzymes. Stress stimulates and increases

metabolic rate that caused to produce high free radicals and also oxidative damage [6]. Stress decreases antioxidant enzymes but probiotic improved them. Antioxidant properties of probiotics have previously been reported [15] but mechanism is unknown.

## Conclusion

In summary, the immobilization similar to other stresses increased inflammation and oxidation. Probiotic could improve inflammatory responses and antioxidant properties. It could be recommended to use the probiotics as daily supplement in patients related with stress such as depression and similar conditions.

## Ethical Considerations

### Compliance with ethical guidelines

Approval for this study was obtained from Hormozgan University of Medical Sciences Research Committee (HUMS-2017-122539).

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### Authors' contributions

All authors contributed in data analysis, drafting and revising the paper and agreed to be responsible for the aspects of this work.

### Conflict of interest

The authors declared no conflict of interest.

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