ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

The Multistrain Probiotic Enhances the Healing Process of Stress-Induced Lesions of the Gastric Mucosa of Rats.

Oleksandr V Virchenko[1]*, Tetyana M Falalyeyeva[1], Tetyana V Beregova[1], and Savytska Ya Maryana[2].

[1]Taras Shevchenko National University of Kyiv, Kyiv, Ukraine.
[2]Danylo Halytskyi Lviv National Medical University, Lviv, Ukraine.

ABSTRACT

It is well known the beneficial effects of probiotics for maintenance of homeostasis of gut but their role in stomach is not well elucidated. Several works suggest antiulcer activity of probiotics. In this study the effects of multistrain probiotic (MP) on the healing of stress-induced gastric lesions of rats and the mechanisms of its action were investigated. The rats were subjected to 3-hour water immersion restraint stress (WIRS) and then treated with aqueous solution of MP (*Lactobacillus* + *Lactococcus (*6×10^{10} CFU/g), *Bifidobacterium* (1×10^{10}/g), *Propionibacterium* (3×10^{10}/g), *Acetobacter* (1×10^{6}/g)) at a dose of 140 mg/kg (1.4×10^{10} CFU/kg) orally twice a day for 4 subsequent days. The influence of MP on stress-induced lesions in gastric mucosa (GM), serum levels of adrenocorticotropic hormone (ACTH), corticocterone, proinflammatory (interleukin (IL) 1β and IL-12B p40) and antiinflammatory (IL-4 and IL-10) cytokines was investigated.The treatment with MP significantly accelerated the healing rate in gastric mucosa of rats. MP enhanced recovery of the ACTH and corticosterone concentrations to the values of intact rats and decreased the proinflammatory cytokines content in the rat serum after WIRS. These data suggest that one of the mechanisms of the therapeutic effect of MP on lesions in the GM caused by stress is the impact on the stress hormones and cytokine profile.

Keywords: stress, gastric lesions, multistrain probiotic, cytokines.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

A lot of studies have shown the benefits of probiotics in therapy of the gastroenterological diseases [1]. It is obtained the promising results regarding treatment of inflammatory bowel disease with probiotics [2, 3]. As for upper gastrointestinal tract it was established effectiveness of probiotics in *Helicobacter pylori* (*H. pylori*) eradication. It was found to probiotic bacteria, especially the *Lactobacillus* genus, inhibit urease activity, growth of bacteria *H. pylori* and reduce inflammation in the gastric mucosa caused by *H. pylori* [4-8]. Others point out that the introduction of probiotics does not lead to the eradication of *H. pylori* but together with antibiotics and proton pump inhibitors probiotics realize stronger therapeutic effect [9, 10]. However, some studies didn't reveal any positive effect of probiotics on the eradication rate [11]. So, the scientific issue about the probiotic use in *H. pylori*-infection treatment is not completely solved.

Furthemore, except impact on the *H. pylori* infection pobiotics were shown to reduce the lesions of the gastric mucosa (GM) in some studies. Lam EK, et al. showed that treatment with Lactobacillus rhamnosus GG enhanced 60% acetic acid-induced gastric ulcer healing via the attenuation of cell apoptosis to cell proliferation ratio and increase in angiogenesis in the gastric mucosa [12]. Similar results were obtained by Uchida M. et al. (2010) using yogurt containing Lactobacillus gasseri OLL 2716 [13] and by Dharmani P. et al. using the probiotic mixture VSL#3 [14]. Senol A. et al found that pretreatment with the probiotic mixture, including 13 different bacteria, attenuates the aspirin-induced gastric lesions [15]. Konturek P.C. et al. established that pretreatment with Escherichia coli Nissle protects GM against water restraint stress erosions due to antiinflammatory and vasodilatory actions involving HSP70, prostaglandins and sensory afferent neurons [16]. Our team previously reported the preventive effect of Bifidobacterium animalis VKL and VKB on the stress-induced ulceration [17]. So, the question about the probiotic usage in the management of ulcer is important and under the attention of scientists. However, most of the studies are concerned to the estimation of the ulcer prophylaxis with probiotics. Effects of therapeutic introduction of probiotics were investigated only on the model of acetic-acid induced ulcer. Thus, more researches are needed to evaluate the influence of probiotics on the treatment of ulcers induced by various factors (stress, ethanol, nonsteroidal antiinflammatory drugs, etc.). In current work we investigate the effects of multistrain probiotic (MP) "Symbiter® acidophilic concentrated" on the stress-induced gastric lesions healing in rats. To reveal the mechanisms of such influence it was determined the effects of MP on the content of stress hormones (corticosterone and adrenocorticotropic hormone(ACTH)) and the content of proinflammatory (interleukin (IL) 1β and IL-12B p40) and antiinflammatory (IL-4 and IL-10) cytokines in rat serum under stress conditions.

MATERIALS AND METHODS

Reagents

Rat ACTH ELISA Kit were obtained by Cusabio (Wuhan, China), corticosterone Mouse/Rat ELISA and rat IL-1 β ELISA kits were obtained from Biovendor (Czech Republic). A mouse monoclonal IgG_1 IL-4 Antibody (OX81), a mouse monoclonal IgG IL-10 Antibody (2G101H7) and rabbit polyclonal IgG IL-12B p40 Antibody (H-306) were supplied by Santa Cruz Biotechnology (Santa Cruz, CA, United States). Polyclonal Anti-Mouse IgG (whole molecule) – peroxidase conjugate antibody and polyclonal Anti-rabbit IgG (whole molecule) – peroxidase conjugate antibody were purchased from Sigma-Aldrich (St.Louis, United States).

Multicomponent probiotic (MP) used in this study was supplied by Scientific and Production Company "O.D. Prolisok". It contains of 14 probiotic strains of *Lactobacillus* + *Lactococcus* (6×10^{10} CFU/g), *Bifidobacterium* (1×10^{10}/g), *Propionibacterium* (3×10^{10}/g), *Acetobacter* (1×10^{6}/g) genera.

Animals

The study was carried on 100 male albino Wistar rats weighing 200-250 g in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the general ethical principles of animal experiments, approved by the First National Congress on Bioethics Ukraine (September 2001). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Taras Shevchenko National University of Kyiv (Protocol number: 19/2013). The rats were kept in collective cages in controlled conditions of temperature (22 ± 3°C), light (12h light/dark cycle) and relative humidity (60 ± 5%). The animals were fed laboratory chow and tap water *ad libitum*.

ISSN: 0975-8585

Stress procedures and animal grouping

Animals were divided into 10 groups of 10 rats each (Table 1). For 24 h before the onset of stress, animals were housed in cages that had wire-net bottoms to avoid coprophagy and had free access to tap water. Rats were subjected to 3-hour water-immersion restraint stress (WIRS) by Takagi et al., 1964 [18]. One day prior to the experiment, the rats were not fed, but they had free access to water. For immobilization rats were placed in a perforated cylindrical metal camera that was put down vertically into the water for 3 hours so that the water level reaches the jugular fossa of animals. Water temperature was set to 22-23° C.

After the stress animals except intact and stress-control were treated with water or aqueous solution of multiprobiotic in a volume of 0.5 ml/200 g orally twice a day. MP was administered at a dose of 140 mg/kg (1.4×10^{10} CFU/kg). Treatment was started in an hour after WIRS.

Evaluation of gastric mucosal lesions

After the stress procedures, animals were released from the camera and were decapitated. The stomachs were then harvested and opened along the lesser curvature. The severity of mucosal lesions was grossly inspected and digitally photographed. The length and width of each lesion, including epithelial cell damage, glandular disruption, vasocongestion, hemorrhage and deep necrosis, were measured by stereoscopy and the total area of the lesions in one stomach was assessed by planimetry [13]. The ulcer estimation was performed by protocol-blind researcher.

After that gastric tissues were fixed in 10% formalin, dehydrated and imbedded in paraffin wax. Paraffin sections of 5 µm were cut and stained with hematoxylin and eosin. Histological changes were checked under a microscope **XS-4130 MICROmed**.

Table 1: The division of rats into the research groups

Group name	Treatment	**Duration (hours) between stress exposure and rats examination**
Intact	Rats weren't exposed to stress and treated	-
Stress-control	Rats were exposed to stress but not treated	0
Water 24h	Rats were exposed to stress and treated with water (two-time introduction)	24
Probiotic 24h	Rats were exposed to stress and treated with probiotic (two-time introduction)	24
Water 48h	Rats were exposed to stress and treated with water (four-time introduction)	48
Probiotic 48h	Rats were exposed to stress and treated with probiotic (four-time introduction)	48
Water 72h	Rats were exposed to stress and treated with water (six-time introduction)	72
Probiotic 72h	Rats were exposed to stress and treated with probiotic (six-time introduction)	72
Water 96h	Rats were exposed to stress and treated with water (eight-time introduction)	96
Probiotic 96h	Rats were exposed to stress and treated with probiotic (eight-time introduction)	96

Measurement of ACTH and corticosterone level

After sacrifice rats blood was collected from the heart into centrifuge tubes without anticoagulant and leaved for 20-30 minutes at room temperature to complete the formation of a clot. Then, blood samples were centrifuged at 1000 g for 15 minutes and the supernatant (serum) were harvested in separate disposable microtubes, frozen at -20° C and used for further studies.

ISSN: 0975-8585

Serum ACTH and corticosterone content were determined at the 1st-3rd days after WIRS by ELISA. The contents of ACTH and corticosterone were expressed in pg/mL and nmol/L accordingly.

Measurement of interleukines level

Serum IL-1β, IL-12B p40, IL-4 and IL-10 were measured at the 1st-4th days after WIRS by ELISA with kits or using antibodies. The level of IL-1β was expressed in pg/mL and others – in units of optical density (absorbance units, a.u.). All samples were analyzed in two repetitions.

Statistical analysis

Statistical analysis of data was carried out by the "Statistica 8.0" software package. For multiple comparisons factorial ANOVA analysis was used. For the investigation of the data distribution type Shapiro-Wilk's W criterion was used. *Post-hoc* analysis included Mann – Whitney U-test for nonparametric data and Student's t-test for parametric data. Mean of value (M) and standard error of the mean (m) were calculated for parametric data; median (Me) and quartiles were calculated for nonparametric data. Significant difference was considered at $P \leq 0.05$ for ANOVA and for *post-hoc* tests it was more rough because of multiple comparisons and set as $P \leq 0.01$.

RESULTS

Effect of multiprobiotic on water immersion restraint stress-induced gastric mucosal lesions

It was shown that 3-hour WIRS caused a lot of erosive-ulcerative lesions in GM of rats. The total size of ulcers was 6.3±1.1 mm^2, with number of 8.3±1.9 per one stomach. The length of erosions was 2.9±0.7 mm, their amount was equal to 1.3±0.3. Factorial ANOVA showed the gradual decrease of ulcer square for four days after WIRS. For example, the total square of ulcers at 4th day was lower by 70% ($P<0.01$) compared to stress-control (Figure 1). The number of ulcers significantly reduced by 82% ($P<0.01$) compared to stress-control just at the 1st day after stress and didn't changed for the next three days (Figure 2). That suggested the increase of the mean size of each separate ulcer at the 1st day after WIRS.

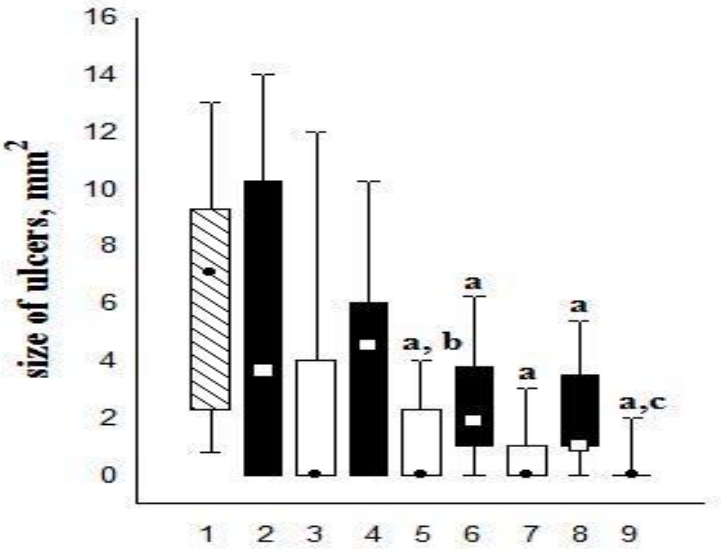

Figure 1: Effects of multiprobiotic on the ulcer area in the gastric mucosa of rats induced by water-immersion restraint stress. 1 – stress-control, 2, 4, 6, 8 – water 24h, 48h, 72h, 96h accordingly, 3, 5, 7, 9 – probiotic 24h, 48h, 72h, 96h accordingly (10 rats in each group). Data are represented as median, box is 25-75%, and whiskers are min-max. [a]P<0.01 *vs* stress-control group, [b]P<0.05 *vs* water 48h group, [c]P<0.05 *vs* water 96h group.

ISSN: 0975-8585

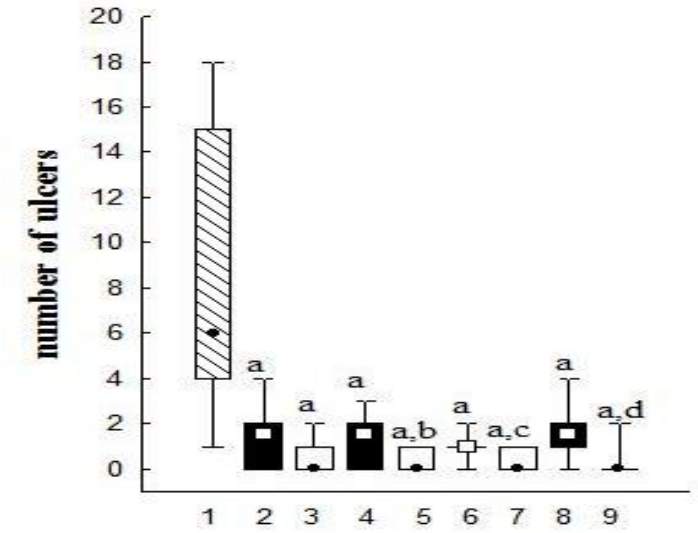

Figure 2 Effects of multiprobiotic on the number of ulcers in the gastric mucosa of rats induced by water-immersion restraint stress. 1 – stress-control, 2, 4, 6, 8 – water 24h, 48h, 72h, 96h accordingly, 3, 5, 7, 9 – probiotic 24h, 48h, 72h, 96h accordingly (10 rats in each group). Data are represented as as median, box is 25-75%, and whiskers are min-max. [a]P<0.01 *vs* stress-control group, [b]P<0.01 *vs* water 48 h group, [c]P<0.01 *vs* water 72h group, [d]P<0.01 *vs* water 96h group.

Meanwhile the length and number of erosions enlarged by 3.74 times (P<0.01) and 2.15 times (P<0.01) correspondingly at the 1st day after stress (Figure 3, 4). At the following days the size of erosions didn't changed but their amount continued to rise (Figure 3, 4). Therefore, it was found that healing of ulcers was associated with appearance of new erosions. The obtained results demonstrated healing process in GM after stress exposure. The original photos of the stomach under transillumination and parallel microphotograph are given (Figure 5). Immediately after WIRS there were deep ulcers with haemorrhages in the GM (Figure 5B). It was registered necrotic tissue at the bottom of ulcer and infiltration of polymorphonuclear inflammatory cells by histological procedures (Figure 5F). In the subsequent four days the haemorrhages were not found (Figure 5C). The histological analysis detected mainly superficial lesions of the GM (Figure 5G).

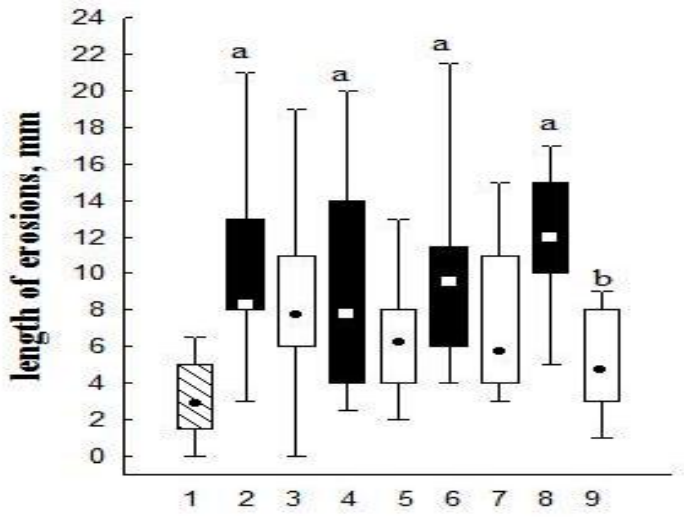

Figure 3 Effects of multiprobiotic on the size of erosions in the gastric mucosa of rats induced by water-immersion restraint stress. 1 – stress-control, 2, 4, 6, 8 – water 24h, 48h, 72h, 96h accordingly, 3, 5, 7, 9 – probiotic 24h, 48h, 72h, 96h accordingly (10 rats in each group). Data are represented as median, box is 25-75%, and whiskers are min-max. [a]P<0.01 *vs* stress-control group, [b]P<0.01 *vs* water 96h group.

ISSN: 0975-8585

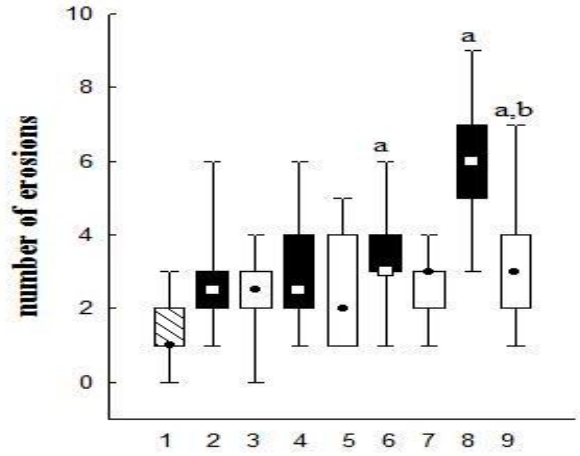

Figure 4: Effects of multiprobiotic on the number of erosions in the gastric mucosa of rats induced by water-immersion restraint stress. 1 – stress-control, 2, 4, 6, 8 – water 24h, 48h, 72h, 96h accordingly, 3, 5, 7, 9 – probiotic 24h, 48h, 72h, 96h accordingly (10 rats in each group). Data are represented as median, box is 25-75%, and whiskers are min-max. [a]P<0.01 *vs* stress-control group, [b]P<0.01 *vs* water 96h group.

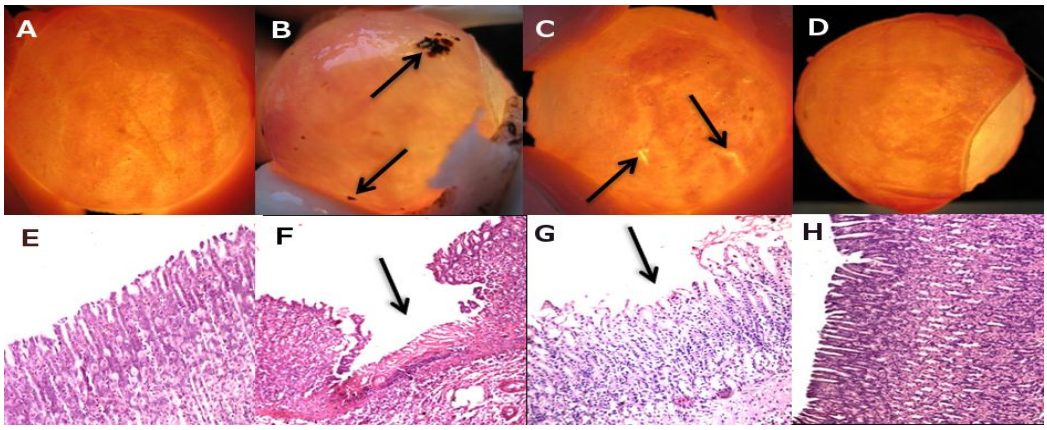

Figure 5: Images of the transilluminated stomachs and light microscopic micrographs of the gastric mucosa stained with haematoxylin and eosin. A, E – intact group; B, F – stress-control; C, G – water 96h group; D, H – probiotic 96h group.

The treatment with MP significantly accelerated the healing rate in the GM of rats. At the 2nd day after WIRS MP reduced the size of ulcers by 77.8% (P<0.01) (Figure 1). The amount of ulcers was decreased by 71.4% (P<0.01) by MP at the 2st day (Figure 2). Such tendency was observed in all experimental days. It was also established the influence of MP on the erosions healing. However significant difference was revealed only on the 4th day after WIRS (Figure 3, 4). A most of microphotographs were similar to those of intact control group (Figure 5 D, H). The GM of rats treated with MP had fewer lesions and most of them were superficial. The inflammation in GM was also less expressed.

Effect of multiprobiotic on water immersion restraint stress-induced changes of adrenocorticotropic hormone and corticosterone level

It was found that the concentration of ACTH in the serum of intact rats was 23 ± 9,7 pg/ml, and the concentration of corticosterone – 27 ± 8,3 nmol/l (Figure 5, 6). As a result of stress corticosterone level increased by 2.2 times (p<0.01), while the concentration of ACTH decreased by 7.9 times (p<0.01), that confirmed the negative feedback between the level of corticosterone in the blood and the level of secretion of ACTH by a pituitary gland (Figure 5, 6) [19].

After 24 hours from the WIRS concentration of corticosterone in the blood serum of rats decreased by 5.7 times (P<0.01) compared with intact controls, which may indicate adrenal depletion under the influence of

stress factors (Figure 5). The ACTH concentration after 24 hours was reduced by 3.2-fold (P<0.01) compared with intact rats, which may indicate depletion of the pituitary gland. However, the concentration of ACTH was 2.5 times (P<0.01) higher compared with measured immediately after WIRS (Figure 5). For the next 2 days after WIRS it was established a gradual recovery of the level of ACTH and corticosterone to the intact control values. So, after 48 hours from the WIRS concentration of ACTH was 1.9 times (P<0.01) lower, and corticosterone – 3.4 times (P<0.01) lower compared with intact controls. Within 72 hours after the WIRS ACTH concentration in serum of rats treated with water did not differ significantly from that of intact rats, but the corticosterone concentration was lower by 1.6-fold (P<0.01) the level of the intact control (Figure 5, 6). So, for three days after the stress ACTH and corticosterone levels were reduced compared with intact rats, indicating the depletion of the endocrine glands, and gradually restored to normal levels.

MP enhanced recovery of the functioning of the hypothalamo-pituitary adrenal (HPA) system under stress conditions, that was confirmed by a more rapid return of ACTH and corticosterone concentrations to the values of intact rats (Figure 5, 6). The level of ACTH under the treatment of MP on the 1st day after the WIRS did not differ from the intact control and the corticosterone concentration was restored to the level of intact animals in 3 days after stress exposure. Thus, the effect of MP on the content of stress hormones is one of the mechanisms of its gastroprotective effect. Indeed, we have found significant erosive and ulcerative lesions of the GM on the 1st-3rd days after WIRS despite of small level of ACTH and corticosterone in the blood of rats. Therapy with MP facilitated the restoration of basal levels of ACTH and corticosteroids, which correlated with acceleration of the stress-induced lesions healing in the GM.

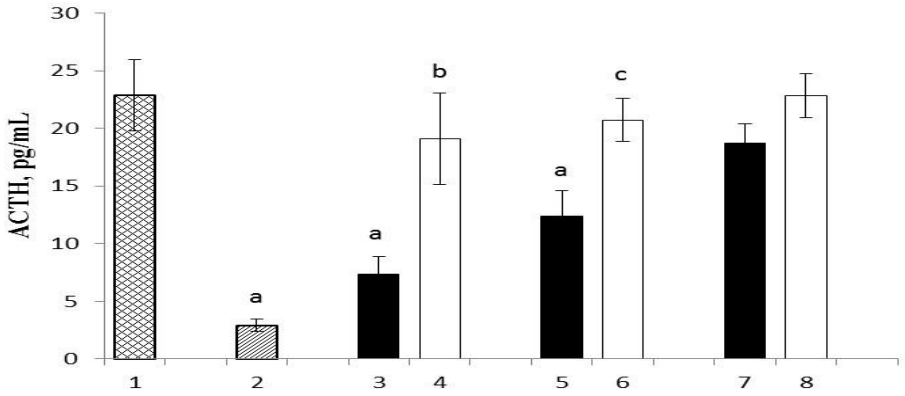

Figure 6: Effects of multiprobiotic on the adrenocorticotropic hormone level in serum of rats under water-immersion restraint stress. 1 – intact group, 2 – stress-control, 3, 5, 7 – water 24h, 48h, 72h accordingly, 4, 6, 8 – probiotic 24h, 48h, 72h accordingly (7 rats in each group). Data are represented as mean±SE. [a]P<0.01 *vs* intact group, [b]P<0.05 *vs* water 24h group, [c]P<0.05 *vs* water 48h group.

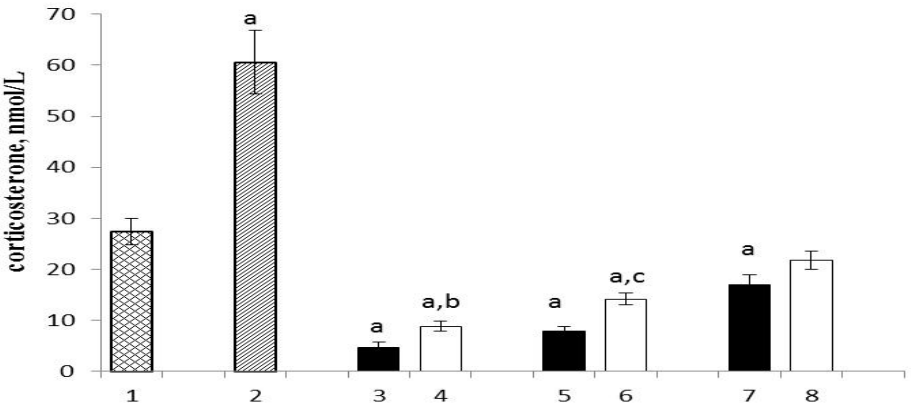

Figure 7: Effects of multiprobiotic on the corticosterone level in serum of rats under water-immersion restraint stress. 1 – intact group, 2 – stress-control, 3, 5, 7 – water 24h, 48h, 72h accordingly, 4, 6, 8 – probiotic 24h, 48h, 72h accordingly (7 rats in each group). Data are represented as mean±SE. [a]P<0.01 *vs* intact group, [b]P<0.05 *vs* water 24h group, [c]P<0.05 *vs* water 48h group.

ISSN: 0975-8585

Effect of multiprobiotic on water immersion restraint stress-induced changes of serum interleukins level

ELISA analysis was performed to determine the immunomodulatory properties of MP under conditions of stress. Although, the level of IL-1β didn't significantly exceed intact control values immediately after stress, but it was revealed the difference of IL-1β level between water 48h group and stress-control group (563±51 pg/ml *vs* 403±68 pg/ml, P<0.01) that suggested more intensive release of that cytokine at the 2nd day after WIRS. Contrary the level of IL-12B p40 rised by 155% (P<0.01) immediately after stress and then gradually restored to the control values in the subsequent days (Table 2). Thus, it was found that after the subjecting to the concentration of proinflammatory cytokines IL-1β and IL-12B p40 significantly increased and remained higher compared to intact animals within 4 days after WIRS stress in serum of rats which were treated with water (Table 2).

Table 2: The level of proinflammatory and antiinflammatory interleukins in rats serum under water immersion restraint stress and therapeutic administration of multiprobiotic

Group name	Interleukin 1β, pg/ml	Interleukin 12Bp40, a.u.	Interleukin 4, a.u.	Interleukin 10, a.u.
Intact	314±71	0.105±0.015	0.076±0.009	0.199±0.018
Stress-control	403±68	**0.268±0.025**[a]	0.076±0.007	0.222±0.015
Water 24h	**449±66**[a]	**0.394±0.056**[a]	**0.172±0.040**[a]	0.235±0.008
Probiotic 24h	414±57	**0.311±0.034**[a]	**0.270±0.054**[a,b]	**0.374±0.063**[a,b]
Water 48h	**563±51**[a1,a2,d]	**0.233±0.035**[a]	**0.141±0.023**[a]	0.145±0.035
Probiotic 48h	409±61[c]	**0.179±0.032**[a,c]	0.084±0.020[c]	0.171±0.013
Water 72h	**511±66**[a]	**0.293±0.016**[a]	0.116±0.021	0.211±0.025
Probiotic 72h	319±53[d]	**0.258±0.030**[a]	0.087±0.023	0.190±0.016
Water 96h	**471±54**[a]	0.137±0.023	**0.122±0.017**[a]	0.214±0.020
Probiotic 96h	334±41[e]	0.082±0.006[e]	0.072±0.007[e]	0.174±0.012

Data are represented as mean±SE. n=7 in each group. [a1]P<0.01 *vs* intact group, [a2]P<0.01 *vs* stress-control group, [b]P<0.01 *vs* water 24h group, [c]P<0.01 *vs* water 48h group, [d]P<0.01 *vs* water 72h group, [e]P<0.01 *vs* water 96h group. Bold type – significant compared with intact, red text color – significant effect of multistrain probiotic. a.u. – absorbance units.

The concentration of anti-inflammatory IL-4 was also higher compared to control at all the days of observation after the WIRS except for the 3rd, indicating a compensatory function of the immune system in terms of the stress. At the same time, the content of IL-10 in serum of rats after stress did not change (Table 2).

Treatment with MP significantly reduced the concentration of proinflammatory IL-1β and IL-12B p40 after stress. For example, under the MP administration content of IL-1β after WIRS did not differ from the level of intact controls at the 3rd and 4th day and decreased by 27.4% (P<0.01), 37.6% (P<0.01), 29.1% (P<0.01) compared to water 48h, 72h and 96h groups accordingly. IL- 12B p40 was decreased by 21.1% (P<0.01) at the 2nd day and by 40.1% (P<0.01) at the 4th day after WIRS.

It was also found a strong effect of MP on the concentration of antiinflammatory cytokines. MP elevated the IL-4 concentration by 57% (p<0.01) and IL-10 by 59% (p<0.01) compared to the group of rats treated with water at the 1st day after WIRS. However, in the following days, the concentration of anti-inflammatory cytokines IL-4 and IL-10 in the group of rats administered with MP did not differ from that of intact animals suggesting the significance of the antiinflammatory interleukins excretion immediately after stress exposure. Thus, the obtained data indicate an anti-inflammatory effect of MP under conditions of stress-induced lesions of the GM.

DISCUSSION

Our study conclusively showed the effectiveness of the MP in the treatment of gastric lesions induced by WIRS. MP significantly accelerated the healing of ulcers in gastric mucosa. That was obvious at the 2nd day after WIRS, and at the 4th day the ulcers was almost completely healed.

It is well known that gastric ulcer is accompanied with tissue inflammation and the significant rise of proinflammatory cytokine level [20]. Healing of ulcer encompasses inflammation, cell proliferation, epithelial

regeneration, gland reconstruction, formation of granulation tissue, neovascularization, interactions between various cells and the matrix and tissue remodeling, resulting in scar formation [21]. Acute stress also leads to the increase of proinflammatory cytokines [22-26]. So, such an excessive release of proinflammatory cytokines induced by both stress and tissue damage trigger activation of macrophages, which in turn produce increased amounts of cytokines and chemokines, which attract neutrophils to the ulcer area. Neutrophils release proteolytic enzymes that destroy the tissue, resulting in ulcer impairment in conditions of stress [20]. In our work it was established the strong anti-inflammatory effect of MP which was registered at the 1st day after WIRS. The secretion of IL-4 and IL-10 contributed to decrease of proinflammatory IL-1β and IL-12B p40 in serum of rats that can attenuate inflammation induced by stress. These results are consistent with other studies that have shown the reduction of the concentration of proinflammatory cytokines under various pathologies of the digestive system and co-administration of probiotic strains [27-29]. Thus , Lin-Lin Chen et al. (2009) found that probiotics can reduce the content of IL-1β levels in experimental colitis [27]. Rodes et al. (2013) revealed that Bifidobacterium longum subsp. infantis reduces the concentration of tumor necrosis factor-α and increases the concentration of anti-inflammatory IL-4 in a model of human intestinal microbiota [28]. Bermudez-Brito et al. (2013) demonstrated a reduction in proinflammatory cytokines level produced by human dendritic cells infected with Salmonella typhi, under the influence of Bifidobacterium breve CNCM I-4035 [29].

It is known that the HPA system activates under the conditions of stress that leads to the elevated secretion of ACTH and corticosterone [30, 31]. In our study after 3 hour from the onset of stress corticosterone increased, while ACTH significantly decreased, that confirmed the inhibition of the ACTH release by corticosterone [19, 32]. Such intensive increase of glucocorticoid in serum resulted in severe damaging of GM of rats [33, 34]. At the following 4 day after WIRS the corticosterone and ACTH level was less than in intact rats. That suggested depletion of glands of the HPA system. Filaretova et al. (2002) show that the basal level of glucocorticoids contributes to normal functioning of GM and their decrease can cause gastric injury [35]. Indeed, in all four days after WIRS we diagnosed the significant lesions of GM. MP restored the functioning of HPAsystem and normalize the level of ACTH and corticosterone that is the mechanism of its therapeutic effect on the stress-induced lesions.

On the other hand the secretion of proinflammatory cytokines under the stress conditions also enhances the level of ACTH and corticosterone [36-38]. Such hyperactivation of HPA system leads to the deeper depletion of its pituatury and adrenal glands and thereafter aggravation of ulcers. MP showed anti-inflammatory properties in conditions of stress. And consequent fall in proinflammatory cytokine resulted in the partial suppression of hyperactivation of the HPA system induced by immune system. That created favorable conditions for the healing of stress-induced lesions in the GM of rats (Figure 8).

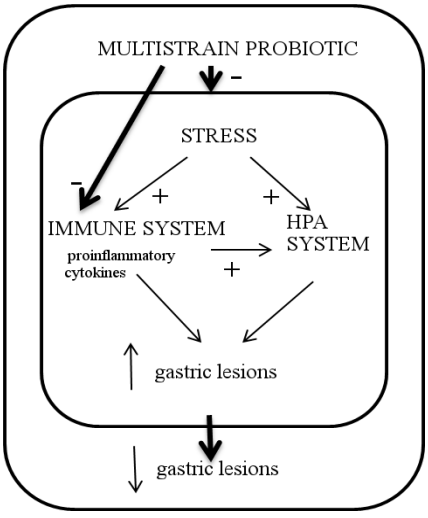

Figure 8 Multistrain probiotic (MP) decreases the content of proinflammatory cytokines in rat serum under the stress conditions and therefore attenuates the inflammation in the gastric mucosa and prevents the hyperactivation of hypothalamo-pituitary adrenal (HPA) system with consequent depletion of the pituatury and adrenal glands. This results in more intense healing of stress-induced gastric lesions.

Summing up the results, we can conclude that the administration of the probiotic strains reduces the immune response and eliminate stress hyperactivation under stress creating favorable conditions for stress-induced lesions healing in the GM

ACKNOWLEDGMENTS

The authors express their sincere thanks to Dr. Yankovsky Dmitro Stanislavovych for the help, advice and financial support for this work.

REFERENCES

[1] Singh VP, Sharma J, Babu S, Rizwanulla, Singla A. J Pak Med Assoc 2013; 63(2): 253-257.
[2] Scaldaferri F, Gerardi V, Lopetuso LR, Del Zompo F, Mangiola F, Boskoski I, Bruno G, Petito V, Laterza L, Cammarota G, Gaetani E, Sgambato A, Gasbarrini A. Biomed Res Int; 2013: 435268.
[3] Fava F, Danese S. World J Gastroenterol 2011; 17(5): 557-566.
[4] Dajani AI, Abu Hammour AM, Yang DH, Chung PC, Nounou MA, Yuan KY, Zakaria MA, Schi HS. Saudi J Gastroenterol 2013; 19(3): 113-120.
[5] Wang ZH, Gao QY, Fang JY. J Clin Gastroenterol 2013.
[6] Lesbros-Pantoflickova D, Corthesy-Theulaz I, Blum AL. J Nutr 2007; 137(3 Suppl 2): 812S-818S.
[7] Ziemniak W. J Physiol Pharmacol 2006;57(3): 123-141.
[8] Yasar B, Abut E, Kayadibi H, Toros B, Sezikli M, Akkan Z, Keskin O, Ovunc Kurdas O. Turk J Gastroenterol 2010; 21(3): 212-217.
[9] Navarro-Rodriguez T, Silva FM, Barbuti RC, Mattar R, Moraes-Filho JP, de Oliveira MN, Bogsan CS, Chinzon D, Eisig JN. BMC Gastroenterol 2013; 13: 56.
[10] Myllyluoma E, Veijola L, Ahlroos T, Tynkkynen S, Kankuri E, Vapaatalo H, Rautelin H, Korpela R. Aliment Pharmacol Ther 2005; 21(10): 1263-1272.
[11] Yoon H, Kim N, Kim JY, Park SY, Park JH, Jung HC, Song IS. J Gastroenterol Hepatol 2010; 26(1): 44-48.
[12] Lam EK, Yu L, Wong HP, Wu WK, Shin VY, Tai EK, So WH, Woo PC, Cho CH. Eur J Pharmacol 2007; 565(1-3): 171-179.
[13] Konturek PC, Brzozowski T, Burnat G, Szlachcic A, Koziel J, Kwiecien S, Konturek SJ, Harsch IA. J Physiol Pharmacol; 61(4): 429-436.
[14] Dharmani P, De Simone C, Chadee K. PLoS One 2013; 8(3): e58671.
[15] Senol A, Isler M, Karahan AG, Kilic GB, Kuleasan H, Goren I, Saritas U, Kaya S, Ciris M, Akturk O, Aridogan BC, Demirin H, Cakmakci LM. Turk J Gastroenterol 2011 Feb; 22(1): 18-26.
[16] Konturek PC, Sliwowski Z, Koziel J, Ptak-Belowska A, Burnat G, Brzozowski T, Konturek SJ. J Physiol Pharmacol 2009; 60 Suppl 6: 41-48.
[17] Spivak M, Lazarenko LM, Falalieieva TM, Virchenko OV, Neporada KS. Fiziol Zh 59(2): 23-30.
[18] Takagi K, Kasuya Y, Watanabe K. Studies on the Drugs for Peptic Ulcer. Chem Pharm Bull (Tokyo) 1964; 12: 465-472.
[19] van der Pompe G, Antoni MH, Duievenvoorden HJ, Heijnen CJ. Int J Behav Med 1997;4(2):145-169.
[20] Arakawa T, Watanabe T, Tanigawa T, Tominaga K, Fujiwara Y, Morimoto K. World J Gastroenterol 2012 Sep 21; 18(35): 4811-4822.
[21] Tarnawski AS, Ahluwalia A. Curr Med Chem 2012; 19(1): 16-27.
[22] Rohleder N, Aringer M, Boentert M. Ann N Y Acad Sci; 1261: 88-96.
[23] Speaker KJ, Fleshner M. BMC Physiol 2012; 12: 8.
[24] Johnson JD, Campisi J, Sharkey CM, Kennedy SL, Nickerson M, Greenwood BN, Fleshner M. Neurosci 2005; 135(4): 1295-1307.
[25] Segerstrom SC, Miller GE. Psychol Bull 2004; 130(4): 601-630.
[26] Steptoe A, Hamer M, Chida Y. Brain Behav Immun 2007; 21(7): 901-912.
[27] Chen LL, Wang XH, Cui Y, Lian GH, Zhang J, Ouyang CH, Lu FG. World J Gastroenterol 2009; 15(3): 321-327.
[28] Rodes L, Khan A, Paul A, Coussa-Charley M, Marinescu D, Tomaro-Duchesneau C, Shao W, Kahouli I, Prakash S. J Microbiol Biotechnol 2013; 23(4): 518-526.
[29] Bermudez-Brito M, Munoz-Quezada S, Gomez-Llorente C, Matencio E, Bernal MJ, Romero F, Gil A. PLoS One 2013; 8(3): e59370.
[30] Ohta Y, Kaida S, Chiba S, Tada M, Teruya A, Imai Y, Kawanishi M. J Clin Biochem Nutr 2009; 45(3): 347-354.
[31] Dronjak S, Gavrilovic L, Filipovic D, Radojcic MB. Physiol Behav 2004; 81(3): 409-415.

[32] Khalid BA, Lim AT, Fraillon DR, Funder JW. J Clin Invest 1982 A; 70(2): 443-452.
[33] Filaretova L, Morozova O, Bagaeva T, Podvigina T. J Physiol Pharmacol 2009 ; 60 Suppl 7: 79-86.
[34] Somasundaram K, Ganguly AK. Hepatogastroenterol 1985; 32(1): 24-26.
[35] Filaretova LP, Bagaeva TR, Podvigina TT, Morozova O. Ross Fiziol Zh Im I M Sechenova 2002 ; 88(5): 602-611.
[36] Berkenbosch F, van Oers J, del Rey A, Tilders F, Besedovsky H. Science 1987; 238(4826): 524-526.
[37] Bernton EW, Beach JE, Holaday JW, Smallridge RC, Fein HG. Science 1987; 238(4826): 519-521.
[38] Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W. Science 1987; 238(4826): 522-524.