Research Article

A synbiotic mixture ameliorated depressive behavior induced by dexamethasone or water avoidance stress in mice model

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ABSTRACT
Objective: Stress and glucocorticoid drugs disrupts the hypothalamic–pituitary–adrenal axis that is one major causes of depression. The benefits of probiotics may extend to systems beyond the gastrointestinal tract; i.e. the central nervous system. Thus, a synbiotic (probiotic+prebiotic) mixture effect was investigated on dexamethasone (Dex) and stress induced depression.

Methods: Male albino mice were used, the forced swimming test (FST) measured despair, while sucrose preference test measured anhedonia. The synbiotic regimen (12.5 × 10⁶ CFU) was inserted in drinking water for 7 days. Dex was administered subcutaneously either single-dose on the test day or for 7 days. Water avoidance stress (WAS) induction, 1 hour each day for 4 days.

Results: Drinking synbiotic reduced the immobility time during FST (54±7 sec vs, 111±6 sec water group, p<0.001). Dex injection significantly increased the immobility time (single-dose=166±6 sec, and 7 day=174±9 sec) compared to the control groups, while adding synbiotic to their drink reduced it (single dose= 81 ± 6.6 sec, and 7 days= 84 ± 14 sec), indicating that synbiotic reversed Dex induced depression. WAS increased immobility time (148±11 sec, vs sham 99±6 sec, p<0.001) in FST test, by adding the synbiotic treatment following WAS, the immobility time decreased (81 ± 6.5 sec).The synbiotic groups also had a higher percentage of sucrose preference.

Conclusion: The synbiotic mixture prevented the effects of WAS, acute or sub-acute Dex induced depression in mice. Therefore, probiotics might be useful and safe supplements to prevent depression related to stress or glucocorticoid therapies that deserves further evaluation.

Keywords: Probiotics, Depression, Forced swimming test, Glucocorticoid, Stress

INTRODUCTION
Probiotics are defined as ‘living microorganisms, which when administered in adequate amounts induce beneficial effects on the host.’¹ Gut bacterial microorganisms have two dominant phyla, Bacteroidetes and Firmicutes.¹ While Bifidobacteria, Lactobacillus and Bacteroides types represent the most important beneficial probiotics.² Various evidence suggest that probiotics
have therapeutic importance in the treatment of several gastrointestinal (GI) disorders such as inflammatory bowel disease, diarrhea, and irritable bowel syndrome. More recently it has been advocated that the effects of probiotic bacteria may extend to systems beyond the GI tract; i.e. central nervous system (CNS) disorders. Animal studies have also suggested that probiotics could have a modulating role in neuroendocrine and neurochemical responses outside the gut. In animal depression models probiotic treatment has proved to be beneficial. Species of Lactobacillus genus are particularly considered to have antidepressant effect. B. infantis relieved depression in rat models of depression, probably by reduction of pro-inflammatory cytokines, and regulation of tryptophan metabolism and CNS neurotransmitters. The prebiotics, such as oligosaccharides, stimulate the proliferation of nonpathogenic intestinal microflora. It has been proved that prebiotics also have neurotropic effects by increasing brain derived neurotropic factor expression in rats, probably by the gut hormones.

The microbiome–gut–brain axis refers to the connection that evidently exists between the intestinal microbiota, the gut, and the CNS. Additionally, research documents suggest that alteration of the gut microflora in mice affects the hypothalamic–pituitary–adrenal (HPA) axis reaction to stress and anxiety behavior. Probiotics (L. rhamnosus and L. helveticus) not only prevented the elevated serum corticosterone and the increase in HPA axis activity following maternal separation in rats, but also prevented depression behavior. That is, probiotic organisms can adjust the HPA axis dysregulation induced by early life stress. The importance is that there is high comorbidity between GI functional illnesses and stress-related psychiatric illnesses, such as anxiety and depression. On the other hand pathogenic bacteria in rodents could cause anxiety-like behaviors, mediated through the vagal afferent nerves. Depression is often related to stress, possibly a hypersensitive HPA axis results in chronically elevated cortisol levels as well as cortisol release in response to lower levels of stress. Synthetic glucocorticoids (GC) are widely used to treat various allergic, inflammatory, and autoimmune disorders. These drugs may induce adverse psychiatric effects (also known as steroid-induced psychosis), including depressed mood. Furthermore, administration of dexamethasone (Dex) and corticosterone have produced depression-like behaviors in animal models. Previously the antidepressant effects of a synbiotic (Syn; probiotic + prebiotic) mixture was proved in animal model of depression. The mixture was chosen on the basis that Lactobacilli and Bifidobacteria have antidepressant effects in animal studies thus a manufactured premixed product was chosen that comprised these genus and also a prebiotic, fructo-oligosaccharides. GCs and stressful life events are risk factors for developing major depression, which are strongly linked to impairments in the HPA axis and serotonin (5-HT) neurotransmission, conversely the beneficial effects of probiotics on the 5-HT system and HPA axis has been proven. The aim of the following study was to first, induce depression in mice by Dex single dose or multiple doses and then verify the effect of the Syn cocktail on depressive behavior. Second, to induce depression by stress in mice and ultimately verifying the effect of the Syn cocktail on depressive behavior.

MATERIALS AND METHODS

Animals

Male albino mice weighing 25±2 g were nurtured at room temperature 23± 2 °C with free access to standard mice chow and their relevant drink according to the experiment, on a 12-12 h light-dark cycle (lights on at 6 AM). Six animals were housed in each cage, and 24 h before the test they were placed in the experimental room for acclimatization. The total number of animals used with considering the expiration were 80 that were divided in 12 groups of animal each containing
6 animals. All the experiments were performed during 8-13 in daylight in the pharmacology laboratory. All animal procedures were performed in accordance with guidelines for the Care and Use of Laboratory Animals Issued by The National Ethical Committee (Ethical No: IR.MUI.RESEARCH.REC.1398.160). All the efforts in the experiments were made to reduce the number of animals used in each experiment and to minimize animal suffering. Animals were manipulated either by Dex administration or by inducing stress.

**Drug administration and the Syn cocktail**

Dex (8 mg/2 ml ampule, Raha Industry, Iran) 250 mcg/kg was injected SC as single dose 3 hours prior the tests, or 15 mcg/kg for 7 consecutive days and tested the day after, while control animals received normal saline.6 Dex was freshly prepared in normal saline and injections were adjusted for a volume of 10 ml/kg mice body weight.

The Syn cocktail was prepared from a premixed product comprised of 10^9 CFU probiotic strains and a prebiotic (Lactobacillus casei, L. acidophilus, L. rhamnosus, L. bulgaricus, Bifidobacterium breve, B. infantis, Streptococcus thermophile, Fructooligosaccharides; a production of Zisttakhmir industry, Iran). Animals had free access to the Syn cocktail solution 12.5 × 10^6 CFU; 22 in drinking water that was prepared freshly each day, control animals drank tap water ad libitum. The amount of the Syn cocktail solution or drinking water ingested by the animals were measured daily for 7 days and the tests were performed on the next day.

**Water avoidance stress (WAS)**

The test was performed in a Plexiglas tank comprised of a block affixed in the middle of the floor. The tank was filled with room temperature shallow water (22 °C) within 1 cm of the top of the block. The animals were placed on the block for a period of 1 h daily for 4 consecutive days.23 The group of animals that had WAS and drank tap water throughout the experiment are referred to the WAS group. The synbiotic cocktail administrated animals were subject to WAS either during the first 4 days, referred to WAS1, or during the last 4 days of ingesting the cocktail, referred to WAS2.

The sham group consisted of animals that were placed similarly for 1 h daily for 4 days on the same platform in a waterless container.

**Locomotor test**

The first experiment was carried out in order to analyze the animal motor activity in an open arena (Borj Sanat, Iran) that was divided into 16 zones by red beams. Mice were allowed to explore the field for 3 min, by passing through the beams the number of zone entries were counted automatically while rears on hind-legs were recorded manually. The sum of zone entries (horizontal exploration) and rears (vertical exploration) were calculated as total activity for each animal.

**Forced swimming test (FST)**

Mice were forced to swim in 25 °C water in a glass 2-liter beaker (diameter 12.5 cm, depth 12 cm) for 6 min. The immobility time was measured during the last 4 min of the 6 min trial after habituation was performed within the first 2 min. The immobility time is when no additional activity is observed other than that required to keep the animals’ head above the water denoting a phenotype of depression. Swimming behavior, defined as horizontal movement around the beaker which involved at least two limbs; and, climbing behavior, defined as upward movements of the forepaws against the beaker wall were also recorded.24 The experiment was recorded by a camera and analyzed later. After the experiment the animals were dried carefully to avoid hypothermia and returned to their home cage.

**Sucrose preference test**
This test measured another depression phenotype in rodents that is anhedonia. The test was conducted in three days: on the first day two bottles of sucrose solution (5 % w/v) were applied in the animals’ home cage, and on the second day one bottle of sucrose solution was replaced with water. After the habituation period, mice had access to two bottles containing 100 ml of sucrose solution and 100 ml of tap water that was finally measured after 24 h, and the percentage for sucrose preference was calculated according to the sucrose solution and water consumption. A decrease in sucrose preference measured to a level below 65% was taken as a criterion for anhedonia.25

**Data processing and statistical analysis**
Results are expressed as group mean ± SEM. The results were analyzed by one-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison tests and p values smaller than 0.05 were considered significant. The software programs used for data analyzing and making graphs were Excel 2010 and the GraphPad Prizm 6.

**RESULTS**

**Daily food, drink intake, and body weight change**
As shown in Table 1, there was no significant difference in food consumption between groups. Drinking was significantly higher in Dex treated animals that drank the Syn cocktail. The daily Syn consumption was then calculated according to the daily drink intake per body weight that varied between 4.5-8.5 × 10^6 CFU/g. There was a great rise in the percentage of body weight change in Dex treated mice that drank Syn (p<0.001 vs control and vs Dex alone group), also in the WAS2 group that ingested Syn (p<0.001 vs control and p<0.05 vs WAS group).

**Effect of Dex and the synbiotic mixture on depressive behavior**
As it is presented in Figure 1A drinking the Syn cocktail reduced the immobility time during FST that was obviously different from its control group that drank tap water (54±7 sec vs 111±6 sec, p<0.001). Dex acute or sub-acute injection increased the immobility time (166±6 sec and 174±9 sec respectively, p<0.001 compared to their corresponding control groups) that clearly indicated animal depressive behavior. While adding the Syn cocktail to their drink significantly reduced the immobility time, indicating that the Syn drink reversed Dex induced depressant effects. As Figure 1B shows the Syn mixture has significantly increased the swimming time in normal animals (167±8 sec vs water group 93±5 sec, p<0.001) or those treated with Dex (acute 153±6 sec and sub-acute 145±8.5 sec, p<0.001 compared to their corresponding Dex alone groups). While the climbing behavior was not different among the various groups (Figure 1C). Table 2 shows the results of sucrose preference test that are in line with the FST results. By adding the Syn mixture to drinking water sucrose preference rose up to 82 %, while Dex acute or sub-acute treatments reduced the preference to levels under 60 %, i.e. no preference of the sucrose solution over water that indicates anhedonia in mice. Adding the Syn mixture to the drinking water clearly increased the sucrose preference to above 72 % in various groups. As shown in Table 3, animals’ locomotor activity in the open arena was not noticeably different between the groups.

**Effect of WAS and the synbiotic mixture on depressive behavior**
Figure 2A shows that by exposing the animals to WAS the immobility time increased during the FST (148±11 sec, vs sham group 99±6 sec, p<0.001) that indicated animal despair behavior. While adding the Syn mixture to drinking water reduced immobility time in the WAS1 group (81±6.5 sec, p<0.001 compared to WAS alone), but the Syn cocktail was not effective when in the WAS2 group (143±6 sec). That might indicate that the Syn mixture was not able to prevent the stress induced rise in the immobility during FST. As shown in Figures 2B,C in the WAS...
group swimming time and climbing time were significantly lower than the sham group. Adding the Syn cocktail to drinking water obviously increased the swimming time (Figure 2B) in the WAS1 group (142±18 sec, vs WAS alone group 68±10 sec, p<0.001), while climbing was lower than the sham group (Figure 2C). The sucrose preference test results were parallel with the FST results (Table 2). As shown in the table WAS reduced sucrose preference to 59% while in the sham group it was 84%. Meanwhile, by adding Syn mixture in the WAS1 group sucrose preference increased to 90% while it was 60% in the WAS2 group. Animals’ locomotor activity in the open arena was not noticeably different between the various groups (Table 3).

**DISCUSSION**

Our results for the first time showed that a Syn mixture is able to remedy and also prevent Dex induced depression in mice during FST, which was also confirmed by the sucrose preference test. We also observed that the Syn mixture treated depression induced by WAS1, but it was not useful to prevent depression induced by WAS2. The sucrose preference test also showed the parallel results as anhedonia was treated following the ingestion of Syn after WAS1, but it did not prevent anhedonia induced by WAS2.

Changes in the body weight showed that Dex reduced the body weight although the amount did not reach a significant level. As in human, GCs show a powerful catabolic effect on experimental animals. The body weight increased in animals that ingested the Syn mixture but not statistically significant, although there was not a noticeable rise in their food consumption. It has been proven that different *Lactobacillus* species could induce different effects on weight depending on the host. In addition, certain *Lactobacillus* species along with other species are related to weight gain and obesity. Therefore at least in part the *Lactobacillus* present in the cocktail may have caused the weight gain. Wang and colleagues have proven the opposite result, since *L. paracasei, L. rhamnosus*, and *B. animalis* have reduced weight in high fat diet fed mice. But when the Syn cocktail was consumed following Dex treatment or WAS it caused a significant body weight rise, and to some extent more food intake. Various mechanisms could have caused weight gain, apart from the cocktail, increased food consumption could also be responsible in these groups. Fructooligosaccharides are composed of fructose units that occur naturally in plants and they induce a low sweetness amount; although they are calorie free, and non-cariogenic. It seems unlikely that the fructooligosaccharides has induced the weight gain. During FST by placing mice in the water the animal gradually loses hope to escape the stressful environment, thus the immobility time reflects a measure of "behavioral despair". FST is a reliable tool in drug discovery not only in industrial settings that high extent screening of new compounds are essential, but also in complementary depression medicine research. In the same trend as previous results, the Syn cocktail presented antidepressant effects in mice by reducing the immobility time. Evidently the selective 5-HT reuptake inhibitor (SSRI) drug, fluoxetine, decreases immobility time while increasing swimming behavior. The 5-HT level was not measured in our experiment but the Syn mixture changes during the FST was similar to the SSRI drug since the swimming time was augmented that might be related to the serotonergic system. This statement is supported by a previous research that reported following treating rats with *B. infantis*, there was a drop in the 5-hydroxyindoleacetic acid level in the frontal cortex indicating that 5-HT degradation was reduced, possibly due to decreased monoamine oxidase activity. Single dose and long term Dex administration induced despair behavior during the FST, and anhedonia was deduced since the sucrose preference reduced to levels under 60%. This was proven earlier since it was shown that Dex dose dependently increased the immobility time during the FST. By adding the Syn mixture the immobility time in the FST was reduced not
only when Dex was injected as single dose but also when it was administered for seven days. This showed that Syn mixture could have preventive effects on Dex induced mental disorders. It has been shown previously that repeated corticosterone injections induce depressive behavioral and dysregulation of the HPA axis, since corticosterone could have blocked the HPA axis response that normally occurs after animal is dropped in the water during FST testing.\(^{31,32}\) GC receptor function may be impaired or it may become resistant to GCs in depression thus causing HPA axis hyperactivity in depression and thereby causing the high GC levels.\(^{33}\) Evidently, the gut microbiota plays an imperative role in the regulation and development of the HPA axis.\(^{7}\) Thus it could be deduced that probably the Syn mixture consumption could prevent HPA alterations induced by Dex and therefore preventing its depressive behavior in the FST. In order to further evaluate the possible effect of probiotic on HPA alteration induced depression a stress model was accomplished.

WAS is a well-recognized method that represents an effective psychological stressor with conspicuous boosts of adrenocorticotropic hormone and corticosterone within 30 min.\(^{34}\) We observed that WAS induced despair behavior by increasing the immobility time during FST, meanwhile anhedonia was assumed since there was no preference of sucrose over water consumption. During WAS1 that the Syn formulation was started with WAS and continued for more three days, depressive symptoms have gone away in mice. However during WAS2 that was imposed on the last four days of the Syn formula ingestion the beneficial effects were not observed. On the other hand, Ait-belgnaoui and colleagues realized that a 2-week treatment with the probiotic formulation (consisting \textit{L. helveticus} R0052 and \textit{B. longum} R0175) mitigated HPA axis and autonomic nerve system response to WAS as revealed by decreased levels of corticosterone, and noradrenaline in stressed mice plasma.\(^{23}\) They administered the probiotic mixture orally at a concentration of $10^9$ CFU/day to C57Bl6 mice and they induced WAS at the end of this period. Although in our observational study corticosterone plasma level were not assessed but behavior studies interestingly showed that the Syn mixture could overcome depression induced by WAS but it could not prevent it, during FST or the sucrose preference test in mice. In addition WAS caused a decrease in the swimming time and the Syn ingestion significantly increased it in WAS1 experiment. Since it has been proven previously that swimming during the FST is related to the serotonergic pathway,\(^{24}\) therefore the Syn mixture may have overcome WAS induced depression by altering the serotonergic system. This believer is supported by previous studies that chronic stress causes depression-related behavior through neurotransmitter changes in the CNS, suppression of hippocampal neurogenesis, as well as HPA axis dysfunction that manipulates 5-HT activity and exacerbates the effects of stress.\(^{35}\) The brain and gut communication is achieved by various interrelated systems, for example through the hormones of the HPA axis, or through neural pathways of the autonomic nervous system (which involves the vagus nerve and the adrenergic system), also the immune cytokines.\(^{36}\)

It has been shown that, various forms of stress, change the composition of the intestinal microbiota in animals for example after a stressful experience the number of \textit{Lactobacillus} and \textit{Bifidobateria} in the gut were reduced.\(^{37}\) Conversely, treatment with probiotic bacteria reduces the detrimental effects of stress.\(^{8}\) Therefore our results were in favor of previous reports that there is a high amount of comorbidity between CNS and GI disorders, where it has been reported that large number of those being treated for irritable bowel syndrome also suffer from psychiatric illness.\(^{38}\) This behavioral study clearly revealed that the Syn formulation can prevent the GC drug, Dex, induced depression in addition it could remedy WAS induced depression. Although interpreting
animal data to human should be done judiciously, but probiotics that could be easily inserted in the diet could be a useful alternative therapy in stress or GC related depression, while avoiding the harmful side effects of common antidepressants. The Syn effect on depressive behavior may be related to a number of aspects including: ameliorating GC or stress induced alteration in stress hormones, brain plasticity, and neurogenesis that warrants further studies.

References
Table 1. Daily food intake, drinking, Syn ingestion, and percent body weight change.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Daily food intake mg/g body weight</th>
<th>Daily drink ml/g body weight</th>
<th>Daily synbiotic CFU/g body weight</th>
<th>Body weight rise (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>181.6±18</td>
<td>0.42±0.01</td>
<td>0</td>
<td>2.1±0.7</td>
</tr>
<tr>
<td>Syn</td>
<td>168.2±25</td>
<td>0.36±0.01</td>
<td>4.5×10⁶</td>
<td>3.8±0.4</td>
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<td>Dex</td>
<td>158±14</td>
<td>0.5±0.04</td>
<td>0</td>
<td>0.9±0.7</td>
</tr>
<tr>
<td>Dex+Syn</td>
<td>197±23</td>
<td>0.68±0.05*</td>
<td>8.5×10⁶</td>
<td>9.9±1***,ddd</td>
</tr>
<tr>
<td>WAS</td>
<td>132±5</td>
<td>0.54±0.03</td>
<td>0</td>
<td>2.9±0.7</td>
</tr>
<tr>
<td>WAS1+Syn</td>
<td>178±22</td>
<td>0.58±0.03</td>
<td>7.2×10⁶</td>
<td>4.5±0.7</td>
</tr>
<tr>
<td>WAS2+Syn</td>
<td>173±24.4</td>
<td>0.56±0.01</td>
<td>7×10⁶</td>
<td>7.7±0.7***,s</td>
</tr>
</tbody>
</table>

Syn cocktail (1.2.5×10⁶ CFU), Dex sub-acute (15 mcg/kg). Stress was imposed by WAS during 4 days and daily measurements were done during this period, while other groups were evaluated for 7 days. The synbiotic cocktail administrated animals were subject to WAS either during the first 4 days (WAS1) or during the last 4 days (WAS2) of ingesting the cocktail. ***p<0.001 compared with the control group, ddd p<0.001 compared with the Dex group, and s p<0.05 compared with the WAS group.
Table 2. The sucrose preference test results.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sucrose preference (%)</th>
<th>Groups</th>
<th>Sucrose preference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>65</td>
<td>Sham</td>
<td>84</td>
</tr>
<tr>
<td>Syn</td>
<td>82</td>
<td>WAS</td>
<td>59</td>
</tr>
<tr>
<td>Control (acute)</td>
<td>65</td>
<td>WAS1+Syn</td>
<td>90</td>
</tr>
<tr>
<td>Dex (acute)</td>
<td>53</td>
<td>WAS2+Syn</td>
<td>60</td>
</tr>
<tr>
<td>Control (sub-acute)</td>
<td>75</td>
<td>Dex (acute)+Syn</td>
<td>95</td>
</tr>
<tr>
<td>Dex (sub-acute)</td>
<td>58</td>
<td>Dex (sub-acute)+Syn</td>
<td>72</td>
</tr>
</tbody>
</table>

Syn cocktail (12.5×10⁶ CFU) for 7 days, Dex acute (250 mcg/kg) single dose and or sub-acute (15 mcg/kg) for 7 days and the control groups received normal saline. Stress was imposed by WAS during 4 days. The synbiotic cocktail administrated animals were subject to WAS either during the first 4 days (WAS1) or during the last 4 days (WAS2) of ingesting the cocktail. The sham group animals were placed in a waterless container. Number of animals in each group was 6. Percentage of sucrose preference = (sucrose consumption/sucrose consumption+ water consumption) ×100

Table 3. Total activity during the locomotor test.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total activity (count)</th>
<th>Groups</th>
<th>Total activity (count)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>165±16</td>
<td>Sham</td>
<td>230±14</td>
</tr>
<tr>
<td>Syn</td>
<td>192±17</td>
<td>WAS</td>
<td>213±7</td>
</tr>
<tr>
<td>Control (acute)</td>
<td>173±12</td>
<td>WAS1+Syn</td>
<td>179±16</td>
</tr>
<tr>
<td>Dex (acute)</td>
<td>155±6</td>
<td>WAS2+Syn</td>
<td>171±20</td>
</tr>
<tr>
<td>Control (sub-acute)</td>
<td>170±13</td>
<td>Dex (acute)+Syn</td>
<td>199±14</td>
</tr>
<tr>
<td>Dex (sub-acute)</td>
<td>150±15</td>
<td>Dex (sub-acute)+Syn</td>
<td>188±13</td>
</tr>
</tbody>
</table>

Syn cocktail (12.5×10⁶ CFU) for 7 days, Dex acute (250 mcg/kg) single dose and or sub-acute (15 mcg/kg) for 7 days and the control groups received normal saline. Stress was imposed by WAS during 4 days. The synbiotic cocktail administrated animals were subject to WAS either during the first 4 days (WAS1) or during the last 4 days (WAS2) of ingesting the cocktail. The sham group was placed in a waterless container. Total activity count during locomotor test = (horizontal +vertical) exploration. Number of animals in each group was 6. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey’s comparison test. The differences between various groups did not reach statistical significance.
Figure 1. Effect of the synbiotic cocktail and dexamethasone on behavior during the forced swimming test. (A) The immobility time, (B) the swimming time, and (C) the climbing time.
Synbiotic cocktail (Syn; $12.5 \times 10^6$ CFU) for 7 days, Dex acute (250 mcg/kg) single dose and control A normal saline, Dex sub-acute (15 mcg/kg) for 7 days and control S received normal saline. Number of animals in each group was 6. Results are expressed as group mean ± SEM and analyzed by ANOVA followed by Tukey’s comparison tests. vvv $p < 0.001$ compared with the water drinking group, *** $p < 0.001$ compared with control A or S groups, ddd $p < 0.001$ compared with Dex alone acute or sub-acute group.

![Graph A: Immobility time (sec)](image)

![Graph B: Swimming time (sec)](image)
Figure 2. Effect of the synbiotic cocktail and stress on behavior during the forced swimming test. (A) The immobility time, (B) the swimming time, and (C) the climbing time. Syn cocktail (12.5×10^6 CFU) for 7 days. Stress was imposed by WAS during 4 days. The sham group animals were placed in a waterless container. The synbiotic cocktail administrated animals were subject to WAS either during the first 4 days (WAS1) or during the last 4 days (WAS2) of ingesting the cocktail. Number of animals in each group was 6. Results are expressed as group mean±SEM and analyzed by ANOVA followed by Tukey’s comparison tests. VVV p < 0.001 compared with the water drinking group, * p<0.05, *** p < 0.001 compared with the sham group, sss p < 0.001 compared with WAS alone group.