A synbiotic mixture augmented the efficacy of doxepin, venlafaxine, and fluvoxamine in mice model of depression

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ABSTRACT

Objective: Currently available antidepressant drugs have notable downsides; in addition to their side effects and slow onset of action their moderate efficacy in some individuals, may influence compliance. Previous literature has shown that probiotics may have antidepressant effects. Introducing complementary medicine in order to augment the efficacy of therapeutic doses of antidepressant drugs seems to be very important. Therefore the effect of adding a synbiotic cocktail in drinking water was assessed in mice model of despair following administering three antidepressant drugs belonging to different classes. Methods: The marble burring test (MBT), and forced swimming test (FST) were used as animal model of obsessive behavior and despair. The synbiotic cocktail was administered in mice drinking water (6.25×10⁶ CFU) for 14 days and the tests were performed on the days 7 and 14 thirty minutes after injecting the lowest dose of doxepin (1 mg/kg), venlafaxine (15 mg/kg), and fluvoxamine (15 mg/kg). Results: After 7 days of the synbiotic ingestion immobility time decreased in FST for doxepin (92 sec ± 5.5) and venlafaxine (17.3 sec ± 2.5) compared to their control group (drinking water) but fluvoxamine could decrease immobility time after 14 days of ingesting the synbiotic (70 sec ± 7.5). The synbiotic cocktail pre-administration improved the MBT test response for venlafaxine, while it did not change the results for the other two drugs. Conclusion: Adding synbiotic to drinking water improved the efficacy of discrete antidepressant drugs particularly during the FST. Probiotics could be useful complementary medicine for drug resistant depressed individuals.

Keywords: probiotic, synbiotic, depression, antidepressant, forced swimming test, complementary medicine
INTRODUCTION

Probiotics are live organisms that when they are chronically ingested in adequate amounts they could induce beneficial effects on host. Single strain probiotics or their combinations are useful for a variety of diseases, including gastrointestinal (GI) disorders such as inflammatory bowel disease, diarrhea, irritable bowel syndrome (IBS), and allergy. This is now even spreading to some ailments of the central nervous system (CNS). Gut bacterial microorganisms have two dominant genera, Bacteroidetes and Firmicutes, while other types have lower abundance, for instance Proteobacteria, Actinobacteria, and Fusobacteria. Evidently, the brain-gut axis is a bidirectional homeostatic route that its aberration may cause important pathophysiological results. The high risk of co-existing psychiatric symptoms such as anxiety with GI disorders for instance IBS provides further evidence of the importance of this axis. The brain-gut axis is connected not only by complex systems including, the vagus nerve, endocrine, immune, and humoral links but also the gut microbiota in order to maintain GI stability and to connect cognitive and emotional areas of the brain with GI functions.

Major depressive disorder is the most common mood disorder that affects 5% of the population each year. Several mechanisms have been involved in the pathogenesis such as altered monoaminergic system (serotonergic, noradrenergic, and dopaminergic), and glutamatergic systems, increased inflammation, HPA axis aberrations, and decreased neuroplasticity. Probiotics have shown antidepressant effects in animal models of depression. Species of Lactobacillus genus are particularly characterized as anti-depressants. A probiotic mixture of Lactobacillus strains (L. rhamnosus and L. helveticus) ameliorated depression by regulating corticosterone level in rat pups. Likewise, chronic ingestion of L. rhamnosus strain (JB-1) in mice alleviated
depressive behavior and caused regional alteration in GABA receptor expression since these changes were not observed in vagotomized mice the vagus nerve was considered critical for the brain-gut axis.\textsuperscript{12} \textit{Bifidobacterium} species also induces physiologic changes in favor of antidepressants effects in animal studies, for instance \textit{B. infantis} ameliorated depressive behavior in the maternal separation model.\textsuperscript{13} In a systematic review regarding the effects of probiotics on depression in human most of the studies found positive results on all values of depressive symptoms; but, there was wide differences in the strain of probiotic, the dosing, and duration of treatment.\textsuperscript{14} The possible mechanisms that were considered for antidepressant effects of \textit{B. infantis} by using in rat model were reduction of pro-inflammatory cytokines, alteration of tryptophan metabolism and CNS neurotransmitters.\textsuperscript{15} The combination of \textit{Lactobacilli} and \textit{Bifidobacteria} were tested in depression following post-myocardial infarction (MI) in rats. Administration of \textit{L. helveticus} and \textit{B. longum} together had positive effects on post-MI depression through reduction of pro-inflammatory cytokines (for instance IL-4) and the intestinal permeability restoration.\textsuperscript{16,17} In addition, prebiotics such as oligosaccharides that stimulate the growth of nonpathogenic intestinal microflora such as \textit{Lactobacilli} and \textit{Bifidobacteria} also have neurotropic effects.\textsuperscript{18}

Although drug therapy of depression is usually safe and effective but it is still not ideal because the latency time for clinical results is quite long (about 3-5 weeks) and there are still concerns regarding side effects such as loss of libido and weight gain. Additionally, there are a significant number of patients that do not respond well to antidepressant drugs, psychotherapy and electroconvulsive therapy.\textsuperscript{19} Thus the aim of the following study was to first, observe the effect of a synbiotic (probiotic + prebiotic) cocktail on behavior in animal model of depression. Since, it has been previously shown \textsuperscript{11-15} that the probiotics \textit{Lactobacilli} and \textit{Bifidobacteria} have antidepressant effects in animal studies a manufactured premixed product was chosen that comprised these genus and also a prebiotic, fructooligosaccharides. Second, in order to observe the effect of the synbiotic cocktail on the efficacy of antidepressant drugs from different classes sub-threshold doses of doxepin a tricyclic antidepressant (TCA), venlafaxine a
serotonin-norepinephrine reuptake inhibitor (SNRI), and fluvoxamine a selective serotonin reuptake inhibitor (SSRI) were chosen.

MATERIALS AND METHODS

Animals

Male Swiss mice weighing 23-26 g were housed at 21± 2 °C in a 12 h light-dark cycle with the lights on at day time 6 AM-6 PM. Tap water or the synbiotic cocktail and standard food pellets were available ad libitum. For each experiment 6 mice were used that were housed 3 per cage. Tests were performed in the behavior laboratory after 24 hours that mice have become acclimatized to the environment. In order to minimize circadian rhythm influence, all experiments were performed between 8 AM-1 PM in the pharmacology laboratory. All animal procedures were approved by the Ethics Committee of Isfahan University of Medical Science and performed in accordance with National Institute of Health Guide for the Care and Use of Laboratory Animals (Ethical No: IR.MUI.REC.1395.3.864).

The synbiotic cocktail and drug therapy

The synbiotic cocktail comprised of 10⁹ CFU (Lactobacillus casei, L. acidophilus, L. rhamnosus, L. bulgaricus, Bifidobacterium breve, B. infantis, Streptococcus thermophiles, Fructooligosaccharides (a prebiotic); a production of Zisttakhmir industry, Iran). Animals had free access to the synbiotic cocktail solution in drinking water that was prepared freshly each day in three concentrations: 6.25, 12.5, and 25 × 10⁶ CFU (based on pilot studies); control animals had tap water ad libitum. The amount of the synbiotic cocktail solution or normal drinking water ingested by the animals were measured daily. The therapy continued for 14 days and the tests were performed on the 7th and 14th day.

The antidepressants were first administered intraperitoneally (IP) alone 30 min before starting the first test. Two doses were applied for each drug according to previous
studies and finally the lowest effective dose (data not shown) was chosen to be applied following the synbiotic mixture administration. The selected antidepressants were doxepin 1 mg/kg (Razak, Iran), venlafaxine 15 mg/kg (Sigma-Aldrich, India), and fluvoxamine 15 mg/kg (Sigma-Aldrich, India). All the drugs were freshly prepared in normal saline and injections were adjusted for a volume of 10 ml/kg mice body weight. The selected dose of each antidepressant drug was administered on the 7th and 14th day following the synbiotic therapy ($6.25 \times 10^6$ CFU), 30 min before performing the tests.

**Marble burying test (MBT)**

The first test was the MBT, this is a method used to evaluate anxiety behavior, obsessive compulsive behavior. With minor modification from method presented by Njung’e and Handley 1991. Mice were separately placed in plastic cages (42×24×12cm) containing 12 glass opaque marbles (1 cm diameter) that were distributed evenly over 5 cm deep sawdust without food or water for 30 min. The number of marbles at least two-thirds buried (MB) was counted after 30 minutes.

**Forced swimming test (FST)**

After performing the MBT each mice was subjected to FST. With some modification mice were forced to swim in a glass beaker (diameter 14 cm) containing 25°C water for 6 min. The depth was about 15 cm to prevent the mice from escaping and touching the bottom of the glass beaker with their paws or tail. After 2 minutes of adaptation the total immobility time was measured by a chronometer in the last 4 min of the trial. Immobility is defined as the time while animal was floating staying still when no additional activity was observed other than that required to keep the animal’s head above the water. Finally the mice were dried carefully and returned to their home cage.

**Data processing and statistical analysis**

Results were 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 less 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 drinking intake

According to table 1, daily measurements of the synbiotic cocktail ingestion showed that approximately a dose of 2.4-9.2 ×10⁶ CFU/g body weight of synbiotic was ingested.

Effect of the synbiotic cocktail on MBT and FST

The number of marbles buried during the MBT, showed that consuming 12.5×10⁶ CFU synbiotic after 7 days or 14 days has reduced obsessive behavior in mice (figure 1A). The percentage of marbles buried after 30 min on the 7th and 14th days of ingesting the middle concentration of the synbiotic cocktail was 25 % and 41 % respectively that was significantly (p < 0.05) different from the corresponding control group (50 % and 58.3 % on days 7th and 14th respectively; p < 0.05). The lowest synbiotic concentration only reduced MB to a significant amount on the 7th day compared to the control animals (33.3 % vs 50 %; p < 0.05). However consuming the highest synbiotic concentration showed opposite results on the MB behavior, since animals buried more marbles measured after 7 (87%, p < 0.001 vs control) and 14 days of therapy. During the FST the animals that ingested the synbiotic showed antidepressant behavior as presented in figure 1B. After the mice ingested the synbiotic cocktail for a week the immobility time measured during the FST reduced in a dose dependent manner since the highest cocktail concentration (25 ×10⁶ CFU) caused the lowest immobility time (68.1 sec ± 2.5 vs 142.3 sec ± 4.3, p < 0.001). After 14 days of synbiotic ingestion, animals dose dependently showed less immobility time compared with the control animals (139.6 sec ± 5.4); the animals that ingested the highest concentration of the synbiotic cocktail had the lowest immobility time (58.3 sec ± 15, p < 0.001).
**Effect of the synbiotic cocktail pretreatment on mice response to antidepressants during MBT and FST**

The lowest concentration of the synbiotic cocktail (6.25 × 10^6 CFU) was chosen to observe the animals behavior during MBT and FST on the 7th and 14th day after injecting the lowest dose of each antidepressant drug (figure 2). The antidepressant drugs have all significantly reduced the number of MB compared to the control group (figure 2A, p < 0.05). Synbiotic ingestion had significantly influenced venlafaxine effects on the MB behavior since the animals significantly buried less marbles compared to animals that drank water (percentage of MB decreased from 25 to 7 %). But consuming the synbiotic cocktail did not have any considerable effect on MB behavior of animals injected with doxepin or fluvoxamine. On the other hand drinking the synbiotic cocktail increased the antidepressant effects of the lowest dose of each of the drugs (figure 2B). The immobility time reduced dramatically in animals that had drank the synbiotic cocktail either for 7 days (17.3 sec ± 2.5) or for 14 days (60 sec ± 16) prior the administration of venlafaxine (p<0.001, vs the venlafaxine alone group 146 sec ± 1).

The results for synbiotic drinking prior to doxepin injection followed the same trend after 7 days the immobility time was 92 sec ± 5.5 and after 14 days it was 70 sec ± 7.4 that decreased significantly compared to the doxepin alone group 100 sec ± 19 (p < 0.001).

Synbiotic effect on fluvoxamine was slightly different as only after 14 days immobility time decreased significantly compared to the drug administered alone (70 sec ± 7.5 vs 100 sec ± 9, p < 0.001).

**DISCUSSION**

Our results showed that the synbiotic cocktail could mitigate the immobility time in mice FST that denotes its possible antidepressant effects. Interestingly only after a week of ingesting the synbiotic cocktail response to the lowest dose of the antidepressants increased dramatically. Although the MB behavior showed variable results. The MB behavior appears to be just a form of digging, but the behavior in mice has been used as a model of anxiety disorders including, obsessive compulsive disorder (OCD).\(^{24}\) This
method is also useful for evaluating the mechanisms of action of drugs.\textsuperscript{25} Venlafaxine (SNRI), doxepin (TCA), and fluvoxamine (SSRI) in our set of experiment clearly reduced the number of marbles buried that was parallel with previous literature.\textsuperscript{22, 25} The FST is a reliable tool in drug discovery in industrial settings that high quantity screening of new compounds are necessary, also in research regarding complementary medicine.\textsuperscript{23} By leaving mice in the water container the animal gradually loses hope to escape the stressful environment, thus the immobility time reflects a measure of "behavioral despair". In the same trend as previous results, all antidepressant drugs of different classes that were tested in our study presented antidepressant effects in mice by reducing the immobility time.\textsuperscript{22}

The daily preparation of synbiotic mixture in mice drinking water was based on previous literature that \textit{B. infantis} was administered by dissolving a powder containing \num{10^{10}} bacterial cells, in 100 ml of the rats drinking water every morning.\textsuperscript{26} There is also evidence that dead probiotic bacteria or just integral components of the bacterial cell such as peptidoglycan fragments or DNA would also be effective.\textsuperscript{27} Therefore although survivability of the mixture was not assessed before the new batch on the next morning the research showed that the content remained effective. The synbiotic mixture dose dependently reduced the immobility time during the FST, which obviously denotes its antidepressant-like effect in mice. However a dose dependent effect on obsessive behavior was not observed during MBT, although lower doses of the synbiotic cocktail reduced the MB behavior this was not observed with the higher dose. High dose of the synbiotic mixture had opposite effects on the MBT. Different effect of the synbiotic mixture on FST and MBT could be because of the different mechanisms involved in each test. Earlier researches have proven that although MBT and FST are invaluable predictive tests of OCD and antidepressant action, each assay appears to engage completely distinct neurochemical systems.\textsuperscript{28} While FST is more susceptible to compounds that are effective by altering activity in the noradrenergic system, MBT mostly depends on modulation of the serotonergic system.\textsuperscript{26} Although on the downside of our research we did not measure the monoamine neurotransmitters but this has been proven earlier.\textsuperscript{29-30} Reports have shown that chronic gavage of \textit{L. plantarum} in mice
reduced stress induced depression-like behaviors, normalized the HPA axis and immune systems, and modulated the changes in the dopamine and serotonin system in the prefrontal cortex. Additionally probiotics changed behavior and the central nervous system function in naïve adult animals. The consumption of a fermented milk product containing \textit{B. aimalis}, \textit{S. thermophilus}, \textit{L. bulgaricus}, and \textit{L. lactis} in healthy women without psychiatric symptoms as observed by functional magnetic resonance imaging induced robust alterations in the activity of brain regions that control the central processing of emotions and sensations.

Pretreatment with the synbiotic mixture before the administration of venlafaxine (SNRI) had a synergist effect on the MBT, this synergist effect was not observed with doxepin or fluvoxamine. Previous studies on rat model of depression have reported that the ingestion of probiotics has a role in restoring the monoamine levels in important brain regions. Therefore, it is possible that restoring the monoamine level has improved the efficacy of venlafaxine in the MBT. On the other hand the synbiotic cocktail had synergist antidepressant-like effect on venlafaxine and doxepin (TCA); the antidepressant efficacy of fluvoxamine (SSRI) only increased after 14 days of synbiotic ingestion. Several studies have supported the argument that probiotics can exert psychotropic potential, this effect could be mediated by alterations in the monoamines. For instance, it has been shown that \textit{B. Infantis} reverses maternal separation induced depressive-like behavior in rats during FST, also at least part of this antidepressant effect is due to elevation of tryptophan. Previous finding suggest that in naïve mice the probiotic \textit{L. plantarum} could modulate both serotonergic and dopaminergic systems, and interestingly the probiotic has increased dopamine, 3,4-dihydroxyphenylaceticacid, and homovanillicacid but there has been no change in the dopamine turnover rate.

Earlier literature advocate that the vagus mediates the behavioral and neurochemical effects of probiotics. Nevertheless, the antidepressant effects of the probiotic mixture may arise independently of any changes to the monoamines systems, for instance it may be caused by attenuation of pro-inflammatory immune responses.

The results of the following research were not only parallel with the previous literature regarding the antidepressant effects of probiotics but also extended it in several ways:
first, the synbiotic mixture could decrease MB behavior. Second, the cocktail ingested by animals augmented the efficacy of the SNRI antidepressant venlafaxine during MBT. Finally, after 7 days the synbiotic drink it had a synergist antidepressant-like effect on venlafaxine, and doxepin; and increased fluvoxamine efficacy after 14 days. Previous results proved that fluoxetine and escitalopram have antimicrobial activity in vitro, and psychotropic medications differentially influence the composition of gut microbiota in vivo. Therefore adding probiotics may help to improve the gut microbial composition in patient receiving antidepressant therapies. Since interpreting animal results to human must be done with caution, further clinical research is warranted regarding adding synbiotics to drug resistant patients and adding synbiotics in order to reduce antidepressant drug dosage and side effects mainly when SNRI, or TCA are administered.

**Acknowledgement**

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**References**


Table 1. Daily liquid intake.

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<th>Synbiotic cocktail CFU</th>
<th>6.25×10^6</th>
<th>12.5×10^6</th>
<th>25×10^6</th>
<th>0 (Tap water)</th>
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<td>Daily intake ml/g body weight</td>
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<td>0.37</td>
<td>0.37</td>
<td>0.39</td>
</tr>
<tr>
<td>Daily dose CFU/g body weight</td>
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<td>4.625×10^6</td>
<td>9.25×10^6</td>
<td>0</td>
</tr>
</tbody>
</table>

The synbiotic cocktail comprised of (*Lactobacillus casei*, *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *Bifidobacterium breve*, *B. longum*, *Streptococcus thermophiles*, *Fructooligosaccharides* (a prebiotic)).
Figure 1. The effect of 3 concentrations of the synbiotic cocktail ingestion for 14 days on mice behavior. A) The marble burring test (MBT) the number of marbles at least two-thirds buried after 30 min, and B) the total immobility time during the last 4 min in the forced swimming test (FST). The tests took place on days 7 and 14, each animal was
first subjected to MBT and then FST. Number of animals in each group was 6, the control animals drank tap water. Results are expressed as group mean ± SEM and analyzed by one-way ANOVA, followed by Tukey’s posthoc. * p < 0.05, ** p < 0.01 and *** p < 0.001 compared with the control group.

![Graph A](image1)

![Graph B](image2)

**Figure 2.** The effect of drinking the synbiotic cocktail and the lowest dose of antidepressants on mice behavior. A) The marble burying test (MBT) the number of marbles at least two-thirds buried after 30 min are presented, and B) the total immobility time during the last 4 min in the forced swimming test (FST). Number of animals in each
of the groups were 6, animals ingested the synbiotic cocktail (SYN; 6.25×10⁶) for 14
days, control group ingested tap water. The drugs were injected IP 30 min before
testing; each animal was first subject to MBT and then FST. Results are expressed as
group mean ± SEM and analyzed by one-way ANOVA followed by Tukey's post test. **
p < 0.01 and *** p < 0.001 compared with the probiotic free group. # p < 0.01 compared
with the corresponding control groups (the plane bares).