


A probiotic supplement, *Lactobacillus rhamnosus* GG, and kefir separately can improve mood and exhibit potential anti-depressant-like activities in mice

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ABSTRACT

Depression is a common psychiatric disorder that can be continuous or recurrent. It has been previously reported that intestine probiotics play an essential role in the bidirectional communication of the intestine and brain. This study aims to investigate the antidepressant effects of kefir, a probiotic supplement, and *Lactobacillus rhamnosus* GG and their potentials in depression-like behaviour treatment in two-week and four-week treatments. In the present study, BALB/c mice were used for this purpose. The saline- and fluoxetine-treated groups were designed as negative and positive control groups, respectively. The forced swimming and tail suspension tests have been performed to assess the level of depression-like activity. We have observed that two-week treatment reduces the duration of depression-like activities, and four-week treatment enhances the antidepressant properties. Overall, our results suggest that kefir, *L. rhamnosus* GG, and the investigated probiotic supplement have antidepressant-like properties.

KEYWORDS

probiotics, kefir, *Lactobacillus rhamnosus*, depression, anti-depressant activities

1. INTRODUCTION

Depression is a prevalent psychiatric disease that can be continuous or recurrent, significantly reducing a person's ability to carry out their daily routine (Huang et al., 2016). More recent antidepressants, selective serotonin reuptake inhibitors including fluoxetine and serotonin-norepinephrine reuptake inhibitors, are more reliable drugs than tricyclic antidepressants and monoamine oxidase inhibitors (Li et al., 2020).

The World Health Organization announced that probiotics, as living microorganisms, benefit the host if consumed in specific amounts (Huang et al., 2016). Intestine probiotics have been repeatedly reported to play an essential role in the bidirectional communication between the intestine and brain (Emge et al., 2016). Intestinal microorganisms can produce serotonin and gamma-aminobutyric acid (Mu et al., 2016). Animal studies have shown that probiotics consumption may increase the level of tryptophan in the plasma and decrease the serotonin concentration in the frontal cortex of the brain. Furthermore, probiotics can reduce dopamine metabolites and thus reduce the symptoms of depression (Desbonnet et al., 2008).

Kefir is a liquid produced from milk by microorganisms, and contains many bacterial species, such as *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, etc. Kefir is known for its beneficial probiotic properties and, therefore, is suggested to be used regularly by patients and neonates (Nielsen et al., 2014).

Feeding mice with kefir helped them improve depression in the long run by altering the effect of microbiota in the gut, metabolism changes, absorption of tryptophan and serotonin, and reducing depression-like symptoms (Sun et al., 2019). *Lactobacillus rhamnosus*, as one of the most important microorganisms in kefir, has many beneficial mental effects (Cheng et al., 2019).

In some types of mental disorders, such as depression, the amounts of some trace elements in the serum are reduced. It seems that their amount affects the duration of the disease, and their measurement as a diagnostic point of treatment can be advantageous. The most important of these trace elements are zinc (Zn) and copper (Cu) (Maes et al., 1999). Serum Zn levels are lower in depressed patients than healthy individuals, and it is associated with the severity of the disease



(Szewczyk et al., 2008). Besides, an inverse correlation between Cu intake and depression status has been shown (Li et al., 2018). The absorption of Cu and Zn ions by the lumen increases in the presence of microbiota. Therefore, we can see an effective relationship between the presence of microbiota and the increased amounts of these ions. Since these ions are involved in mental health, a direct effect can be found between the microbiota-Cu, -Zn, and depression (Namkung et al., 2006, Pérez et al., 2011, Ding et al., 2019). The amount of Cu can affect the amount of serotonin, which might affect the symptoms of depression (Jones et al., 2007). Zn can also affect serotonin levels, and subsequently, serotonin has impact on the depression symptoms (Maes et al., 1999).

Considering the side effects of antidepressant drugs (Tarleton et al., 2016), this is essential to study how to prevent and treat depression in other ways. In the present study, we aimed to study the antidepressant effects of kefir, a probiotic supplement, and *L. rhamnosus* GG, compare their effects, and investigate their potential use in depression treatment.

2. MATERIALS AND METHODS

2.1. Drugs, supplements, and other materials

Fluoxetine 10 mL kg^{-1} (Pars Darou Company, Tehran, Iran) was used daily as an antidepressant drug. A probiotic supplement including safe strains of *L. rhamnosus*, *Lactobacillus casei*, *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium longum*, and *Streptococcus thermophilus* (Zist Takhmir Pharmaceutical Company, Tehran, Iran) with a final concentration of 1.5×10^8 CFU/mL/day was used. Kefir as a probiotic drink was used daily (1.5×10^8 CFU/mL/day). The strain of *L. rhamnosus* GG ATCC 53101 (used with a final concentration of 1.5×10^8 CFU/mL/day) was purchased from the Iranian Probiotics Association (Tehran, Iran).

2.2. Animals

In this experimental study, newborn male BALB/c mice were used, provided by the Laboratory Animals Center, Babol University of Medical Sciences. The present study was done under the ethical principles and the national norms and standards for conducting medical research in Iran (IR.MUBABOL.HRI.REC.1397.064).

2.3. Behavioural models of depression

Maternal separation and chronic mild stress models were used to induce depression in mice. The repetitive 3-h daily separation starting from the second till the 12th postpartum day has been suggested as the most recommended separation model (Vetulani, 2013). Furthermore, the chronic mild stress model has been used (Willner, 2017).

2.4. Depression assessment methods

Forced swimming and tail suspension tests were performed on male BALB/c mice of P49 (postnatal day 49) age to assess the level of depression.

2.4.1. Forced swimming test. A 50 cm high cylindrical container having a diameter of 40 cm was filled with $25 \pm 2^\circ\text{C}$ water up to the height of 25 cm to run the forced swimming test.



Swimming time was considered 8 min in this test. The initial 2-min time was considered adaptation time to the environment for all mice and was not included in the test results (Abbasi-Maleki et al., 2020).

2.4.2. Tail suspension test. Mice were hanged from their tails at the height of 40 cm to run a 6-min tail suspension test. Immobility behaviour was defined as no struggles or paw movements, and their durations were measured as immobility time (Pałucha-Poniewiera et al., 2017).

2.5. Experimental design

Kefir, a probiotic supplement, *L. rhamnosus* GG, and saline as a control group were used separately to investigate their effect on depression-like behaviour in each group of depressed mice ($n = 8$). A fluoxetine-treated group was used as a positive control group. The exact amounts of treatments were administered daily via their drinking water for 30 days. The experimental study in each group involved evaluating/assessing the effects of both sub-chronic and chronic treatments.

2.6. Measurement of zinc and copper

The animals' serums were collected at the end of the experiments (after four weeks of treatments). The serum levels of Zn were detected with a Zinc kit (ZishChem Diagnostics, Iran, sensitivity $4.0 \mu\text{g dL}^{-1}$, detection limit $400 \mu\text{g dL}^{-1}$) using a spectrophotometer (UNICO 2100). Zn in the sample was chelated by 5-Br-PAPS (2-(5-bromo-2-pyridylazo)-5-(N-propyl-N sulphopropylamino)-phenol) present in the reagent, then the formation of this complex was measured at a wavelength of 560 nm.

The serum Cu concentrations were measured by atomic absorption spectrophotometry (PG 990, China, equipped with a graphite furnace). All samples were diluted 1/20 using Triton-X100 0.1% for Cu determination. $10 \mu\text{L}$ of the diluted samples were later injected into the graphite furnace. Working standard solutions were prepared from the stock standards ($1,000 \text{ mg L}^{-1}$, Merck) of Cu for the calibration curve.

2.7. Statistical analysis

P-values less than 0.05 were considered significantly different. The data were analysed by the one-way ANOVA test following Newman-Keuls as a post-hoc test or by two-way ANOVA following Tukey's multiple comparisons test as a post-hoc analysis applying the Graph Pad Prism (version 6) software.

3. RESULT AND DISCUSSION

3.1. Forced swimming test

The swimming time of depressed groups was significantly lower than that of the healthy group (Fig. 1A, $P < 0.001$). No significant difference was observed in the swimming times of different depressed groups.

An increase was observed in the swimming time in depressed groups after the 2-week treatment. The fluoxetine group indicated the highest swimming time, followed by probiotic



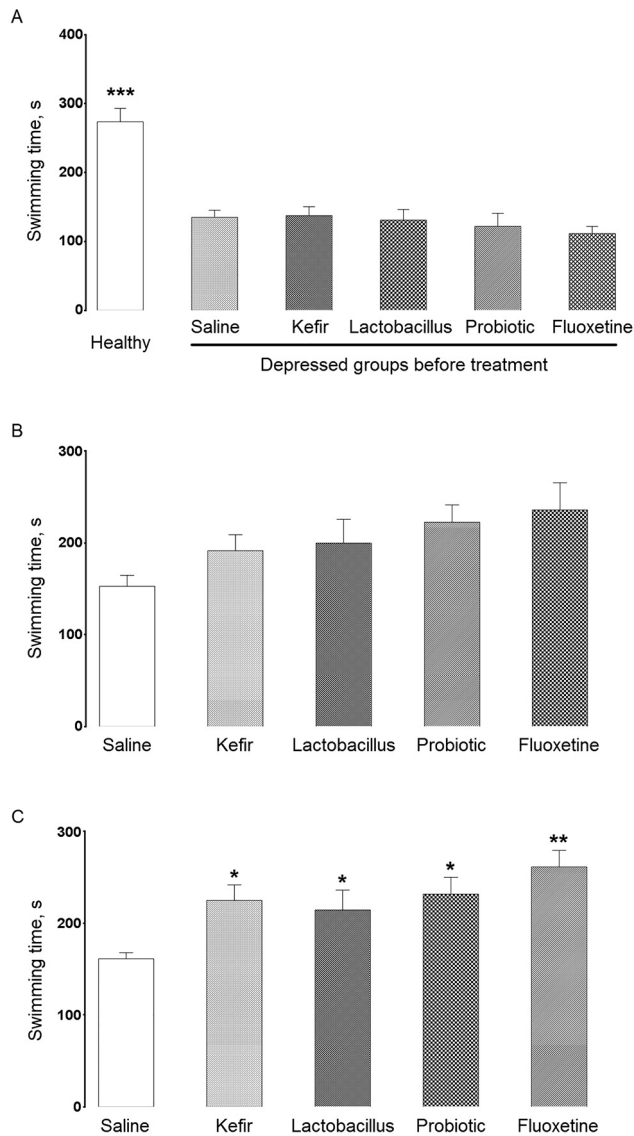


Fig. 1. (A): Swimming time in depressed groups before treatment. (B): Swimming time in depressed groups after two-week treatment. (C): Swimming time in depressed groups after four-week treatment. Each column shows the mean \pm SEM for $N = 8$ rats in each group.

* $P < 0.05$ and ** $P < 0.01$ different from the saline group

supplement, *Lactobacillus*, and kefir. An increasing trend in swimming time was observed after treatments in depressed mice compared to control mice, although it was not statistically significant (Fig. 1B). The swimming time in all groups increased after the four-week time treatment, except the saline one. Among these groups, the fluoxetine treated group showed a



significantly higher increase in the swimming time ($P < 0.01$). Interestingly, the kefir-treated group ranked third in the fourth treatment week, while it ranked fourth in the second treatment week. This indicates that several genera of bacteria associated with kefir have been able to exert even more potent effects in long-term treatments (Fig. 1C).

3.2. Tail suspension test

The first section of this test showed similar results to the forced swimming test, as the struggling times of depressed groups were significantly lower than that of the healthy group ($P < 0.001$; Fig. 2A). It is noteworthy that the differences among depressed groups were not significant. The fluoxetine-treated group also showed the longest struggling time in this test. The struggling time increased after two weeks; however, it was not significant (Fig. 2B).

Interestingly, a significant increase was observed in the struggling time of all groups except the saline group after four-week treatments ($P < 0.001$), similar to the previous test. It is important to note that the increase in struggling time in the *Lactobacillus* group in the third and fourth weeks was lower than that of other groups ($P < 0.05$), but the overall increase in the struggling time of this group was significant after four-week time treatment versus the control group (Fig. 2C).

The results of tail suspension and forced swimming tests in the same groups indicate a significant difference in immobility behaviour after applying subchronic and chronic treatments, which are more notable after a four-week time (the chronic treatment) comparing the pre- and post-treatment situations. Furthermore, the significant increases in the struggling and swimming times of the kefir-treated group after four weeks of treatment were also evident in this section. The two-way ANOVA analysis revealed that this trend in depression-like behaviour in different groups is significant in forced swimming [Time effect: $F(2, 68) = 52.48$, $P < 0.0001$; Interaction: $F(8, 68) = 2.788$; $P < 0.01$] and tail suspension tests [Time effect: $F(2, 68) = 91.39$, $P < 0.0001$; Interaction: $F(8, 68) = 5.009$; $P < 0.0001$].

3.3. Process of weight gain

There was no significant difference regarding the weight of the groups during treatments, and all groups have gained weight in the same pattern during one month [Time effect: $F(5, 170) = 164.9$, $P < 0.001$; Interactions: $F(20, 170) = 1.471$, $P = 0.1$; Fig. 3].

3.4. Measurement of zinc and copper

Zn levels increased in the kefir-, *L. rhamnosus*-, and probiotic supplement-treated groups and decreased in the fluoxetine-treated group versus the control group, but changes were not statistically significant. Cu levels were statistically higher than in those of saline- and fluoxetine-treated groups ($P < 0.01$; Fig. 4).

3.5. Discussion

Most chemical antidepressants used in clinical settings can increase norepinephrine or serotonin levels, or both, in the brain. *L. rhamnosus* has been shown to increase the expression of the serotonin transporter (SERT) gene in the intestine (Wang et al., 2015). The antidepressant effects of *L. rhamnosus* observed in this study was evident by comparing the swimming and



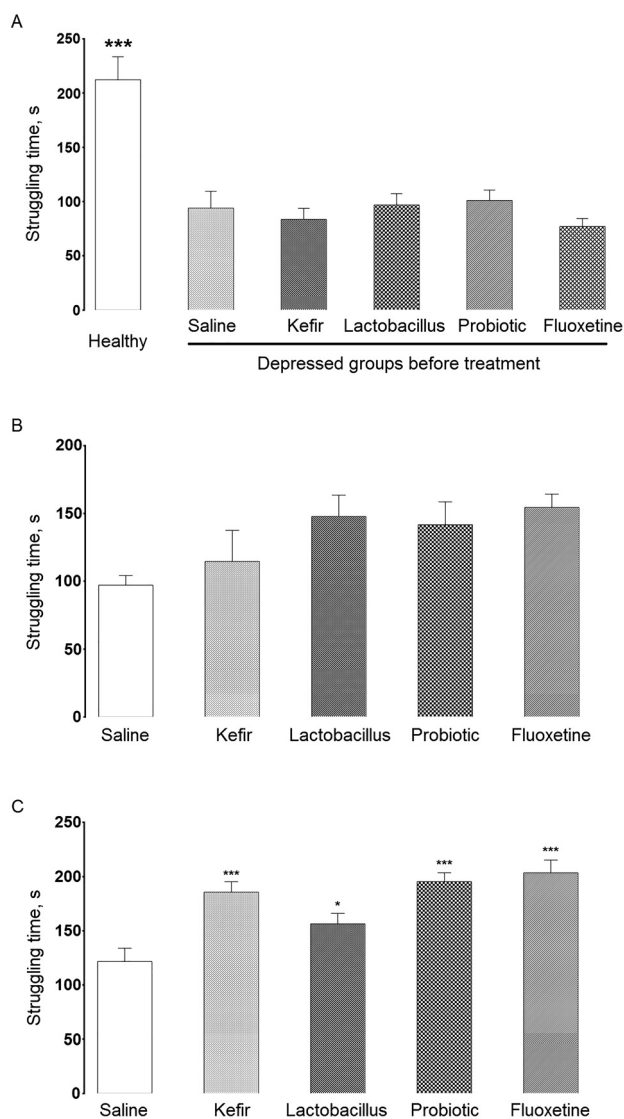


Fig. 2. (A): Struggling time (Tail suspension test) before treatment. (B): Struggling times after two-week treatments; struggling times have been increased, but not significantly. (C): Struggling times after four-week treatments. Each column shows the mean \pm SEM for $N = 8$ rats in each group.

* $P < 0.05$ and *** $P < 0.001$ different from the saline group

struggling times of the *L. rhamnosus*-treated group versus the saline group. These effects might be due to increased SERT expression, increasing serotonin uptake from the intestine, leading to reduction of depression-like symptoms.



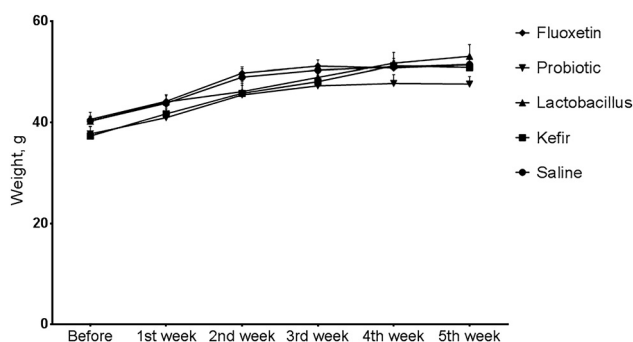


Fig. 3. Comparison of the weights of the experimental groups during treatments. There was no significant difference among different treatments. All groups have gained weight with the same pattern in the timeframe. Each dot shows the mean \pm SEM for $N = 8$ rats in each group

The neurotransmitter carrier of serotonin in the gut can lead to the uptake of digested serotonin in food. Serotonin can also be produced by the microbiota in the gut, which is eventually absorbed into the bloodstream by SERT. The uptake of serotonin into the bloodstream and its transport to the brain can replenish the brain and neurons serotonergic stores (Gershon, 2003).

The intestinal microbiota can affect the host nervous system through the intestinal nervous system (ENS). The ENS is considered “second brain” because of its complexity and independence for regulating the intestine activities (Furness, 2012).

L. casei can increase serotonin levels in the intestine, which may ultimately affect the brain's serotonin levels (Jenkins et al., 2016; Hara et al., 2018). *B. breve* also elevates serotonin levels in the intestine and ultimately in the brain, indicating its positive effect on depression treatment (Savignac et al., 2014; Jenkins et al., 2016; Cao et al., 2018).

L. bulgaricus, *S. thermophilus*, and *L. acidophilus* can hydrolyse proteins using their proteases (Courtin et al., 2002; Azcarate-Peril et al., 2005; Delorme et al., 2010). Increased hydrolysis of proteins can increase the tryptophan amino acid level, and eventually, as a precursor of serotonin, tryptophan can elevate cerebral serotonin levels and may thus have antidepressant effects. The combination of *L. acidophilus* and *B. longum* increases the expression of serotonin transporter genes in the intestine (Cao et al., 2018). The presence of *B. breve* along with *L. acidophilus* and *B. longum* can increase not only the level of serotonin in the intestine but also the expression of serotonin transporter by increasing its uptake. These three bacteria appear to have synergistic effects on treating depressive symptoms. These suggest that probiotic supplements can improve depression-like symptoms, confirming the results of this study.

Kefir proteins are predominantly tryptophan-rich. This amino acid can be converted into serotonin in the brain and thereby increase brain serotonin levels and reduce the symptoms of depression (Chandan et al., 1982).

Zn level has significantly increased in depressed patients treated with citalopram (Schlegel-Zawadzka and Nowak, 2000) but not in the present study. Although the exact correlation between depression-like activities and Cu levels has not been yet determined, the difference between the probiotic versus control groups shows a significant increase, which requires further investigations to figure out the relationship between Cu levels and depression-like activities.



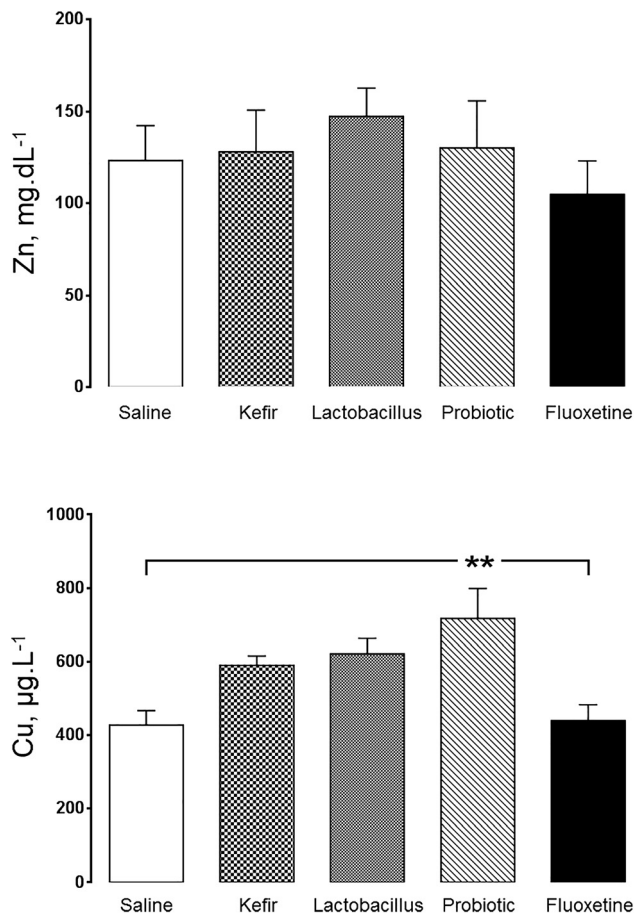


Fig. 4. Measurements of copper and zinc. Each column shows the mean \pm SEM for $N = 8$ rats in each group.

** $P < 0.01$ different from the saline and fluoxetine groups

4. CONCLUSIONS

Probiotics can help improve the symptoms of depression-like behaviour. It is also suggested that the use of multiple genera and species of bacteria in dairy products such as Kefir can relieve the symptoms of depression-like behaviour without harmful side effects on the body. Therefore, using these suggested supplements along with antidepressants is recommended. It may reduce the required dose of these drugs and eventually reduce their side effects. In the future, replacing antidepressants with probiotics may be a great step forward in alleviating depression-like symptoms.

Conflict of interest: The authors declare that there is no conflict of interests.



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