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Effect of probiotics as adjuvant therapy of antidepressants in male Wistar rats with anhedonia behavior

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ABSTRACT

Background: Depression is one of the most common causes of disability in the world, and one third of patients do not respond to currently available antidepressants, so a strategy is needed in its management. There have been many studies looking for a relationship between the role of the gut and mental health. Probiotics are the focus of much research in the field of psychiatry today due to their alleged role in improving mental health through gut health.
Methods: This research was a true experimental study with a posttest-only design with a control group design. Brain-derived neurotrophic factor (BDNF) plasma levels, serum serotonin (ST) serum levels, and sucrose preference test (SPT) values were analysed in 39 male Wistar rats with anhedonic behavior.
Results: Average BDNF plasma level in treatment group was 1.63 ng/mL with a standard deviation of 0.56 ng/mL, while the mean BDNF plasma level in control group was 0.89 ng/mL with a standard deviation of 0.46 ng/mL. There was a significant difference in that the BDNF plasma levels in the control group were lower than in the treatment group, with a $p<0.001$ (CI = 95%). The median ST serum level in the treatment group was 25.78 with an IQR of 9.61 ng/mL, while the median ST serum level in the control group was 7.5 with an IQR of 0.38 ng/mL. Statistically, the difference is significant from the p value <0.001. The median SPT score in the treatment group was 84.5 with an interquartile range (IQR) of 12%, while the median SPT value in the control group was 67 with an IQR of 8%.
Conclusion: There was an effect of probiotics as an adjuvant antidepressant therapy in male Wistar rats with anhedonic behavior.

Keywords: probiotic, depression, anhedonia, brain-derived neurotrophic factor, serotonin.
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INTRODUCTION

Depression is one of the most common causes of disability in the world. Many antidepressants are available, but one third of patients do not respond to the currently available antidepressants, so it is necessary to develop a strategy in the management of patients with depression.[1] There have been many studies looking for a relationship between the role of the gut and mental health, especially depression. Probiotics are the focus of much research in the field of psychiatry today due to their alleged role in improving mental health through gut health. Depression is a common condition found worldwide, with an estimated 3.8% of the world's population.[1] Depression has the highest lifetime prevalence (17%) of all psychiatric disorders.[2,3] More than 75% of people in low- and middle-income countries do not receive treatment for depression. Lack of resources, effective treatments, trained service providers, knowledge, misconceptions, and stigma about people with depression are barriers to improving mental health.[1]

Treatment of depression is not only with medication and psychotherapy, but also with lifestyle modifications such as exercise and diet. Several recent research analyzes support a relationship between what a person eats and the risk of depression.[4] An imbalance in the representation of gut bacteria (dysbiosis) is known to cause various diseases including depression.[5] This is conceptualized through the gut-brain axis (GBA), a bidirectional "dual pathway" system that contributes to the maintenance of gastrointestinal function, and connects emotional and cognitive centers in the brain with the gut, so that probiotics are thought to be beneficial in reducing depressive symptoms.[6,7]

Anhedonia as one of the core symptoms of depression that is most often resistant to treatment, is used in behavioral tests in mice that represent an animal model of depression, assessed by the sucrose preference test (SPT).[6,7] Anhedonic behavior is reduced motivation to reward which results in significant weight loss. Brain-derived neurotrophic factor (BDNF) and serotonin (5-hydroxytryptamine/5-

HT/ST) have recently been studied extensively for their role in depression.[7] BDNF plasma levels, serum serotonin levels, and SPT values which will be the focus of this study have been commonly studied in relation to depression in experimental animals so that they are able to represent the concept of this study.

Nearly all of the human genes associated with disease have counterparts in mice. Mice are used to model several human behaviors because some of their brain structures resemble more primitive elements of the human brain,[8] so researchers chose mice to be used as experimental animals in this study. Although there have been many studies that have focused on GBA on its role in mental health, especially depression, this relationship is still controversial. This study aims to know the bidirectional link and the effect of probiotics as an antidepressant adjuvant therapy in the treatment of depression which can be recommended for their use later.

METHODS

Research design

This research was a true experimental study with a posttest-only design with a control group design. This study was divided into 2 groups, the treatment group and the control group. The treatment group was male Wistar rats with anhedonic behavior who received antidepressants and probiotics as an antidepressant adjuvant therapy, while the control group was male Wistar rats with anhedonic behavior who received antidepressants only. BDNF plasma levels, serotonin (ST) serum levels, and sucrose preference test (SPT) values will be assessed once on the 14th day.

Location and time of research

The research was conducted at the Integrated Biomedical Laboratory Unit, Faculty of Medicine, Universitas Udayana in the period February 2022-July 2022.

Data source determination

Population

The target population in this experimental study were all Wistar male rats (*Rattus norvegicus*) with anhedonic behavior. Anhedonic behavior is the reduction or loss of response to pleasure, in this study dexamethasone was induced to cause anhedonic behavior. Dexamethasone induction is the administration of dexamethasone 1.5 milligrams per kilogram (mg/kg) body weight (BB) mixed with normal saline (sodium chloride/ NacL 0.9%) to achieve the required dose, administered subcutaneously. The affordable population were Wistar strain male rats (*Rattus norvegicus*) obtained from the Department of Pharmacology, Faculty of Medicine, Universitas Udayana and received anhedonic behavior induction.

Sample

The sample used in this study was taken from an affordable population of male rats (*Rattus norvegicus*) of the Wistar strain that met the inclusion criteria. Samples were cultured in the Department of Pharmacology, Faculty of Medicine, Universitas Udayana until the body weight reached 200-250 grams. Samples that met the inclusion criteria were then transferred to the Integrated Biomedical Laboratory, Faculty of Medicine, Universitas Udayana for acclimatization for 7 days. Samples were divided into 2 groups, samples in each group underwent research for 14 days. Inclusion criteria: Wistar rat, body weight ranges from at least 150-200 grams after dexamethasone injection, male gender, with anhedonic behavior.

Sample size

The required sample size from this calculation method is 19 rats per group. 20% of the 19 rats were added because of the possibility of dropping out, so 23 rats per group were needed. The total sample required is 46 rats.

Research procedure

Preparation, induction, and intervention in experimental animals

The samples were male Wistar rats (*Rattus norvegicus*) with a weight of 150-200 grams. Rats were housed in animal care facilities at the Integrated Biomedical Laboratory, Faculty of Medicine, Universitas Udayana. Preparation, induction, and intervention in experimental animals using the following procedures:

a. Samples that met the research requirements (inclusion criteria) were acclimatized for 7 days in the laboratory before the study started, the room was maintained at a temperature of 25 ± 20°C with a cycle of 12 hours light-12 hours dark (lights turned on at 8 a.m. and turned off at 8 p.m.), was fed and watered *ad libitum*.
b. After acclimatization, each sample received an injection of 1.5 mg/kgBW/ day of dexamethasone mixed with NaCl to achieve the required dose, given subcutaneously at the same hour every day for 7 days. Food and drink are still provided *ad libitum*.
c. After 7 days of receiving dexamethasone injection, the sample is then divided into 2 groups which will receive different treatment. The treatment group received escitalopram and probiotics, while the control group received escitalopram alone. Escitalopram dose of 10 mg/kgBB/day dissolved in water to reach the required dose, administered intraorally using a sonde at the same time every day for 10 days (day 1 to day 10). Probiotics at a dose of 10^7 CFU/rat were administered intraorally using a sonde at the same time every day for 10 days (day 1 to day 10). Food and drink were still provided *ad libitum*.
d. The sample then underwent a sucrose preference test (SPT) for 3 days. SPT assessment was carried out, blood sampling was carried out to check plasma levels of BDNF and serum serotonin on the 14th day. Examination of plasma levels of BDNF and ST serum using the enzyme-linked immunosorbent assay (ELISA) method. Samples were returned to their habitat after the research was completed.

RESULTS

Basic characteristics of research subjects

True experimental research with a posttest-only design with a control group design. A total of 46 male rats (*Rattus norvegicus*) Wistar strain received subcutaneous injection of dexamethasone 1.5 mg/kg/ day for 7 days to induce anhedonia. One of the markers of anhedonia in experimental animals is weight loss. Male Wistar rats with a weight ranging from 150-200

grams were then divided into two groups, namely the treatment group (P) received 10 mg/kg BW escitalopram and probiotics 10^7 CFU/100 gram BW intraorally for 10 days, and the control group received escitalopram 10 mg/kg intraorally for 10 days. A total of 39 male Wistar rats with anhedonic behavior remained until the end of the study, 20 in the treatment group and 19 in the control group. BDNF plasma levels, ST serum levels, and SPT values were assessed once on day 14. BDNF plasma and serum serotonin levels were measured by taking a blood sample from the retro-orbital vein which was then examined using the ELISA method.

Data normality test

BDNF plasma levels, ST serum levels, and SPT values were tested for normality using the Shapiro-Wilk test. The results showed that the data for BDNF plasma levels were normally distributed in each group ($p \geq 0.05$), while the data for ST serum levels in the control group and SPT values in treatment and control groups were not normally distributed ($p \leq 0.05$).

Table 1. Normality test of BDNF plasma level, ST serum level, and SPT value.

Variable	Group	Saphiro-Wilk		
		Statistic	n	p-value
BDNF plasma level (ng/mL)	Treatment	0.961	20	0.573
	Control	0.906	19	0.062
ST serum plasma (ng/mL)	Treatment	0.942	20	0.264
	Control	0.787	19	0.001
SPT	Treatment	0.828	20	0.002
	Control	0.816	19	0.002

BDNF: Brain-derived neurotrophic factor; ST: Serotonin; SPT: Sucrose preference test

Table 2. Homogeneity test of BDNF plasma levels.

Variable	Levene's Test	
	F	P
BDNF plasma level (ng/mL)	0.222	0.640

BDNF: Brain-derived neurotrophic factor

Tabel 3. Effect of probiotics on BDNF plasma levels.

Variable	Group		Mean (95% CI)	p-value
	Treatment (n=20)	Control (n=19)		
BDNF plasma level (ng/mL), mean±SD	1.63±0.56	0.89±0.46	0.74 (0.401-1.071)	<0.001

BDNF: Brain-derived neurotrophic factor

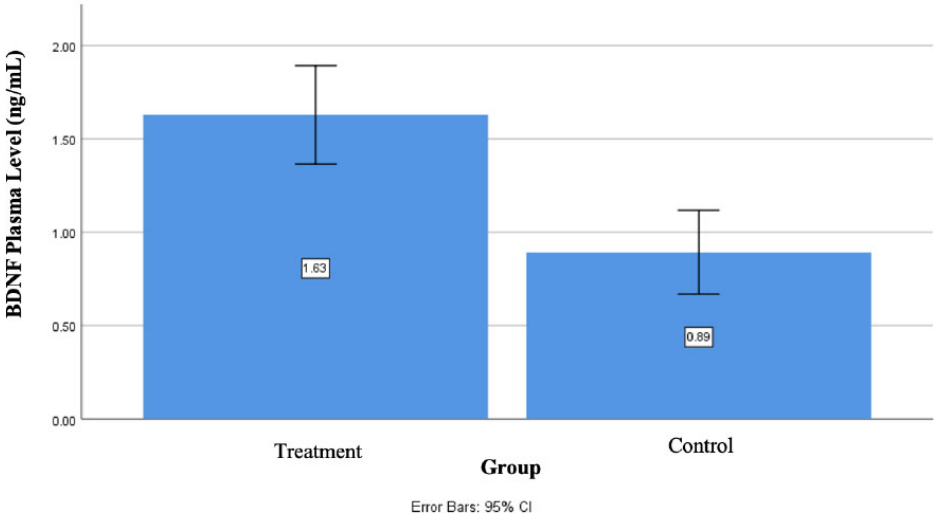

Figure 1. Comparison of mean plasma levels of BDNF between the treatment group and the control group.

Data Homogeneity Test

A homogeneity test was carried out for the variance of BDNF plasma levels because the data were normally distributed in the normality test, the data variance between groups was homogeneous ($p \geq 0.05$).

Effect of probiotics as adjuvant antidepressant therapy on BDNF plasma levels

The mean BDNF plasma level in group P was 1.63 ng/mL with a standard deviation of 0.56 ng/mL, while the mean BDNF plasma level in group K was 0.89 ng/mL with a standard deviation of 0.46 ng/mL. There was a significant difference in that the BDNF plasma levels in group K were lower than in group P, with a $p<0.001$ (CI = 95%). The results of the analysis use the independent t-test because the data is normally distributed.

Effect of probiotics as adjuvant antidepressant therapy on ST serum levels

Data on ST serum levels in control were not normally distributed, so the statistical measures used to compare ST serum levels between groups were the median and the interquartile range (IQR). The median ST serum level in group P was 25.78 with an IQR of 9.61 ng/mL, while the median ST serum level in the control group was 7.5 with an IQR of 0.38 ng/mL. Statistically, the difference was significant as seen from the p value <0.001 and the results of visualizing differences in the range of ST serum levels between groups using a boxplot where it was found that the lowest ST serum levels in treatment group were still higher than the highest ST serum levels in control group. The results of the analysis using the Mann-Whitney test.

Effect of probiotics as adjuvant antidepressant therapy on SPT values

Data on SPT values in treatment group and control group were not normally distributed, so the statistical measures used to compare SPT values between groups were the median and the interquartile range (IQR). The median SPT score in the

treatment group was 84.5 with an IQR of 12%, while the median SPT score in the control group was 67 with an IQR of 8%. Statistically, the difference is significant as seen from the p value <0.001 and the results of visualizing the differences in the range of SPT values between groups using a boxplot where the lowest possible SPT value in the treatment group is still higher than the highest SPT value in the control group. The results of the analysis using Mann-Whitney test.

Table 4. Effect of probiotics on ST serum levels.

Variable	Group		p-value
	Treatment (n=20)	Control (n=19)	
ST serum level (ng/mL), median (IQR)	25.78 (9.61)	7.5 (0.38)	<0.001

ST: Serotonin

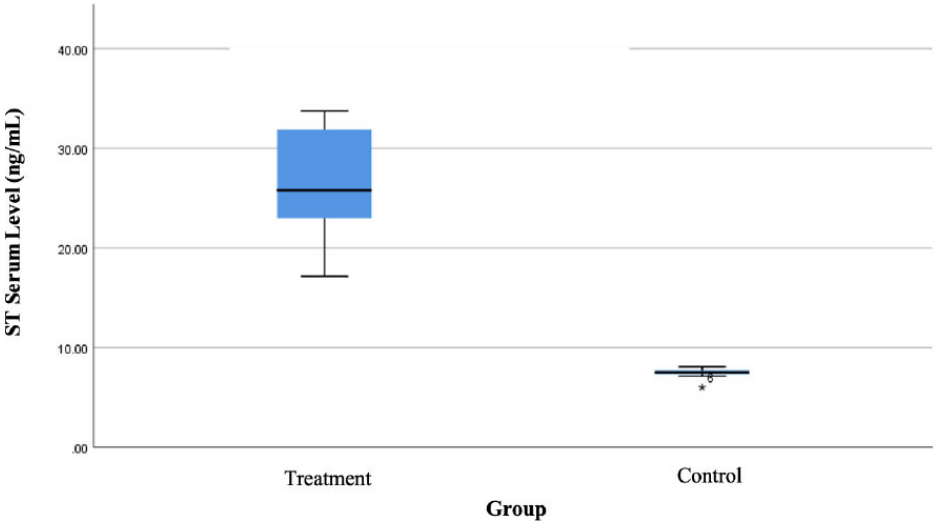

Figure 2. Comparison of ST serum levels between the treatment group and the control group.

Table 5. Effect of probiotics on sucrose preference test (SPT) values.

Variable	Group		p-value
	Treatment (n=20)	Control (n=19)	
Nilai SPT, median (IQR)	84.5 (12)	67 (8)	<0.001

SPT: Sucrose preference test

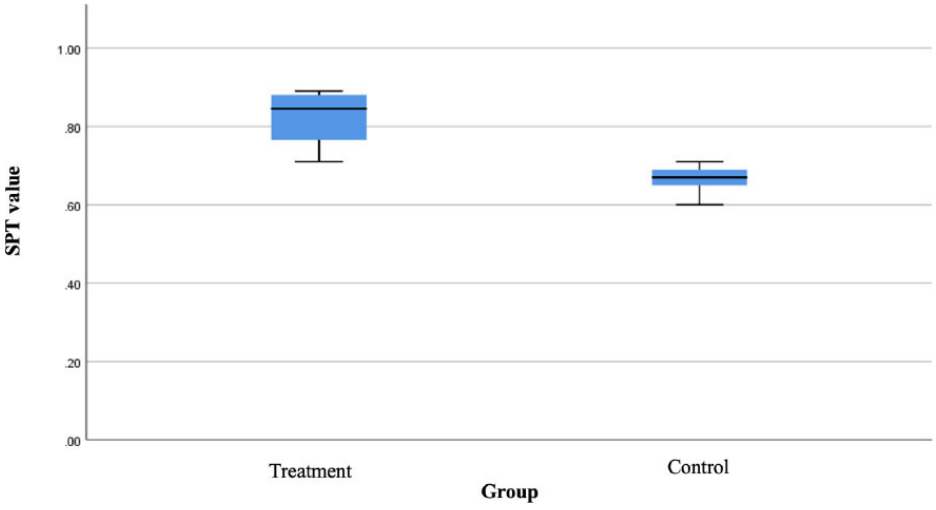

Figure 3. Comparison of SPT values between the treatment group and the control group.

DISCUSSION

Effect of probiotics as adjuvant antidepressant therapy on BDNF plasma levels

BDNF is a protein present in humans encoded by the BDNF gene. BDNF is part of the neurotrophin group that has a lot of influence in supporting the function and development of neurons, with its receptor being Tropomyosin receptor kinase B (TrkB). Brain-derived is widely expressed in areas of the brain with a high level of plasticity, namely in the hippocampus, hypothalamus, and cortex.[9] BDNF plays a role in central nervous system neurons to support the existence of neurons, helps the growth and differentiation of new neurons, increases synaptogenesis, plays a role in neurogenesis, and is able to protect neural stem cells (NSC) and neural precursor cells (NPC).[9]

According to previous studies, BDNF has a significant role in depression.[10] A decrease in BDNF can cause brain dysfunction and lead to depression.[11] Several postmortem studies have shown decreased BDNF expression in the hippocampus and prefrontal cortex in patients with depression. A study evaluating the relationship between chronic stress, BDNF, and depression in sows showed decreased BDNF protein levels, increased cortisol levels and adrenal weight. This effect was more pronounced in stress-induced sows, suggesting that chronic stress progressively lowers BDNF protein levels resulting in degeneration of neurons in brain areas responsible for memory and mood.[12]

Several studies have shown that serum and BDNF plasma levels decrease in depressed patients.[13] Research by Hacimusalar et al. demonstrated low serum BDNF levels as a peripheral marker associated with suicidal ideation in depressed patients.[14] There is evidence to suggest a decrease in BDNF platelet levels in drug-free depressed patients compared to healthy controls. Serum

BDNF levels in antidepressant-naive patients with depression were significantly lower than in treated patients or healthy control subjects, indicating that serum BDNF concentrations increased during antidepressant treatment. This shows the important role of BDNF as a biomarker of disease pathogenesis, which not only acts as a marker of disorders, but also as a predictor of the effectiveness of antidepressants.[13] Kleins et al. conducted a study to evaluate whether blood BDNF levels could represent brain tissue BDNF levels, the results obtained a positive correlation between whole blood BDNF levels and hippocampal BDNF levels in experimental animals.[15]

The results of this study showed that the mean BDNF plasma level in group P was 1.63 ng/mL with a standard deviation of 0.56 ng/mL, while the mean BDNF plasma level in group K was 0.89 ng/mL with a standard deviation of 0.46 ng /mL. There was a significant difference where the BDNF plasma levels in the control group were lower than in the treatment group, with a $p<0.001$. The results of the analysis use the independent t-test because the data is normally distributed. In this study, dexamethasone was induced for 7 days to induce anhedonic behavior at a dose of 1.5 mg/kg BW mixed with 0.9% NaCl until the required dose was reached, administered subcutaneously (SC). Dexamethasone has been shown to be able to induce anhedonic behavior and reduce body weight in experimental animals in previous studies.[16,17,18] Body weight is an indicator of distress in experimental animals and is used as an objective sign of pain and discomfort. In experimental animal studies, >20% weight loss is considered severe distress.[19] The body weight of the experimental animals in this study decreased by an average of 18% after 7 days of dexamethasone induction. This study used 10 mg/kgBW/day of escitalopram dissolved in water and administered intraorally (IO) for 10 days. The reason for using escitalopram is because escitalopram is not affected by food administration, with an elimination half-life of 27-33 hours and is consistent with once-daily administration. The reason for using 10 days is because steady state concentrations will be reached within 7-10 days after administration.[20] This study also did not combine male and female rats because there were differences in body weight and developmental stages. The body weight and developmental stages of the experimental animals used are characteristics that can affect the results of the study. The age and body weight of experimental animals can affect drug metabolism, gene expression, metabolic parameters, and other dependent variables measured in experimental animal studies.[19]

BDNF is produced by neurons, microglia, and astrocytes in the brain plays an important role in the survival, maintenance, differentiation, and synaptic plasticity of neurons. Measurements of blood and BDNF plasma levels are considered to reflect brain tissue BDNF levels, and decreased serum BDNF levels have been widely evaluated in depressed patients.[15,21,22] Structural imaging studies show that serum BDNF levels correlate with hippocampal volume, that is, lower volumes are found in depressed patients compared to healthy controls.[23] Furthermore, animal studies have shown that SSRI administration increased hippocampal BDNF levels and neurogenesis, accompanied by a decrease in depressive-like behavior. These results were confirmed in human studies, in which antidepressant treatment improved the decrease in serum BDNF levels in depressed patients along with improving clinical symptoms.[24,25]

Several factors are known to affect BDNF levels, including exposure to excessive stress, inflammation, and aging. In addition to these factors, the gut microbiota has been reported to play an important role in controlling BDNF levels. Sudo et al. demonstrated that hippocampal BDNF levels decreased in germ-free mice compared to specific pathogen free mice.[26] Intestinal microbiota-derived metabolites, especially short-chain fatty acids, have been confirmed as molecular mediators in the microbiota-gut-brain (MGB) axis, and butyrate is one of the products that can link gut microbiota with brain BDNF regulation. After absorption in the large intestine, butyrate is used by colonocytes to produce energy, some of which then travels through the systemic circulation and reaches the brain through the blood-brain barrier.[15,21] In animal studies, butyrate accelerated BDNF expression in the hippocampus through inhibition of histone deacetylase.[27] Research on animals with a chronic mild stress (CMS) model shows that administration of butyrate-producing bacteria reduces depressive-like behavior with increased levels of BDNF.[28,29]

Effect of probiotics as adjuvant antidepressant therapy on serotonin serum levels

There is a decrease in the neurotransmitter serotonin in depression. In recent years, it has been found that there is a connection between the brain and the digestive tract (gut-brain-axis) for this condition. The existence of a new role related to gut microbiota with depression can be one of the potentials for the development of antidepressants. In a systematic review, daily consumption of probiotic supplements can have a positive effect on improving mood, overcoming anxiety, and improving cognitive symptoms without serious side effects in patients with major depression.[30] This is consistent with studies in experimental animal models which reported similar findings, an improvement in depressive-like behavior in experimental animals receiving probiotics.[31] Another study found a decrease in serotonin levels in experimental animals with depressive symptoms. It is known that low serotonin levels are associated with a lack of *Bifidobacterium infantis* and *Lactobacillus rhamnosus* JB-1, which can also reduce depressive-like behavior by changing the expression of messenger ribonucleic acid (mRNA) receptor gamma aminobutyric acid (GABA).[32] In a clinical study conducted in Australia, it was found that all clinical trial participants showed an improvement in depression symptoms. Participants in the probiotic group showed significantly greater improvement in depressive symptoms compared to the placebo group.[33] A probiotic product consisting of eight bacterial strains given to experimental animals (*Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24,

Lactococcus lactis W19, and *Lactobacillus lactis* W58) has been shown to reduce symptoms depression by 34% (95% CI: 22%-44%).[34] The findings of this study are in line with previous studies, namely serum serotonin levels were higher in the treatment group compared to the control group which was not given probiotics in experimental animal models (p<0.001). This study found that the lowest ST serum level in the treatment group was still higher than the highest ST serum level in the control group.

Several reports have demonstrated that the gut microbiota can influence and modulate emotional behavior, suggesting that probiotics may be useful agents for reducing depressive symptoms. One known mechanism is by inhibiting systemic inflammation.[31] Increased expression of the proinflammatory cytokines IL-1β, IL-6, TNF-α, and IFN-γ, as well as CRP, is frequently found in patients with depression. Dysbiotic conditions cause dysfunction of the intestinal mucosal lining which results in translocation of bacteria to the systemic circulation, causing an increase in systemic inflammation. Systemic inflammation can cause neuroinflammation through the HPA axis, which occurs due to increased expression of microglia and astrocytes.[30,35]

Although several studies have reported a positive effect of probiotics on depression, several studies have not found similar results. Research conducted by Tillmann et al found no significant difference in improving cognition and improving depressive-like behavior in the experimental animal group, namely Flinders Sensitive Line (FSL) rats given probiotics.[36] Another study conducted by Neufeld et al in the BALB/c and Swiss Webster (SW) strains of mice towards the probiotic *L. rhamnosus* showed that although the group of mice with the BALB/c strain gave a positive response to the treatment, the group of mice with the SW strain showed no response.[37] This suggests that the expected antidepressant effect of probiotic administration may not apply to all types of mouse strains.

Effect of probiotics as adjuvant antidepressant therapy on SPT values

In this study, administration of dexamethasone 1.5 mg/kgBW/day to induce anhedonic behavior in samples was carried out by subcutaneous injection (SC) technique. In the first experiment, the researchers tried to inject dexamethasone intraperitoneally, but considering the risk of inflammation of the peritoneum or internal organs, the researchers decided to change the dexamethasone injection technique to subcutaneous (SC). This is supported by the findings of previous studies which show that SC injection has several advantages and can prevent some of the disadvantages of intraperitoneal injection. This is because the SC injection technique can create large volumes of injected material deposits (1 mL per 100 grams of body weight). In addition, the SC injection technique is also relatively simple, with more choices of injection sites compared to intraperitoneal techniques.[38]

Intraperitoneal injection is the most used method of administering compounds to experimental animals. This is because the technique allows injections to be made over a large surface area of the abdominal cavity, accompanied by many surrounding blood vessels which can facilitate rapid absorption of the injected material. This injection technique has several drawbacks, such as repeated injections into the peritoneum and in the long term, there is a risk of causing paralytic ileus or inflammation of the peritoneum which can cause adhesions to the internal organs of the experimental animals. In addition, intraperitoneal injection also has the risk of injuring the internal organ structures of experimental animals at the time of injection, such as injury to the kidneys, intestines, and bladder because the distance between the skin and internal organs is relatively close. The error rate in the intraperitoneal injection technique is quite high, even in experienced researchers, which is 11% to 20%.[38,39]

This study found that the experimental animals that were given probiotics as adjuvant antidepressant therapy had a higher median SPT score than the experimental animals in the control group that were only given antidepressants (84.5% vs. 67%), which was a statistically significant result (p<0.001). This study found that the lowest SPT value in group P who received probiotics as an adjuvant antidepressant therapy was still higher than the highest SPT value in group K. The findings of this study are in line with several previous research findings.

Research Zhao et al. found that experimental animals that received corticosterone along with a probiotic in the form of Lactobacillus plantarum DP189 had the same SPT values as the group that received corticosterone along with fluoxetine, and higher than the group that received corticosterone alone (p=0.008).[40] These results are in line with the study of Mesripour and Rakhshankhah which showed that administering a synbiotic solution (probiotics + prebiotics) could increase SPT values by up to 82% in experimental animals with depressive-like behavior induced by dexamethasone, compared to the control group which was only 60%.[41] Furthermore, research by Li et al. who applied chronic mild stress (CMS) to experimental animals showed a significant decrease in SPT values when compared to the control group who were not given anything and, in that study, SPT values showed a significant increase after administration of prebiotics in the form of fructooligosaccharides (FOS) and galacoligosaccharides (GOS) as well as probiotics in the form of *Bifidobacterium longum* and *Lactobacillus rhamnosus*. In this study it was seen that the group that received *Lactobacillus rhamnosus* showed the highest increase in SPT values, followed by the *Bifidobacterium longum* group and the FOS/GOS group.[42]

Research by Chen et al also showed consistent results. In that study, depressive-like behavior in experimental animals was induced with a 300 mg/mL lead acetate solution for 24 weeks, divided into groups that received probiotics and those without probiotics (Lactobacillus and Bifidobacterium). The results of this study indicated that experimental animals exposed to lead acetate solution had lower SPT values (<50%) compared to experimental animals exposed to lead acetate solution and probiotics (±80%), even approaching SPT values in the control group. not exposed to lead acetate.[43]

Several previous studies have shown that giving probiotics can help improve depressive-like behavior in experimental animals. This is because the administration of probiotics can affect

the composition of the gut microbiota. Probiotics are intestinal microorganisms that provide beneficial effects, namely inducing changes in the composition of the intestinal microbiota. The absence of this commensal microbiota will affect the expression of genes related to monoamine neurotransmitters which can cause changes in several neurotransmitters that play an important role in the occurrence of stress, such as serotonin (5-HT), dopamine (DA), norepinephrine (NE), and several other neurotransmitters. Furthermore, several previous studies have also found the fact that the gut microbiota can play a role in regulating brain development and function through the microbiota-gut-brain axis.[40,43]

CONCLUSION

Giving probiotics as adjuvant antidepressant therapy has an effect on brain-derived neurotrophic factor (BDNF) plasma levels, serotonin serum levels (5-hydroxytryptamine/5-HT/ST), and sucrose preference test (SPT) values which are higher than the control group in rats Wistar male with anhedonia behavior.

CONFLICT OF INTEREST

The authors affirm that there are no conflicts of interest in this study.

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ETHICAL CLEARANCE

This study has obtained ethical clearance from the Ethics Committee of Faculty of Medicine, Universitas Udayana with reference letter number 507/UN14.2.2.VII.14/LT/2022.

AUTHOR CONTRIBUTION

All authors contributed equally in this research and publication of this manuscript.

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