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The Combined Use of SCD Probiotics and Tauroursodeoksikolik Asit (TUDCA) is More Effective in Controlling Anxiety-Like Behavior in Aged Rats

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Abstract
In this study, the effects of TUDCA administration with a SCD probiotics for one week on locomotors activity and anxious behavior were investigated. Male Saprague-Dawley rats aged 24 months were used as the model organism. At the end of the application, open-field and elevated plus maze behavioral tests were performed on the rats. Although SCD probiotics were more effective on anxious behavior, the group in which they were administered together had a significantly greater effect on locomotor activity and anxious behavior. This study provides important evidence that combining SCD probiotics and TUDCA administration may be more beneficial to behavior.

1. Introduction

The gut microbiota (GM) and its metabolites play a role in modulating gastrointestinal functions by affecting intestinal permeability, motility, and sensitivity, mucosal immune function, and the release of neurotransmitters and gastrointestinal hormones from enteroendocrine and enterochromaffin cells [1]. Preclinical data also demonstrates that GM and its compounds influence a variety of behavioral and brain functions, including stress response, emotional reactions, pain modulation, eating behavior, and brain biochemistry [2]. The majority of probiotics are found in the human digestive tract, where they can aid the host by helping to keep the microbiota in balance [3]. Daily life often involves the ingestion of active bacteria preparations containing popular probiotics like Lactobacillus or Bifidobacterium [4]. A great deal of progress has been made in the study of probiotics in recent years, and a great many studies have shown that probiotics play a crucial role in preserving human health. In the case of Crohn's disease and other chronic inflammatory disorders, probiotics can be helpful in managing symptoms. In addition, probiotics may reduce the risk of developing cancer, obesity, and diabetes. There is growing proof that probiotics can help strengthen immune systems and keep people healthy [5]. With different strains of probiotics, a wide variety of effects of gut microbial modulation were reported on emotional behavior, social interactions, learning and memory, and ingestive behaviors [2].

The numerous microbial metabolites of dietary substrates, such as bile acids and short-chain fatty acids (SCFAs), constitute the primary basis for the regulatory activities of the gut microbiota. Bile acids are metabolic products of dietary substrates. Their function is crucial in metabolic processes and immune system modulation [6]. While the liver is responsible for synthesizing primary bile acids from cholesterol, several bacterial species in the intestines including Clostridium and Eubacterium are responsible for converting them into secondary bile acids [7]. Intestinal dysbiosis and inflammation are brought on by bile acid dysregulation or bile acid-activated receptor dysfunction [8]. Regulation of bile acid production and metabolism is a major function of the gut microbiota [9]. In humans, the microbiota in the intestines is the only source of ursodeoxycholic acid (UDCA), a secondary bile acid. The conjugation

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of taurine with UDCA occurs in the liver via enterohepatic circulation [10]. TUDCA has been shown to resolve endoplasmic reticulum stress by functioning as a chemical chaperone [11]. This study also showed that TUDCA improved the gut microbiota in high-fat-fed mice, which in turn reduced the absorption and production of lipids in the small intestine [12]. TUDCA has been found to be neuroprotective in many animal models of neurological illness [13]. In this study, the effects of SCD Probiotics and TUDCA administered to aged rats for one week on anxious behavior and locomotor activity were evaluated.

2. Material and Method

2.1. Animal Studies

The male Sprague-Dawley rat (24-month old) species was used. Four groups were formed: the control group (n = 7) and the group that TUDCA administered group (n = 7) for 7 days; the group that was administered SCD probiotics (n = 7) for 7 days; and the group that TUDCA administered during the SCD probiotics supplementation (n = 7) for 7 days. The usual rat food was provided on an ad libitum basis to the animals. TUDCA was administered intravenously from the tail to each animal at 300 mg/kg [14]. The SCD probiotics supplement was given by oral gavage at a dose of 3 mL (1 x 108 CFU) per day [15]. SCD Probiotic Human Beverage contains *Bacillus subtilis, Bifidobacterium bifidum, Bifidobacterium lognum, Lactobacillus acidophillus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus plantarum, Lactococcus lactis, Saccharomyces cerevisiae*, and *Streptococcus thermophiles* species. One day after the end of the application, open field and elevated plus maze behavioral tests were applied to the rats in the experimental and control groups, respectively.

2.2. Behavioral Tests

2.2.1. Open field test (OF)

The open-field test evaluates the rats' locomotor activity as well as their anxiety levels. The test was done in a 60 x 60 x 50 centimeter square box made of untreated, black-painted wood. The animal was placed at the side wall's middle. Computer-aided video caught the animal's locomotion. The open field was divided into sixteen equal squares by virtual lines, twelve of which made up the arena's periphery zone, and four of which made up its core zone. The technology monitored 10 minute intervals of time and distance in each zone [16].

2.2.2. Elevated plus maze test (EPM)

The EPM test is used to assess the level of anxiety in mice and rats. It consists of a platform (10×10 cm) in the center, two open arms (50×10 cm) and two (50×10 cm) arms covered with plastic glass 30 cm high and consists of in the apparatus designed as a plus, open arms and closed arms face each other. The cross-shaped labyrinth consisting of four arms is at a height of 80 cm from the ground. For each experiment, only one rat is placed in the center of the cross maze and allowed to explore the maze for five minutes. At the end of the given time, the time spent by the mouse in the closed and opens arms and the numbers of crossing between arms are recorded with a computer-aided video system [16].

2.3. Statistics

The statistical analysis results were given as the mean ± value plus the standard error of the mean (SEM). One-Way Anova test was utilized to compare the behavior between the control (Cnt), Tudca (Tudca), SCD probiotics (Prb), and Tudca administration during the SCD probiotics supplementation (TdPrb) groups. The comparison was carried out using the GraphPad Prism 9 program (GraphPad Software, USA). The degrees of significance were denoted as * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

3. Results and Discussion

The effects of aging are most evident on cognitive states and behavior. In particular, there is a significant decrease in learning and memory abilities [17]. This situation also triggers anxious behavior [18]. Today, many different approaches continue to be tested to reverse the effects of aging. As the importance of the role of GM in health and disease conditions was understood, probiotic bacteria that play a role in the regulation of gut microbiota moved to a more important position [19]. There are many different probiotic products available commercially today. TUDCA is another common product that is easily accessible commercially, owing to its regulatory role on intestinal microbiota. However, the positive effects of both probiotic use and TUDCA on cognitive states and behavior make them even more important [13,19]. In this study, the effects of co-administration of TUDCA and SCD probiotics human beverages containing 11 different probiotic bacteria on locomotor activity and anxious behavior in aged rats were investigated. There was no significant difference between the groups in terms of time spent in motion in the periphery in the first 5 minutes of the open field test (p = 0,1629) (**Figure 1A**). However, the animals in the TdPrb group, in which the TUDCA and SCD probiotics were given together, appear to be willing to investigate the test apparatus further. Since the rats used in the study are 24 months old, which corresponds to 60–70 years of age for humans, the differences that are not significant are actually valuable [20]. Despite the fact that there was no statistically significant difference between the groups in terms of the central area studies in the first 5 minutes (p = 0.1096), it is seen that the rats in the Tudca group explored the central area more than the other groups (**Figure 1B)**. At the second 5 minutes, there was a significant difference between the groups in the periphery (p = 0.0497) and it is seen that the rats in the TdPrb group continued to explore the test apparatus more than the other groups (**Figure 1C)**. At the second 5 minutes, there was no significant difference between groups in their preference for the central area (p = 0.1476) (**Figure 1D)**.

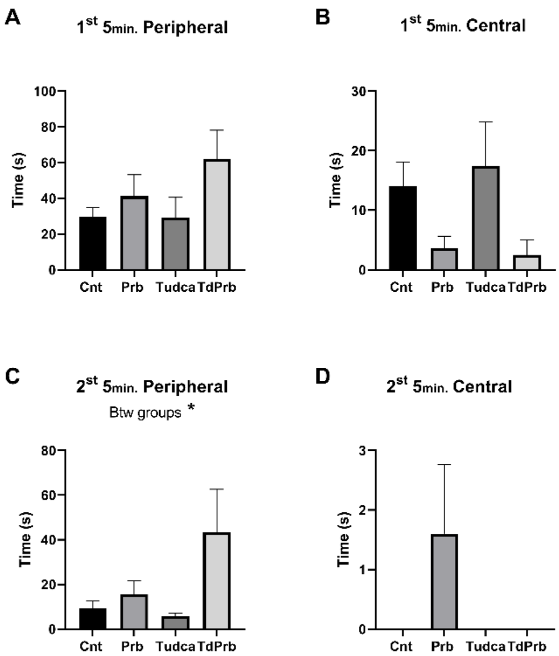

Figure 1. The mean distance moved in the periphery (A & C) and the center (B & D) of the OF during the 1st (upper row) and 2nd (lower row) 5-minute control periods (Cnt), Tudca (Tudca), SCD probiotics (Prb), and Tudca administration during the SCD probiotics supplementation (TdPrb) groups. Error bars represent SEM and asterisks indicate the statistical significance: *p < 0.05.

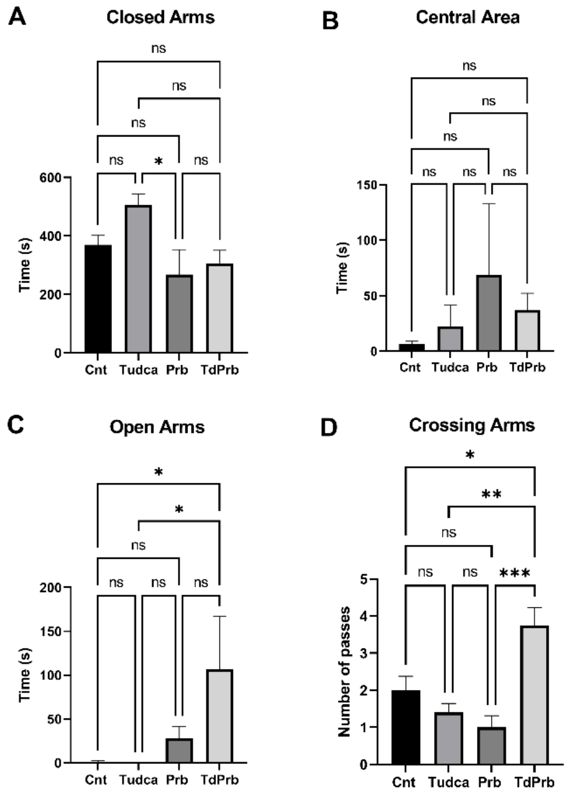

Figure 2. The mean time spent in the EPM's closed arms, open arms, and center zone and the number of passes between closed arms in control (Cnt), Tudca (Tudca), SCD probiotics (Prb), and Tudca administration during the SCD probiotics supplementation (TdPrb) groups. Error bars represent the standard error of the mean and asterisks indicate the significance level: *p < 0.05; **p < 0.01 and *** p < 0.001.

The number of passes between the arms shows the behavior of the animals to search the test apparatus [22]. According to the EPM results, there was a significant difference between the groups in preferring the closed arm (p= 0.0318). There was a significant difference between the Tudca and Prb groups in the comparison of the groups with each other (p = 0.0275) (**Figure 2A)**. This indicates that the SCD probiotics is more effective on anxious behavior than TUDCA. While there was no significant difference between the groups in their preference for the central area (p = 0.5310), it is seen that the Prb group spent more time in this area (**Figure 2B)**. However, there was a significant difference between the groups in their preference for open space (p = 0.0244). In addition, when comparing the groups with each other, it is seen that the TdPrb group, in which TUDCA and SCD probiotics were used together, investigated the open space significantly more than the Tudca (p = 0.0356) and control groups (p = 0.0249) (**Figure 2C)**. The most significant difference between the groups was in terms of transition between arms (p = 0.0009). In the comparison of the groups with each other, it is seen that the rats in the TdPrb group exhibit significant test apparatus research behavior TdPrb vs. Cnt (p = 0.0188), TdPrb vs. Tudca (p = 0.0032), TdPrb vs. Prb (p = 0.0007) (**Figure 2D)**.

Although it is known that both probiotic use and TUDCA separately have positive effects on behavior, this study shows that using both together is more effective on both locomotor activity and anxious behavior. Even though it is not possible to say whether TUDCA or the probiotic used is more effective, the use of SCD probiotics seems to be more effective on anxious behavior. The probiotic content used with TUDCA also plays an important role in seeing this difference since the effects of probiotics depend on a variety of factors, including the probiotic species, its concentration, and duration, as well as the host's age and health state [23]. Species contained in SCD probiotics are known to have a positive effect on behavior. For example, *Bacillus subtilis*, one of the most well-known probiotic bacteria, produces l-tryptophan, and the increase in serotonin in the hypothalamus acts as an antidepressant that reduces anxious behavior [24]. In addition, numerous animal studies indicate that *Bifidobacterium longum* can significantly reduce anxious behavior [25]. *Lactobacillus plantarum* [26], *Lactobacillus casei* [27] and *Lactobacillus fermentum* [28] are also probiotic strains that have a strong effect on anxious behavior. On the other hand, since younger and smaller model organisms can investigate test apparatuses more actively, this allows for more reliable results in behavioral tests. Due to both their advanced age and large size, 24-month-old rats may not be very willing to use research test apparatus such as EPM. However, the significant differences occurring in as little as one week indicate the strong effect of both TUDCA and SCD probiotics bacteria on locomotors activity and anxiety-like behavior.

4. Conclusion and Suggestions

This study evaluated the effects of administering SCD probiotics supplement with TUDCA on anxious behavior and locomotors activity. The SCD probiotics was more effective on anxious behavior. When used together, they are more effective on both locomotors activity and anxious behavior.

Statement of Competing Interests

The authors have no competing interests

Research and Publication Ethics Statement

Research and publishing ethics were followed

Conflict of Interest Statement

There is no conflict of interest between the authors.

Statement of Research and Publication Ethics

This study was carried out with the approval of the Ethics Committee (approval number: 2022/03) from the Saki Yenilli Experimental Animal Production and Practice Laboratory.

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