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Research Article

Levi *Levilactobacillus brevis* (TCI988) Supplementation Enhances Sleep Quality

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Abstract

Levilactobacillus brevis (TCI988) is a probiotic known for improving gut health, enhancing the immune system, and reducing inflammation and improved sleep quality. The aim of this study was to investigate whether *L. brevis* increased deep sleep and improve stress. Neuro-2a cells were treated with TCI988 for 24 hours, and then examined mitochondrial activity and related melatonin genes. In addition, subjects analyzed the GABA concentration and sleep and stress status. TCI988 can increase neuronal cell mitochondrial activity by 33.4% compared to the mock, and increased the expression of SIRTs, TPH1, and DDC by about 1.3, 1.23, and 1.05-fold, respectively, compared to the mock. The serum GABA level of the subjects increased from 125.0 ng/mL to 199.1 ng/mL after consuming TCI988 for 4 weeks, and increased deep sleep time and improved stress. *L. brevis* showed promise as a natural remedy for improving sleep quality.

Keywords: Deep Sleep; GABA; *Levilactobacillus brevis*; Probiotics; Stress

Introduction

Modern people are facing increasingly serious sleep problems, with many finding it difficult to fall asleep or maintain good sleep quality. This is mainly due to the fast-paced and high-stress lifestyle of modern society, as well as the widespread use of electronic devices which emit blue light that disrupts the natural sleep-wake cycle. Recent studies have shown that there is a correlation between gut health and sleep quality, and that consuming probiotics may help improve both [1]. Probiotics, such as *Lactobacillus* and *Bifidobacterium*, are beneficial bacteria that live in the gut and help regulate the immune system and digestive function [2]. In addition, they also produce neurotransmitters like serotonin and GABA, which are important for regulating mood and promoting relaxation [3]. One study published in the Journal of Clinical Sleep Medicine found that consuming a combination of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Lactobacillus rhamnosus* for four weeks improved sleep quality and reduced daytime sleepiness in patients with chronic fatigue syndrome [4]. Another study published in Nutrients found that consuming *Lactobacillus plantarum* for eight weeks improved sleep quality and reduced anxiety in healthy adults [5]. Overall, the evidence suggests that taking probiotics may be a safe and effective way to improve sleep quality, especially for those who suffer from sleep problems related to stress or poor gut health. Further research is needed to fully understand the mechanisms behind this relationship, but the potential benefits of probiotics for sleep should not be overlooked.

Levi *Levilactobacillus brevis* is a probiotic bacteria commonly found in fermented foods and beverages such as sourdough bread, sauerkraut, and beer. It belongs to the lactic acid bacteria family and is known for its ability to produce lactic acid, which helps to preserve and ferment food. *L. brevis* has been studied for its potential health benefits, including improving gut health, enhancing the immune system, and reducing inflammation [6,7]. It has also been shown to have antimicrobial properties against harmful bacteria. Overall, *L. brevis* is a promising probiotic strain that may offer numerous health benefits when consumed as part of a balanced diet. Research has found that *L. brevis* can improve sleep quality by regulating the sleep-wake cycle and promoting relaxation [8]. One study showed that consuming *L. brevis* for four weeks significantly im-

proved sleep quality in healthy adults [9]. Another study found that *L. brevis* supplementation for eight weeks improved sleep quality and reduced fatigue in patients with chronic fatigue syndrome [10]. The mechanism behind these benefits is thought to be related to the ability of *L. brevis* to produce gamma-aminobutyric acid (GABA), a neurotransmitter that plays a key role in regulating the sleep-wake cycle and promoting relaxation. *L. brevis* has been found to increase GABA levels in the brain, which may explain its sleep-promoting effects [11]. Overall, the evidence suggests that *L. brevis* may be a promising natural remedy for improving sleep quality and promoting relaxation. Further research is needed to fully understand the mechanisms behind its effects on sleep, but the potential benefits of this probiotic should not be overlooked.

This study aimed to investigate the potential of *L. brevis* (TCI988, produced by TCI Co., Ltd) to improve sleep both *in vitro* and *in vivo*. The study was conducted in two parts. First, Neuro-2a cells were treated with TCI988 to evaluate its effect on mitochondrial activity and gene expression related to melatonin. Second, a group of 6 participants were recruited to take one capsule of TCI988 daily for four consecutive weeks, and their sleep quality was analyzed before and after the intervention.

Material and Methods

Isolation and identification of TCI988 strain

Sixteen bacteria strains were isolated from twenty samples of different objects including grapefruit, kimchi, loquat, human gut and tempeh through spraying homogisized samples on MRS agar (Difco™) and culture in anaerobic culture chamber in 37°C. Amplification was carried out in a thermal cycler ABI/2720 (Applied biosystems, USA). The reaction mixture contained Taq DNA Polymerase 2 × master mix red with 1.5mM $MgCl_2$ (Ampliqon), 50-100 ng of each bacterium, and primer used are 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3'). DNA fragments were amplified as follows: initial denaturation at 95°C for 5 min, followed by 30 cycles consisting of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a 7 min final extension step at 72°C. The products were stored at 4°C for subsequent Sanger sequencing. The sequenced 16S rRNA genes of each isolate are searched with GenBank DNA database by using the BLAST algorithm. The identities of the isolates were determined on the basis of highest score. Among them, three *Levilactobacillus brevis* (previous name: Lactobacillus brevis) were identified and named *Levilactobacillus brevis* TCI988, *Levilactobacillus brevis* L816 and *Levilactobacillus brevis* L846.

The manufacturing process of TCI988 strain

The yeast peptone, glucose, $MgSO_4$-$7H_2O$, NaCl, KH_2PO_4, K_2HPO_4, manganese gluconate, water for mixing and sterilization. After sterilization, *Levilactobacillus brevis* TCI988 was added, and then the fermentation was carried out. After the fermentation was terminated, took a sample for confirmation. After centrifugation, the bacterium mud was collected, freeze drying protective agents were added before lyophilization process. *Levilactobacillus brevis* TCI988 were homogenized and sieved to harvest fine powder. Those powder were packaged, labeled, and stored below -20°C.

Cell culture

The standard culture medium for Neuro-2a cells is Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The medium should be pre-warmed to 37°C before use. Seed the Neuro-2a cells in a culture dish at a density of 5,000-10,000 cells per cm^2. Allow the cells to adhere to the dish for 24 hours before proceeding with the experiment [12].

Mitochondrial activity

The Mitochondrial Membrane Potential Detection Kit (JC-1) (Beyotime Institute of Biotechnology, China) was used to observe changes in the mitochondrial potential. Briefly, 5 mg/ml JC-1 working solution was added to the medium and incubated for 30 min at 37°C with CO_2. The cells were subsequently washed with PBS to remove the JC-1 probe, and images were obtained via fluorescence microscopy (Olympus BX-61). The ratio of red to green fluorescence was analyzed using flow cytometer (BD Accuri C6 Plus) [13].

Quantification of gene expressions by real-time PCR

The treated Neuro-2a were harvested, and the total RNA was isolated from cells using an RNA purification kit (Geneaid, Taiwan). DNA-free total RNA was reversely transcribed to cDNA using a SuperScript™ Reverse Transcriptase kit (Invitrogen, Life Technologies Co., CA, USA). Quantitative real-time PCR was conducted using an ABI StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, Inc., CA, USA) and the SYBR Green Master Mix (KAPA Biosystems, MA, USA) for the transcript measurements. The gene-specific primers used in this study are listed in table 1. The GAPDH gene was used as a normalization control [14].

Self-controlled trial design

6 adult subjects (20-55 years old adults with insomnia or emotionally stressed) were recruited in this trial. Informed consent was obtained from all subjects before the study at clinical laboratory. Subjects were informed to take one sachet every day (1×10^9 CFU/capsule, 1 capsule/day) for 4 weeks. Subjects were instructed to eat normally and avoid mistakes caused by overeating. The subjects were measured at weeks 0, and 4 in the trial and they were not allowed to take any other supplements during the intervention period. Exclusion criteria: [1] Taking sleeping pills or melatonin supplements, [2] Suffering from psychiatric disorders, [3] Pregnant or lactating women, [4] Known allergy to probiotics, [5] Having heart-related diseases, [6] Having symptoms of sleep apnea.

Gene	Species	Direction	Sequence
SIRT1	Mouse	F	CAACGGTTTCCATTCGTGTG
SIRT1	Mouse	R	GTTCGAGGATCTGTGCCAAT
TPH1	Mouse	F	TTCTGACCTGGACTTCTGCG
TPH1	Mouse	R	GGGGTCCCCATGTTTGTAGT
DDC	Mouse	F	TCGAGAAGATAATCATGCCA
DDC	Mouse	R	TTCTACAGAGGAATGCGCCT
GAPDH	Mouse	F	ACAACTTTGGTATCGTGGAAGG
GAPDH	Mouse	R	GCCATCACGCCACAGTTTC

Table 1: Species-specific quantitative PCR (qPCR) primers.

Preparation of test sample

The One capsule is 500 mg, included 392mg maltodextrin, 100 mg TCI988 (1×10^9 CFU), 4mg silicon dioxide, 4 mg magnesium stearate. Each subject was required to examine the sleep condition at week 0 and 4.

GABA measurement

Serum samples are prepared according to manufacturer's instructions and added to a 96-well microtiter plate containing GABA antibody solution. The plate is incubated at room temperature for 1-2 hours, followed by addition of blocking solution for 1 hour. GABA standards and serum samples are added to the plate and incubated for 1 hour. Biotin-labeled GABA antibody and streptavidin-HRP conjugate are added and incubated for 1 hour. Substrate solution is then added and incubated for 30 minutes. The enzymatic reaction is stopped with a stop solution and absorbance of each well is read using a microplate reader. GABA concentrations are calculated using the standard curve generated from the GABA standards [15].

Sleep measurement

All participants used the Xiaomi Mi Band 2 based on other studies. Xiaomi mi Band2 can be used to detect sleep onset and offset times, time to fall asleep, awake time, total sleep time, overnight heart rate, and sleep stage distribution, among others. Prior to the experiment, participants were educated and trained to ensure that they understood how to use the Mi Band 2. During the experiment, each participant wore the Mi Band 2 and other measuring devices for one night of sleep monitoring. After the experiment, the data recorded by the Mi Band 2 was compared to the data from the control instruments [16].

Self-assessment questionnaire

A stress assessment questionnaire is a standardized assessment tool used to measure an individual's response to stress. Typically, a stress assessment questionnaire includes multiple questions or statements that require the respondent to answer based on their own experiences and feelings. The questionnaire contains standard questions and options and is distributed to the respondents for completion. When analyzing the results, researchers can assess the respondents' stress response based on their answers [17].

Statistical analysis

The comparison of measurement results for the parameters among groups and between groups was analyzed by Student's t test, with $p < 0.05$ considered statistically significant.

Results

TCI988 increased mitochondrial activity and melatonin related genes

To evaluate whether TCI988 can increase mitochondrial activity and gene expression related to melatonin in neuronal cells, Neuro-2a cells were treated with TCI988 for 24 hours. The results had showed that TCI988 can increase neuronal cell mitochondrial activity by 33.4% compared to the mock group (Figure 1A). Tryptophan hydroxylase 1 (TPH1) and dopa decarboxylase (DDC) are genes involved in serotonin synthesis. Serotonin is an important precursor in the melatonin synthesis pathway, which promotes the production of melatonin and improves sleep quality. SIRTs (sirtuins) have been linked to synaptic dysfunction and may play a role in improving synaptic plasticity in depression. The results had showed that TCI988 increased the expression of SIRTs, TPH1, and DDC by approximately 1.3-fold, 1.23-fold, and 1.05-fold, respectively, compared to the mock group (Figure 1B).

TCI988 increased deep sleep in human

To evaluate whether TCI988 can increase deep sleep in human, 6 subjects who self-reported having sleep difficulties or stress were recruited. GABA (gamma-aminobutyric acid) is a neurotransmitter closely related to sleep. GABA inhibits neuronal activity, allowing the brain to enter a relaxed state and promote sleep. Some medications, such as sleeping pills and sedatives, also promote sleep by enhancing the effects of GABA. Additionally, studies suggest that some sleep disorders may be related to abnormalities in

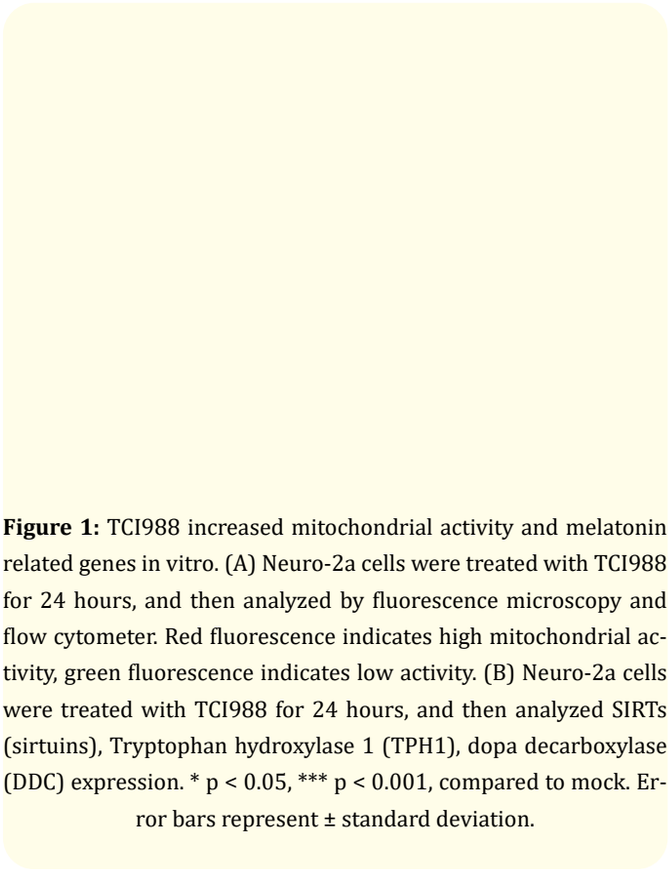

Figure 1: TCI988 increased mitochondrial activity and melatonin related genes in vitro. (A) Neuro-2a cells were treated with TCI988 for 24 hours, and then analyzed by fluorescence microscopy and flow cytometer. Red fluorescence indicates high mitochondrial activity, green fluorescence indicates low activity. (B) Neuro-2a cells were treated with TCI988 for 24 hours, and then analyzed SIRTs (sirtuins), Tryptophan hydroxylase 1 (TPH1), dopa decarboxylase (DDC) expression. * $p < 0.05$, *** $p < 0.001$, compared to mock. Error bars represent ± standard deviation.

GABA receptor function. The results had showed that the serum GABA level of the subjects increased from 125 ng/mL to 199 ng/mL after consuming TCI988 for 4 weeks, representing a change rate of 59.3%. These results suggest that TCI988 has the potential to enhance GABA production (Figure 2A). After 4 weeks of consuming TCI988, the average duration of deep sleep was observed to increase from 133 minutes to 212 minutes, with a change value of 79 minutes (Figure 2B). Additionally, the average proportion of deep sleep among the subjects increased from 35.6% to 48.8%, with a change value of 13.2 (Figure 2C). These results suggest that TCI988 can be beneficial in increasing the amount of deep sleep.

TCI988 had stress relief efficacy

Next, to evaluate whether TCI988 can improve stress, a questionnaire survey will be conducted for assessment. The emotional stress score of the subjects decreased from 42.9 to 32.5 after taking TCI988 for 4 weeks, resulting in a change rate of 10.4% (Figure 3A). The severity of nervousness, frustration, short-temperedness, and impatience was reduced (Figure 3B). The behavioral stress score decreased from 41.3 to 33.8 after taking TCI988 for 4 weeks, resulting in a change rate of 7.5% in week 4 (Figure 3C). The severity of sleep deprivation, lack of energy, and impulsiveness was reduced (Figure 3D). The psychological stress score decreased from 47.1 to 37.1 after taking TCI988 for 4 weeks, resulting in a change rate of 10% (Figure 4A). The severity of confusion, lack of confidence, procrastination, lack of concentration, and forgetfulness was reduced (Figure 4B). The physical discomfort symptoms such as soreness, fatigue, stomach ache, and headache were reduced after taking TCI988 for 4 weeks (Figure 4c).

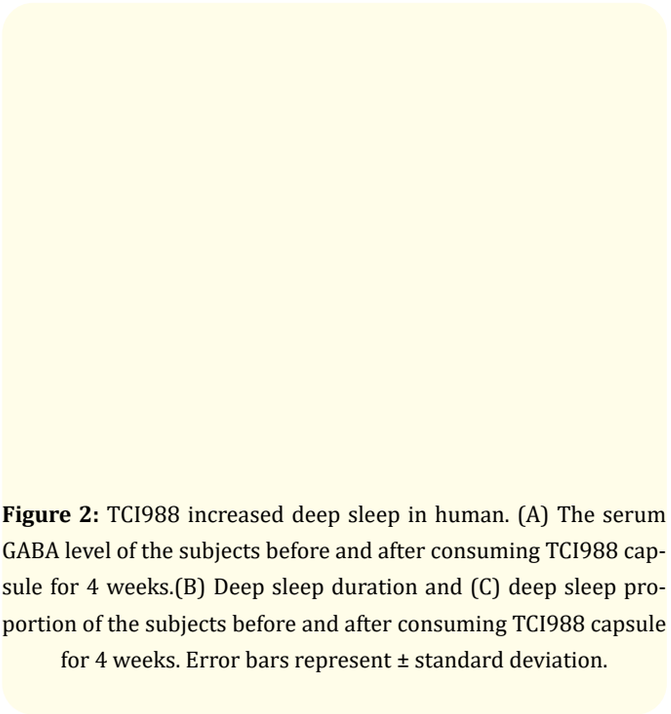

Figure 2: TCI988 increased deep sleep in human. (A) The serum GABA level of the subjects before and after consuming TCI988 capsule for 4 weeks.(B) Deep sleep duration and (C) deep sleep proportion of the subjects before and after consuming TCI988 capsule for 4 weeks. Error bars represent ± standard deviation.

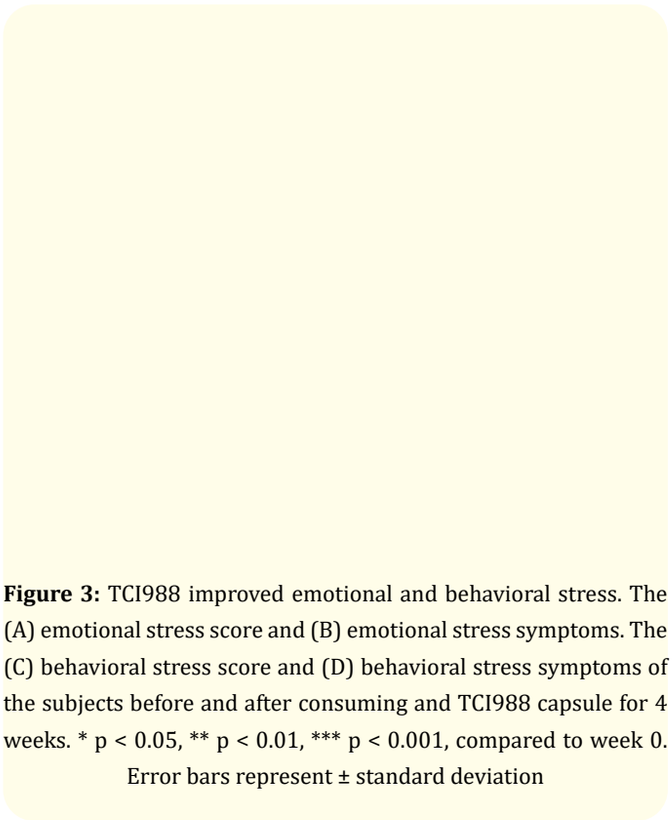

Figure 3: TCI988 improved emotional and behavioral stress. The (A) emotional stress score and (B) emotional stress symptoms. The (C) behavioral stress score and (D) behavioral stress symptoms of the subjects before and after consuming and TCI988 capsule for 4 weeks. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to week 0. Error bars represent ± standard deviation

Figure 4: TCI988 improved psychological stress. The (A) psychological stress score and (B) behavioral stress symptoms. (C)Physical discomfort change of the subjects before and after consuming and TCI988 capsule for 4 weeks. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to week 0. Error bars represent ± standard deviation.

Discussion

In this study, TCI988 can increase mitochondrial activity and melatonin related genes, and had the potential on GABA boosting, deep sleep enhancing and stress relief efficacy. In addition, all the subjects had no evidence on skin irritation, gastrointestinal discomfort and any other discomforts.

Recent studies have investigated the potential of *L. brevis*, a type of probiotic, in promoting better sleep. *L. brevis* has been found to regulate the immune system and improve gut health, which are both important factors that affect sleep quality [18]. In a clinical study, participants who took *L. brevis* supplements for 4 weeks reported improvements in their sleep quality, with significant reductions in the time taken to fall asleep, sleep disturbances, and daytime sleepiness [19]. Moreover, *L. brevis* has been shown to reduce levels of cortisol, a stress hormone that can interfere with sleep, and to increase levels of serotonin, a neurotransmitter that regulates mood and sleep [20]. While these findings are promising, further research is needed to fully understand the mechanism of action of *L. brevis* in improving sleep quality and to determine the optimal dosages and duration of supplementation. Nonetheless, *L. brevis* shows potential as a natural and safe intervention for promoting better sleep and managing sleep-related disorders.

Recent studies have shown that *L. brevis* has the ability to produce gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter that plays a crucial role in regulating neuronal excitability and promoting relaxation. Clinical studies have investigated the effects of *L. brevis* on GABA production and its potential for improving sleep quality [21]. One study found that oral administration of *L. brevis* significantly increased GABA levels in the brain, resulting in improved sleep quality in mice [22]. Another study conducted on human subjects showed that consumption of *L. brevis*-containing yogurt led to increased GABA levels in the blood, which was associated with improved sleep quality and reduced sleep latency. *L. brevis* has been studied for its potential in reducing stress and symptoms of depression [23]. Several clinical trials have shown promising results. In one study, individuals with high levels of psychological distress who consumed *L. brevis* daily for 12 weeks experienced a significant reduction in stress and depressive symptoms [24]. Another study found that *L. brevis* supplementation improved mood and reduced anxiety and depression symptoms in individuals with chronic fatigue syndrome [25]. These findings suggest that *L. brevis* may have a beneficial effect on mental health by reducing stress and symptoms of depression and anxiety.

Studies have also investigated the potential effects of *L. brevis* on mitochondrial activity in the brain. One study found that *L. brevis* supplementation increased mitochondrial respiratory chain complex activity and ATP production in the hippocampus of mice [26]. Additionally, another study showed that *L. brevis* supplementation increased mitochondrial activity and reduced oxidative stress in the brains of rats with induced neurodegeneration [27]. These findings suggest that *L. brevis* may have a positive impact on mitochondrial function in the brain, which is important for maintaining cellular energy and preventing cellular damage. *L. brevis* has been studied for its potential effects on the expression of various genes involved in neurotransmitter synthesis and metabolism. One study found that *L. brevis* supplementation increased the expression of sirtuin 1 (SIRT1) in the hippocampus of mice, which is a gene involved in the regulation of various cellular processes, including inflammation and oxidative stress [24]. Another study showed that *L. brevis* supplementation increased the expression of tryptophan hydroxylase 1 (TPH-1) and dopa decarboxylase (DDC) in the brain of mice, which are genes involved in the synthesis of serotonin and dopamine, respectively [28]. These findings suggest that *L. brevis* may have a positive effect on neurotransmitter synthesis and metabolism by upregulating the expression of key genes involved in these processes. However, further research is needed to fully understand the underlying mechanisms and clinical implications of these findings.

Conclusion

TCI988 has the potential to increase mitochondrial activity and gene expression related to melatonin in neuronal cells, leading to improved sleep quality. The increase in GABA production may also contribute to the observed increase in deep sleep. In addition, TCI988 showed stress relief efficacy by reducing emotional, behavioral, and psychological stress scores, as well as physical discomfort symptoms. These findings suggest that TCI988 may be a

promising therapeutic option for individuals with sleep difficulties, stress, and related disorders. However, further studies are needed to fully understand the underlying mechanisms and long-term effects of TCI988 on human health.

Conflict of Interest

The authors declare no conflict of interest.

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