The protective effects of Probiotic X on sleep pattern and cognitive function in a long-term caffeine-induced chronic insomnia rat model

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<u>Abstract</u>

Background: Chronic insomnia (CI) not only causes physiological and mental issues but also increases the occurrence of chronic disorders in the future, including dementia. Therefore, it is crucial to develop accessible and effective ways to alleviate CI. Probiotic X (X) has been revealed to improve stress-induced anxiety and sleep disturbance in mice, suggesting X may alleviate insomnia. However, previous animal studies have relied on acute caffeine to disrupt sleep. There are still few studies discussing CI animal models. Therefore, establishing a more compatible animal model to human CI is necessary to explore the effects of X. Hypothesis: Probiotic X alleviates symptoms of CI, including sleep disturbance and cognitive decline. Aims: (1) To establish the long-term caffeine-induced CI rat model; (2) To explore the effects of Probiotic X on sleep patterns and cognitive function. **Methods:** X will be dissolved in water and freely available to rats until the end of the experiments. At the third week of X administration, a caffeine-containing osmotic pump will be implanted subcutaneously in rats for four weeks. During the third week of caffeine intervention, electroencephalography, electromyography, and electrocardiography will be recorded to analyze sleep patterns and autonomic function. Subsequently, behavioral tests will be conducted to measure cognitive function. Results: First, we found that 15 mg/kg/day of caffeine (C15) increased waking time and decreased sleeping time. Additionally, C15 enhanced interruptions during quiet sleep and caused reference and working memory damage in the radial arm maze. According to these findings, we adapted 15 mg/kg/day as the longterm caffeine-induced CI rat model. Next, we examined the effects of Probiotic X. We found that X decreased waking time and increased sleeping time in the long-term caffeine treatment. Additionally, X reduced interruptions during quiet sleep, indicating that X could improve long-term caffeine-induced sleep disturbance. Furthermore, X ameliorated reference and working memory damage, indicating that X could improve long-term caffeine-induced cognitive decline. Conclusions: Probiotic X may alleviate sleep patterns, sleep quality, and cognitive function in the long-term caffeine-induced CI rat model. Significance: This study will provide a new animal model for chronic insomnia and validate the benefits of Probiotic X.