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Komagataella phaffii KM71H Attenuates Sleep Deprivation–Induced Anxiety and Cognitive Dysfunction via Neuroimmune–Metabolic and Stress-Related Pathways

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Abstract

Sleep deprivation (SD) disrupts emotional regulation and hippocampal-dependent memory through converging neuroimmune, metabolic, oxidative, and neuroendocrine mechanisms. Psychobiotics have emerged as promising strategies to counteract these alterations by modulating the microbiota–gut–brain axis. *Komagataella phaffii* KM71H has previously demonstrated antidepressant-like and immunometabolic benefits; however, its effects on SD-induced anxiety and cognitive dysfunction remain unexplored. Here, we investigated whether *K. phaffii* KM71H attenuates behavioral, biochemical, and molecular alterations induced by SD in mice. Male mice received oral *K. phaffii* KM71H (1×10^8 CFU) or vehicle for 14 days, and SD was induced using the multiple-platform method. Behavioral performance, oxidative stress markers, corticosterone levels, acetylcholinesterase activity, and the expression of neuroimmune and stress-related genes were evaluated in brain and intestinal tissues. Treatment with *K. phaffii* KM71H prevented the anxiogenic-like phenotype and rescued spatial working memory without affecting locomotion. The yeast reduced reactive species in all evaluated tissues, lowered plasma corticosterone, and normalized acetylcholinesterase activity. Additionally, *K. phaffii* KM71H downregulated key inflammatory and immune-related markers -indoleamine 2,3-dioxygenase, interleukin-6, nuclear factor kappa-B, and Toll-like receptor 4, as well as glycogen synthase kinase-3β, a regulator of synaptic plasticity, and the glucocorticoid receptor, a component of the HPA axis. Together, these findings identify K. phaffii KM71H as a psychobiotic candidate capable of mitigating SD-induced neurobehavioral dysfunction through coordinated modulation of neuroimmune–metabolic and stress-related pathways, supporting its translational potential for sleep-related neuropsychiatric conditions.

Introduction

Komagataella phaffii KM71H, a methylotrophic yeast with emerging psychobiotic properties, has gained attention for its ability to modulate the microbiota–gut–brain axis, a bidirectional communication network linking intestinal microorganisms to the central nervous system (CNS) through neural, immune, and metabolic pathways [1, 2]. By influencing microbial composition and metabolite production, the gut microbiota plays a key role in emotional regulation, stress responses, and intestinal homeostasis [3] and probiotic interventions have been shown to strengthen these interactions [4]

Within this context, *K. phaffii* KM71H has emerged as a promising candidate. Initially identified as a probiotic by França et al. [5], the strain was later shown to attenuate immune activation, oxidative stress, and depressive-like behavior in murine models challenged with lipopolysaccharide (LPS) or acute restraint stress [6]. In addition, this yeast prevents antibiotic-induced dysbiosis, preserves intestinal barrier integrity, modulate inflammatory cytokines, and increase beneficial taxa such as *Bacteroidetes* and *Lactobacillus* [7]. Its oral administration for 14 days is considered safe, producing no hematological, biochemical, or histological alterations, even under inflammatory or dysbiotic conditions [6] *K. phaffii* KM71H reverses depressive-like phenotypes in early-life stress and postpartum models, improves gastrointestinal and antioxidant parameters [8], and mitigates cognitive decline alongside depressive-like behavior in an Alzheimer's disease model [9].

Despite advances in studies on *K. phaffii* KM71H, current knowledge remains limited to depression-centered paradigms, with no evidence on its effects on anxiety-like behavior or cognitive performance outside neurodegenerative contexts—an important gap considering conditions such as sleep deprivation (SD), where anxiety and cognitive deficits are prominent. SD is a growing public health concern driven by modern lifestyle habits [10], and sleep - particularly rapid eye movement (REM) sleep - is essential for emotional regulation, synaptic plasticity, and memory consolidation [11][12]. Consequently, REM sleep reduction or fragmentation increases vulnerability to anxiety and cognitive decline [13, 14], highlighting the need for interventions capable of mitigating these neurobehavioral consequences.

Mechanistically, SD triggers hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis [15], leading to excessive cortisol release that, despite facilitating memory acquisition, impairs recall at supraphysiological levels [16] and contributes to anxiety through inflammatory pathways [17]. Sleep fragmentation further disrupts circadian rhythms by altering endogenous melatonin and cortisol fluctuations, intensifying anxious symptoms [18, 19]. Another key mechanism activated by SD is oxidative stress—damaging lipids, proteins, and DNA and impairing cognition by disrupting memory consolidation and reducing neuronal protein synthesis required for neurogenesis and synaptic plasticity [20]. Additionally, dysregulation of the cholinergic system, particularly acetylcholinesterase (AChE), a key regulator of acetylcholine availability, has been implicated in SD-related cognitive decline and in Alzheimer's disease pathology [21] Together, these mechanisms highlight the complexity of SD-induced neurobehavioral alterations.

Pharmacological treatments for SD-related consequences including antidepressants, antipsychotics, benzodiazepines, non-benzodiazepine hypnotics, AChE inhibitors, and NMDA receptor modulators [22], but these are often associated with adverse effects such as dependence, exacerbation of anxiety, cognitive dulling, and sleep disturbances [22] These limitations reinforce the need for safe, non-pharmacological alternatives.

We hypothesized that K. phaffii KM71H would mitigate SD-induced anxiety and cognitive impairment by converging on oxidative stress, HPA axis dysregulation, cholinergic imbalance, and immune–metabolic signaling (TLR4/NF-κB–IDO–GSK3β pathways) across the gut–brain axis.

Materials and Methods

Komagataella phaffii KM71H Culture

The yeast *K. phaffii* KM71H was cultivated as described by Birmann et al. [6] with a final concentration of approximately 8 log Colony Forming Units (CFU) of *K. phaffii* KM71H in 500 µL of PBS.

Animals

The experiments were conducted using male Swiss mice (25–35 g), obtained from the Central Animal Facility of the Federal University of Pelotas (UFPel). Animals were maintained under standard

environmental conditions (22 ± 1°C and a 12 h light/dark cycle) with free access to food and water. All procedures were carried out between 08:00 and 18:00 h, in accordance with the guidelines of Institutional Animal Care and Use Committee (UFPel), under protocol number 17/2024.

Study Groups and Experimental Design

The animals were randomly divided into four experimental groups with or without SD: G1: PBS + No SD; G2: PBS + SD; G3: *K. phaffii* KM71H + No SD; G4: *K. phaffii* KM71H + SD. The experimental protocol was carried out as shown in Fig. 1. In this protocol, the animals received *K. phaffii* KM71H at a dose of 8 log CFU/animal by intragastric (i.g.) administration or PBS (vehicle, i.g.) for 14 consecutive days. The yeast dose and protocol duration followed Birmann et al. [6].

From day 7 to day 10, SD was performed using the 72 hours water-platform model, as previously described by Marzabadi et al. [23], in which the mice had free access to food and water, and the cages were cleaned daily. On day 15, the open field test (OFT) was performed, followed by the elevated plus maze (EPM) or the Y-maze test, depending on the experimental group. After the behavioral tests, the mice were euthanized by isoflurane overdose. The hippocampi, prefrontal cortex, small intestine, and blood were collected. Biochemical assays included the corticosterone levels, reactive species (RS) levels, corticosterone levels, AChE activity, RT-qPCR and the Bradford assay for total protein quantification.

Behavior Tests

Open Field Test (OFT)

The OFT was performed according to Walsh and Cummins [24] before the other behavioral assessments. This test is used to measure behavioral changes in rodents exposed to new environments and is used to confirm that anxiety-like behavior and cognitive deficits do not occur due to general stimulation of motor activity [25] The mice were individually placed in the center of a box (30 × 30 × 15 cm) divided into nine equal-area quadrants and observed for 5 minutes to record their locomotor activity (number of crossings) and exploratory activity (number of rearings).

Y-Maze Test

The Y-maze test [26] assesses the spatial memory ability of rodents. This test evaluates whether the rodent retains in memory the space it has just explored. Each animal is individually placed at the end of a Y-maze with three arms 35 cm (long) and 8 cm (wide) with 26-cm walls (central position of the apparatus) and is free to explore the three arms for 8 minutes. Briefly, mice were initially placed within one arm (A), and the arm entry sequence (e.g., ABCCAB, where letters indicate arm codes). Alternation was determined from successive entries into the three arms on overlapping triplet sets in which three

different arms are entered. An actual alternation was defined as entries into all three arms consecutively (i.e., ABC, BCA, or CAB but not ABA). An entry was defined as placing all our paws within the boundaries of the arm.

Elevated Plus Maze Test (EPM)

The EPM [27] is a simple method to assess anxiety-like behavior in rodents. The test is conducted on an elevated platform with four arms, two open arms and two closed arms. The animal is allowed to freely explore the maze for 5 minutes, while the duration and frequency of entries into the open arms are recorded, as well as the number of head dips in the open arms. The task evaluates the animal's ability to explore a new environment in elevated and open spaces. Consequently, an anxious state is characterized by increased time spent in the closed arms compared with the open arms.

Biochemical Analyses

Tissue Collection

The animals were anesthetized with isoflurane, and blood samples were obtained via cardiac puncture. The blood was centrifuged at 3.600 × g for 10 minutes at 4°C, and the resulting plasma was used for corticosterone quantification. The small intestine (corresponding to the duodenal region) was excised and prepared for following tests according to Birmann et al. [7]. The hippocampi, prefrontal cortex, and small intestine samples were homogenized in 50 mM Tris-HCl buffer (pH 7.4; 1:10, w/v). The homogenates were then centrifuged at 2.500 × g for 10 minutes at 4°C, and the supernatant was used for the RS (hippocampi and prefrontal cortex) assay. Hippocampi and prefrontal cortex were homogenized in 25 mM sucrose buffer (1:10, w/v) and designated to AChE activity. The protein concentration was measured by the method of Bradford et al. [28] using bovine serum albumin (1 mg/ml) as the standard. For gene expression analysis, the hippocampi, prefrontal cortex, and small intestine were collected and stored at − 80°C until further processing.

Plasma Corticosterone Levels

In this analysis, performed according to Zenker et al. [29] 200 µL of blood plasma, 800 µL of H_2O, and 2 mL of chloroform are added in a tube and shaken for 15 seconds and centrifuged at 2.500 x g for 5 minutes. The aqueous phase is discarded, and the chloroform phase is retained. Next, 1 mL of 0.1 M NaOH is added, the tube is shaken for 15 seconds and centrifuged again at 2.500 x g for 5 minutes. Then, 1 mL of the chloroform phase is transferred to a new tube and mixed with 3 mL of fluorescence reagent. The tube is shaken for 15 seconds and centrifuged at 2.500 x g for 5 minutes. The chloroform phase is removed, and the tube is incubated for 2 hours. Fluorescence is then measured in a spectrofluorometer at 247 nm excitation, 540 nm emission, slit width 5. Plasma corticosterone levels are expressed in ng/mL.

Quantification of Reactive Species (RS) levels

The quantification of RS formed in the hippocampi, prefrontal cortex and small intestine was performed according to the method described by Loetchutinat et al. [30]. Briefly, 10 µL of 1 mM DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) was incubated with 10 µL of tissue sample and 2,990 µL of 10 mM Tris-HCl buffer (pH 7.4). The oxidation of DCFH-DA to dichlorofluorescein (DCF), its fluorescent form, was used to detect intracellular RS. Fluorescence intensity was measured at 520 nm emission and 480 nm excitation, with results expressed in fluorescence units.

Acetylcholinesterase (AChE) activity

AChE activity was assessed according to the method of Ellman et al. [31], using acetylthiocholine as the substrate. In this assay, thiocholine (8 mM, an analog of acetylcholine) reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, 10 mM), reducing it to 5-thio-2-nitrobenzoic acid (TNB). TNB has a yellow coloration proportional to AChE activity and can be measured in a UV spectrophotometer at 412 nm at 30-second intervals. AChE activity was expressed as nmol/mg protein/min.

RNA isolation and quantitative Real-Time PCR (RT-qPCR)

Total mRNA was extracted from the cortex, hippocampi and intestine using TRIzol followed by mRNA quantification. The cDNA synthesis was performed using a High-Capacity cDNA Reverse Transcription kit according to the manufacturer's protocol. The amplification was made with the GoTaq® qPCR Master Mix using the Stratagene Mx3005P real-time PCR. Gene expressions were normalized using GAPDH primer as a reference gene. The analyzed genes and primer sequences used are described on Table 1.

Table 1
Primer sequences used.

Gene	Sequence 5'-3'
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	F: GGGTGAGGCCGGTGCTGAG
	R: TGGGGGTAGGAACACGGAAGG
Glucocorticoid receptor (GR)	F: CGGCGATCTCCACAGCAATG
	R: ACCGCTCCACACATCCTGATTG
Glycogen synthase kinase-3 beta (GSK3β)	F: CGGGACCCAAATGTCAAACT
	R: TCCGAGCATGTGGAGGGATA
Interleukin (IL)-6	F: CCAGAAACCGCTATGAAG
	R: CACCAGCATCAGTCCCAAGA
2,3-dioxygenase (IDO)	F: AATCAAAGCAATCCCCACTG
	R: AAAAACGTGTCTGGGTCCAC
Nuclear factor kappa B (NF-κB)	F: GCTTTCGCAGGAGCATTAAC
	R: CCGAAGCAGGAGCTATCAAC
Toll-like receptor 4 (TLR4)	F: GCCTTTCAGGGAATTAAGCTCC
	R:AGATCAACCGATGGACGTGTAA

Statistical Analyses

Data from behavior and neurochemical analyses are expressed as mean ± standard error of the mean (SEM). The experimental results were analyzed by two-way analysis of variance (ANOVA) followed by Tukey's test for post hoc comparison. The results were considered significant when the probability values were equal or below 0.05 ($p < 0.05$). The statistical analysis was carried out using Graph Pad Prism software version 8.0 (San Diego, CA, USA).

Results

K. Phaffii KM71H attenuates anxiety-like behavior and cognitive decline in a sleep-deprived mice

The data analysis of OFT showed no change in the number of crossings ($F_{(1,24)} = 0.0060 = p = 0.93$) and rearings ($F_{(1,24)} = 0.00015 = p > 0.99$) after the different treatments in mice (Fig. 2A and B).

In the Y-maze test, SD significantly reduced the percentage of spontaneous alternations, indicating impairments in spatial memory and working memory (Fig. 2C). Treatment with *K. phaffii* KM71H, however, increased alternation rates (interaction $F_{(1,24)} = 15.50 = p < 0.001$), reversing the cognitive deficit induced by SD. *K. phaffii* KM71H *per se* did not change the percentage of spontaneous alternations.

As shown in Figs. 2D–F, SD also negatively affected anxiety-like behavior in the EPM, reducing the number of open-arm entries ($F_{(1,24)}$ = 14.40 = p < 0.001), head-dipping behaviors ($F_{(1,24)}$ = 28.04 = p < 0.001), and the time spent exploring the open arms ($F_{(1,24)}$ = 19.51 = p < 0.001). Treatment with *K. phaffii* KM71H increased all these parameters, demonstrating a clear protective effect against the anxiety-like phenotype induced by SD. *K. phaffii* KM71H per se did not change the parameters evaluated in the EPM.

K. Phaffii KM71H reduces plasma corticosterone elevation in sleep-deprived mice

It is possible to observe in Fig. 3 that SD caused an increase in plasma corticosterone levels compared to the control group, and treatment with *K. phaffii* KM71H reversed this alteration ($F_{(1,24)}$ = 28.10 = p < 0.001). Meanwhile, treatment with *K. phaffii* KM71H *per se* did not alter plasma corticosterone levels.

K. Phaffii KM71H attenuates oxidative stress in the brain and intestine in sleep-deprived mice

According to the results shown in Fig. 4, SD caused a significant increase in RS levels in the small intestine (Fig. 4A) ($F_{(1,24)}$ = 9.855 = p = 0.004), prefrontal cortex (Fig. 4B) ($F_{(1,24)}$ = 11.93 = p = 0.002), and hippocampi (Fig. 4C) ($F_{(1,24)}$ = 8.704 = p = 0.007), compared to the control group. On the other hand, after administration of *K. phaffii* KM71H, this increase in RS formation was prevented in the brain and intestinal structures of mice. *K. phaffii* KM71H *per se* did not change RS levels.

K. phaffii KM71H lowers acetylcholinesterase activity in the brains of sleep-deprived mice

According to Fig. 5, SD caused an increase in AChE activity in the prefrontal cortex (Fig. 6A) ($F_{(1,24)}$ = 17.63 = p < 0.001) and hippocampi (Fig. 6B) ($F_{(1,24)}$ = 34.33 = p < 0.001) of the mice when compared to the control group. Conversely, the increase in AChE activity was prevented by treatment with *K. phaffii* KM71H in both evaluated brain regions. *K. phaffii* KM71H *per se* did not alter AChE levels in the brain structures evaluated.

K. phaffii KM71H downregulates mRNA expression of neuroinflammatory and stress-related markers in sleep-deprived mice

Treatment with *K. phaffii* KM71H broadly counteracted the molecular disturbances induced by SD across all tissues analyzed. In the hippocampi, SD promoted the upregulation of IDO ($F_{(1,8)}$ = 58.76 = p < 0.001) and GR ($F_{(1,8)}$ = 111.6 = p < 0.001) (Fig. 6B,E) and NF-κB ($F_{(1,8)}$ = 65.31 = p < 0.001) and IL-6 ($F_{(1,8)}$ = 175.8 = p < 0.001) (Fig. 7B,E), and GSK3β ($F_{(1,8)}$ = 30.69 = p < 0.001) and TLR4 ($F_{(1,8)}$ = 111.9 = p < 0.001) (Fig. 8B,E).

The prefrontal cortex exhibited a similar vulnerability to sleep loss, with significant elevations in IDO ($F_{(1,8)}$ = 13.37 = p = 0.006), GR ($F_{(1,8)}$ = 11.68 = p = 0.009) (Fig. 6C,F) and NF-κB ($F_{(1,8)}$ = 2.346 = p = 0.16) and IL-6 ($F_{(1,8)}$ = 94.22 = p < 0.001) (Fig. 7C,F), GSK3β ($F_{(1,8)}$ = 3.448 = p = 0.10), and TLR4 ($F_{(1,8)}$ = 14.03 = p = 0.006) (Fig. 8C,F).

In the intestinal tissue, SD also triggered marked increases in IDO ($F_{(1,8)}$ = 122.4 = p < 0.001) and GR ($F_{(1,8)}$ = 217.0 = p < 0.001) (Fig. 6A,D) and NF-κB ($F_{(1,8)}$ = 59.71 = p < 0.001) and IL-6 ($F_{(1,8)}$ = 162.0 = p < 0.001) (Fig. 7A,D), and GSK3β ($F_{(1,8)}$ = 1244 = p < 0.001) and TLR4 ($F_{(1,8)}$ = 66.14 = p < 0.001) (Fig. 8A,D), indicating activation of mucosal immune pathways and disruption of host–microbiota signaling.

Administration of *K. phaffii* KM71H reduced the expression of the evaluated markers (IDO, GR, NF-κB, IL-6, GSK3β, and TLR4) in the brain and intestinal tissue of mice, restoring the alterations caused by SD. Treatment with *K. phaffii* KM71H *per se* did not cause alterations in the expression of these markers in the evaluated tissues.

Discussion

The yeast *K. phaffii* KM71H emerged as an effective intervention against SD-induced deficits, providing the first evidence that this strain attenuates anxiety-like behavior and cognitive decline in a SD model. These findings, summarized in Fig. 9, are consistent with its previously described psychobiotic profile, including immunomodulatory, antioxidant, HPA axis–regulating, microbiota-restoring, and gut barrier–protective properties, as well as reported neuroprotective and antidepressant-like effects. [6–9].

SD induced the expected neurobehavioral impairments, characterized by increased anxiety-like behavior in the EPM and reduced working memory in the Y-maze, corroborating evidence that sleep loss disrupts emotional regulation and hippocampal-dependent cognition [32, 33]. In line with studies showing that probiotics such as *Lactobacillus acidophilus* and *L. fermentum* mitigate anxiety and cognitive deficits via anti-inflammatory and antioxidant mechanisms [34], KM71H reversed the anxiogenic and cognitive impairments caused by SD, reinforcing its psychobiotic and therapeutic relevance.

In this study, we show for the first time that *K. phaffii* KM71H modulates cholinergic activity in sleep-deprived mice. SD significantly increased AChE activity in both the prefrontal cortex and hippocampi, in line with evidence linking elevated AChE activity to reduced cholinergic neurotransmission and memory impairment [35]. Treatment with *K. phaffii* KM71H prevented this SD-induced increase in both regions, suggesting that preservation of cholinergic signaling may contribute to the cognitive benefits observed. Given the central role of acetylcholine in REM sleep physiology, memory consolidation, and synaptic plasticity, these findings point to a relevant mechanistic pathway, although additional studies are required to clarify how the yeast modulates AChE regulation within the CNS.

In addition to these findings, the present study demonstrated a significant modulation of GSK3β, a serine/threonine kinase central to synaptic plasticity, neurogenesis, and inflammatory signaling [36]. Overactivation of GSK3β enhances pro-inflammatory pathways via NF-κB and contributes to tau hyperphosphorylation and synaptic dysfunction, processes that drive neuroinflammation and cognitive decline [37]. Here, we show for the first time that *K. phaffii* KM71H reduces GSK3β expression in both intestinal and brain tissues of sleep-deprived mice, indicating that the yeast may confer neuroprotective and immunomodulatory effects through pathways linked to inflammation and neurodegeneration.

Another key central finding is the reduction of IDO activity across all evaluated tissues. Sleep loss–related physiological stress, particularly the elevation of corticosterone, can upregulate IDO, redirecting tryptophan metabolism away from serotonin and melatonin synthesis toward the kynurenine pathway [38]. This metabolic shift has consequences in both the gut and the brain: in the intestine, increased IDO activity disrupts mucosal homeostasis, enhances oxidative and inflammatory signaling, and alters microbiota, host interactions [39]; in the CNS, the resulting imbalance among serotonin, melatonin, and neuroactive kynurenine metabolites contributes to neuroinflammation, synaptic dysfunction, and impairments in cognition and mood [40]. Certain kynurenine-derived metabolites may further influence cholinergic neurotransmission, directly or indirectly, affecting acetylcholine receptor activity [41]. Notably, *K. phaffii* KM71H reduced IDO activity across all evaluated tissues, indicating that the yeast mitigates the SD-induced shift in tryptophan catabolism toward the kynurenine pathway and supports a more balanced metabolic profile.

Consistent with these mechanistic pathways, SD activated the HPA axis, elevating corticosterone and GR expression, a hallmark of stress-axis dysregulation associated with circadian disruption and increased vulnerability to anxiety and CNS disorders [42, 43]. Yeast-treated SD animals showed reduced corticosterone levels and decreased GR expression in both brain and intestinal tissues, indicating attenuation of SD-induced HPA axis overactivation and alignment with evidence that antioxidant and microbiota-targeted interventions can normalize corticosterone under prolonged SD [44] and complement previous findings showing that *K. phaffii* KM71H preserves barrier integrity and reduces hippocampal GR expression, thereby mitigating neuroinflammatory and stress-related pathways [7, 8].

Beyond its central neuroendocrine effects, sustained corticosterone elevation also disrupts peripheral systems, particularly the intestinal barrier, initiating inflammatory cascades that shape the microbiota–gut–brain axis under SD conditions [45]. Corticosterone-mediated impairment of tight junctions facilitates the translocation of bacterial endotoxins such as lipopolysaccharide (LPS) into systemic circulation, where LPS activates TLR4 and triggers the canonical NF-κB–dependent inflammatory pathway [46]. Subsequent NF-κB activation promotes the expression of pro-inflammatory cytokines, including IL-6, thereby amplifying systemic and neuroimmune dysfunction associated with SD [44]. Notably, *K. phaffii* KM71H attenuated these inflammatory responses, reducing TLR4, NF-κB, and IL-6, even in animals exhibiting elevated corticosterone, demonstrating a robust anti-inflammatory effect. These results align with findings from Birmann et al. [6], where the yeast decreased intestinal TLR4 mRNA and hippocampal IL-1β expression, further supporting its capacity to mitigate systemic inflammation.

From an oxidative perspective, insufficient sleep imposes a substantial burden on both central and peripheral tissues, as SD compromises antioxidant defenses and increases RS in the brain and the gut due to prolonged wakefulness and elevated metabolic demand [47–51]. Supporting the contribution of gut physiology to redox balance, a recent study showed that probiotic supplementation in middle-aged mice enhanced antioxidant capacity in multiple tissues, reduced oxidative damage, reshaped microbiota

composition, and increased short-chain fatty acids (SCFAs), highlighting the interaction between microbial modulation and systemic antioxidant effects [52].

Consistent with evidence linking SD-induced oxidative stress to gut dysbiosis and elevated RS in intestinal tissues [51], probiotics such as *Lactobacillus plantarum* have been shown to reduce RS and improve cognition in animal models [53]. In line with these findings, the present study demonstrates that *K. phaffii* KM71H markedly reduced RS levels in the small intestine, prefrontal cortex, and hippocampi of SD-exposed mice, thereby protecting neural tissue from oxidative and inflammatory damage.

Despite the broad behavioral, biochemical, and molecular evidence presented, this study has important limitations. Microbiota composition was not assessed, preventing identification of the microbial shifts that may contribute to the observed effects. Additionally, oxidative stress was evaluated solely through RS quantification, without complementary antioxidant markers that could refine the understanding of redox pathways. These gaps limit mechanistic depth and highlight the need for future studies integrating microbiome profiling and expanded oxidative and inflammatory analyses.

Given the rising prevalence of sleep loss–related neuropsychiatric symptoms in humans, the broad immunometabolic and neuroprotective actions observed here underscore the translational potential of *K. phaffii* KM71H as a safe, low-cost, microbiota-targeted intervention. Together, these findings establish a solid foundation for advancing this yeast strain toward more detailed mechanistic investigations and eventual clinical evaluation.

Conclusion

Taken together, our findings demonstrate that *K. phaffii* KM71H effectively counteracts the neurobehavioral and molecular disturbances induced by SD. Notably, the central axis of this study lies in the coordinated modulation of IDO, GSK3β, and AChE, highlighting a convergent immunometabolic–cholinergic mechanism underlying the protective effects of the yeast. Beyond improving anxiety-like and cognitive outcomes, KM71H attenuated SD-associated disruptions in inflammatory, metabolic, and stress-responsive pathways which are functionally interconnected within the microbiota–gut–brain axis. Collectively, these results position *K. phaffii* KM71H as a multi-target psychobiotic with strong therapeutic relevance and establish a robust foundation for future mechanistic and translational investigations.

Declarations

Author Contribution

R. Xavier performed the experimental design, behavioral tests, biochemical assessments, and data analysis and wrote the manuscript. L. Savegnago performed data analysis, writing, reviewing, and editing of the manuscript, supervised the experiments, acquired funding, and is the corresponding author*. R. L. Oliveira performed data analysis, writing, reviewing, editing of the manuscript and supervised the

experiments. L. N. Pujol performed the behavioral tests and the biochemical assessments. F.S.S. Sousa, S. L. Lanius, F.K. Seixas, and T. Collares performed the qRT-PCR analyses. F. R. Conceição and R.R. Rodrigues performed the cultivation and preparation of *K. phaffii*. L. Savegnago and F.R. Conceição were responsible for funding acquisition and reviewing and editing the manuscript.

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Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Code Availability

Not applicable.

Ethics Approval

The experiments with animals were carried out in accordance with the Institutional Animal Care and Use Committee of the Federal University of Pelotas (approval number of the ethics committee: 17/2024), affiliated with the National Council for Animal Experimentation Control (CONCEA).

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing financial or non-financial interests.

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Figures

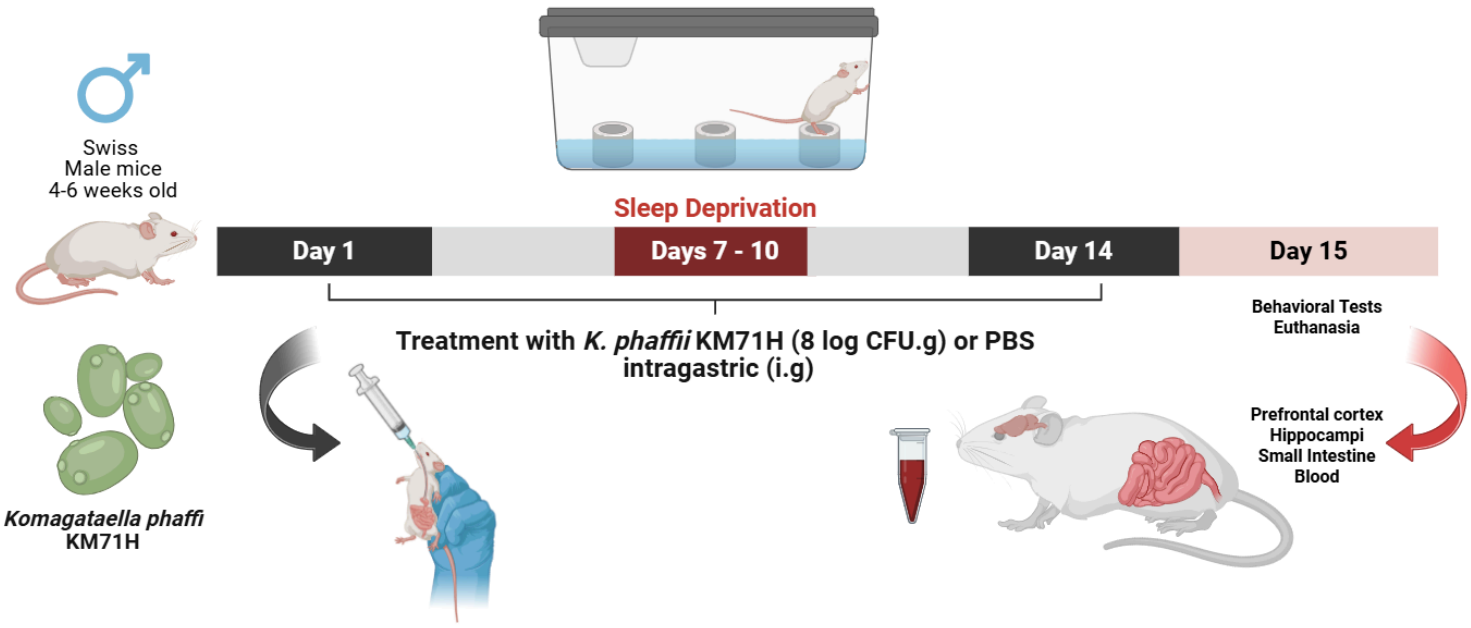


Figure 1

Experimental design.

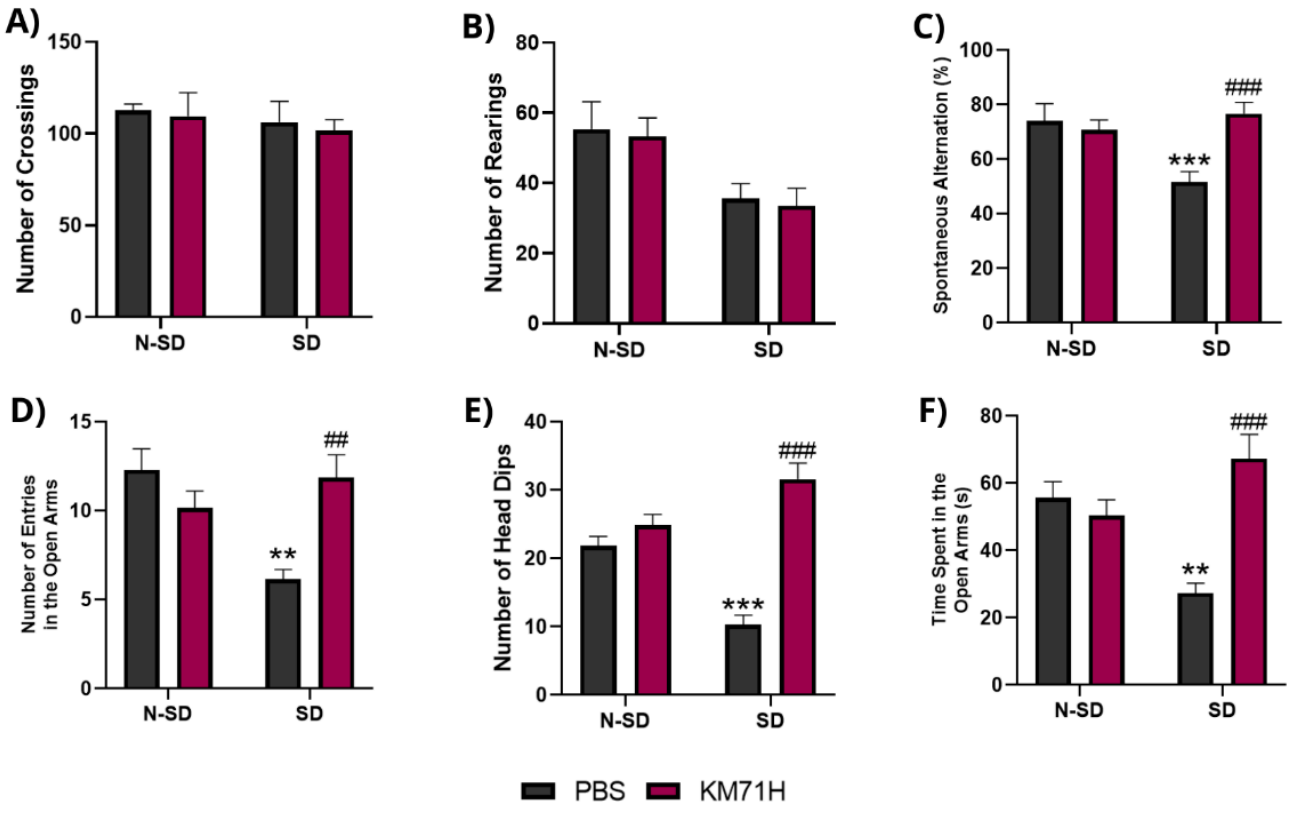


Figure 2

Effects of *K. phaffii* KM71H treatment on behavioral parameters. (A–B) Number of crossings and rearings in the open field test. (C) Spontaneous Alternations (%) in the Y-maze test. (D) Number of open-arm entries, (E) head-dips, and (F) time spent in the open arms (s) in the EPM test. Data are expressed as mean ± standard error of the mean (SEM) (n = 7). **p < 0.01; ***p < 0.001 vs. control group; ##p < 0.01; ###p < 0.001 vs. SD group. PBS: phosphate-buffered saline; N-SD: non sleep-deprived; SD: sleep deprivation. Statistical analyses were performed using two-way ANOVA followed by Tukey's post hoc test.

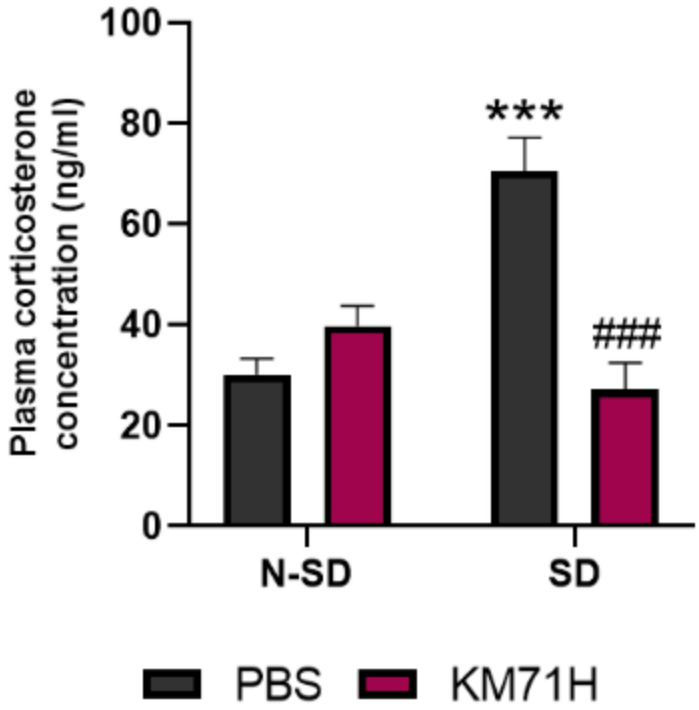


Figure 3

Effect of *K. phaffi* KM71H treatment on the quantification of plasma corticosterone levels in mice. Data are expressed as mean ± standard error of the mean (SEM) (n = 7). ***p < 0.001 compared with the control group, and ###p < 0.001 compared with the SD group. PBS: phosphate-buffered saline; N-SD: non sleep-deprived; SD: sleep deprivation. Statistical analyses were performed using two-way ANOVA followed by Tukey's post hoc test.

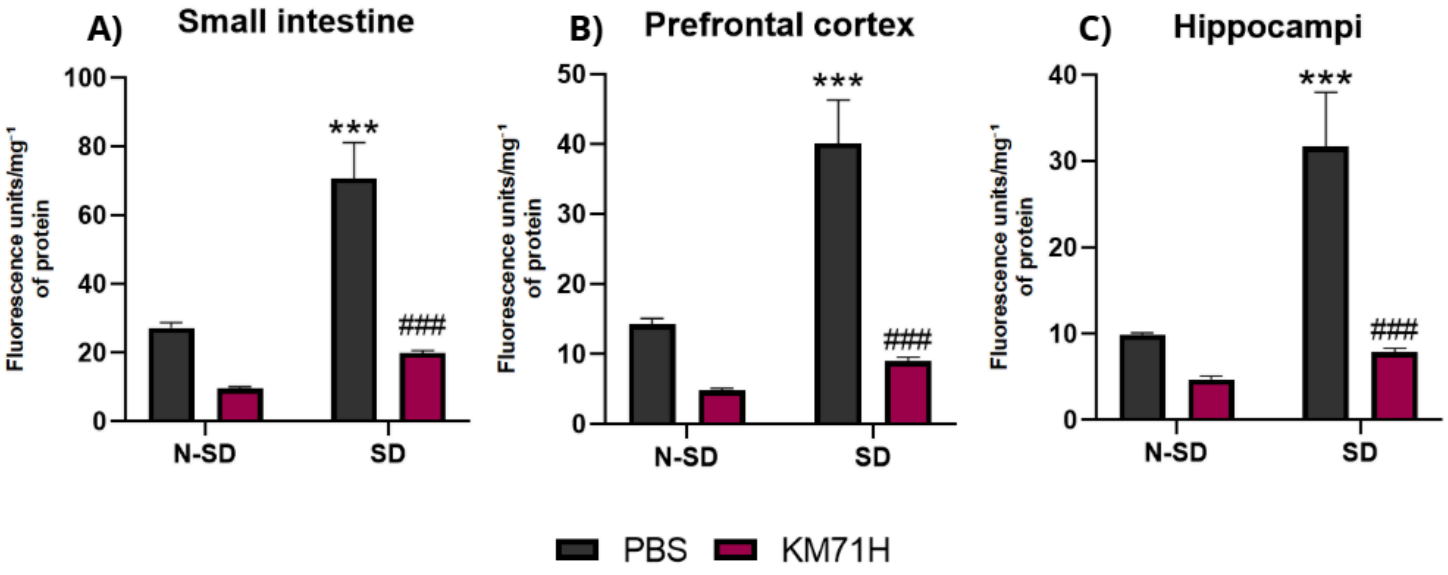


Figure 4

Effect of *K. phaffii* KM71H treatment on the quantification of reactive species levels generated in the small intestine (A), prefrontal cortex (B), and hippocampi (C). Data are presented as mean ± standard error of the mean (SEM) (n = 7). ***p < 0.001 compared with the control group and ###p < 0.001 compared with the SD group. PBS: phosphate-buffered saline; ; N-SD: non sleep-deprived; SD: sleep deprivation. Statistical analyses were performed using two-way ANOVA followed by Tukey's post hoc test.

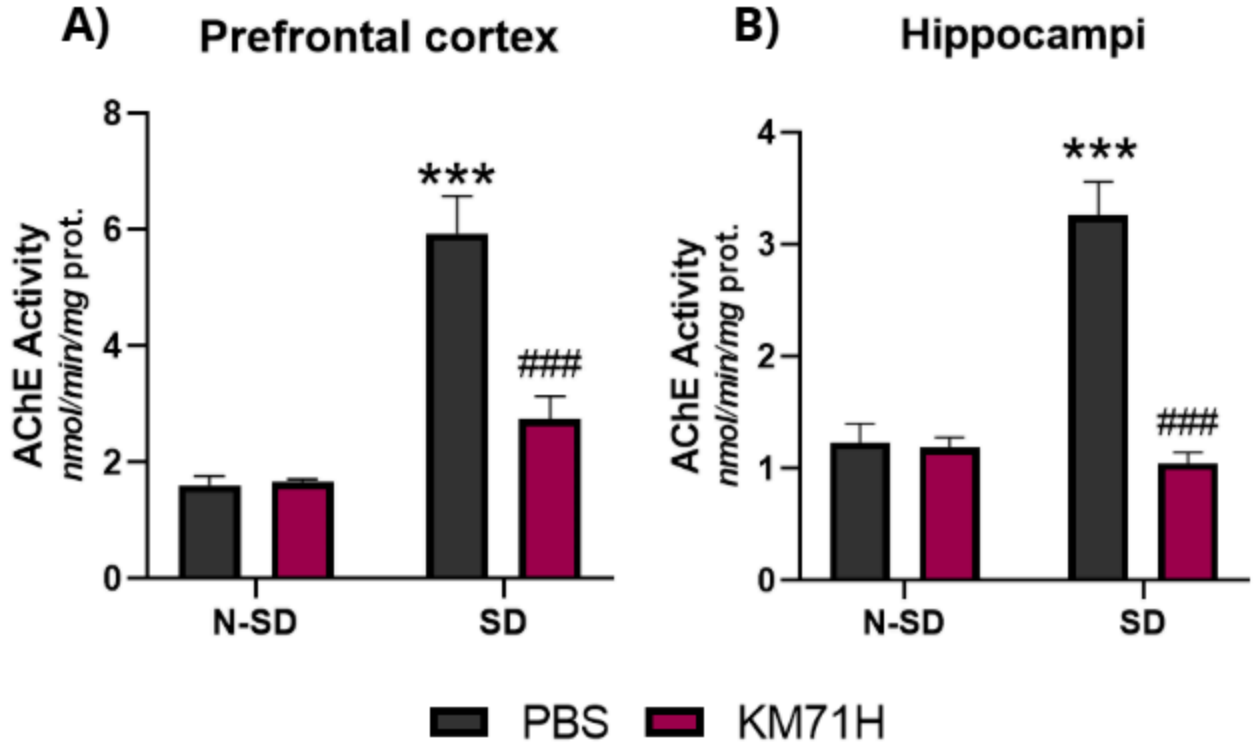


Figure 5

Effect of *K. phaffii* KM71H treatment on acetylcholinesterase enzymatic activity in the prefrontal cortex (A) and hippocampi (B) of mice. Data are expressed as mean ± standard error of the mean (n = 7). ***p < 0.001 compared with the control group, and ###p < 0,001 compared with the SD group. PBS: phosphate-buffered saline; N-SD: non sleep-deprived; SD: sleep deprivation. Statistical analyses were performed using two-way ANOVA followed by Tukey's post hoc test.

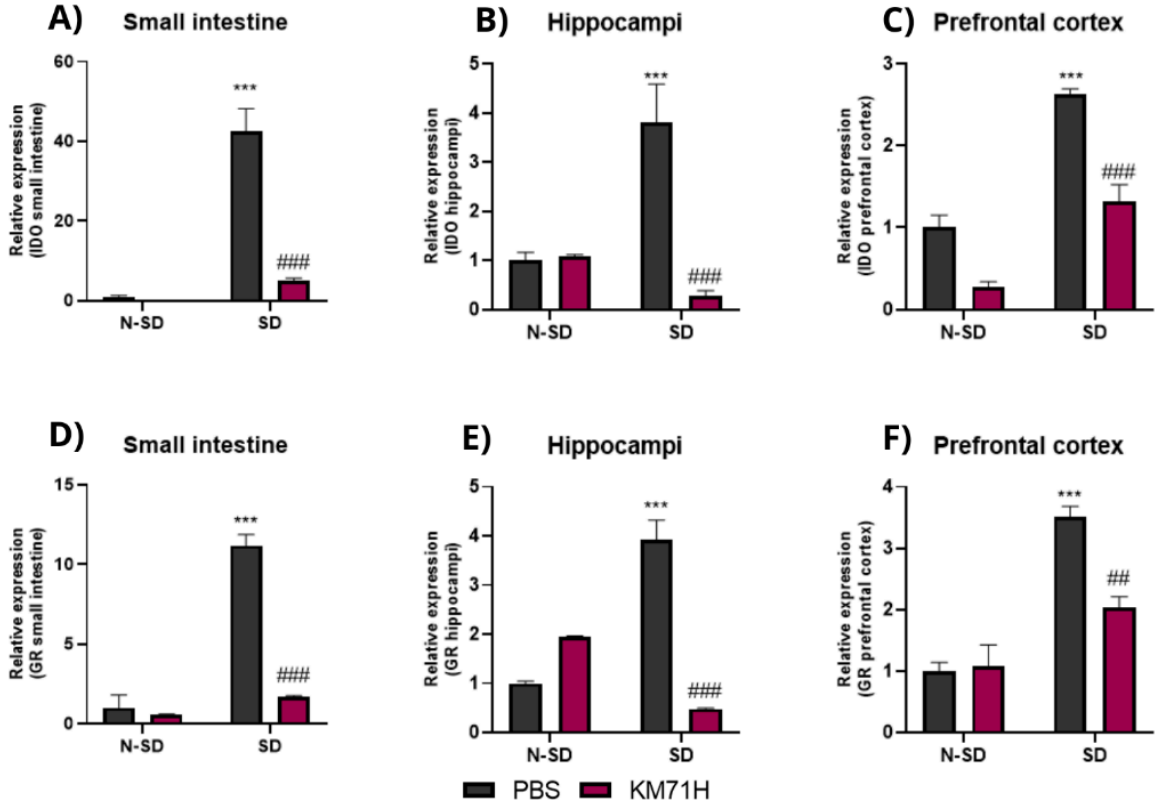


Figure 6

Relative mRNA expression of indoleamine 2,3-dioxygenase (IDO) and glucocorticoid receptor (GR) in the small intestine (A,D), hippocampi (B,E), and prefrontal cortex (C,F). Data are expressed as mean ± SEM (n = 3). ***p < 0.001 vs. compared with the control group, and $^{##}$p < 0.01, $^{###}$p < 0.001 compared with the SD group. PBS: phosphate-buffered saline; N-SD: non sleep-deprived; SD: sleep deprivation. Statistical analyses were performed using two-way ANOVA followed by Tukey's post hoc

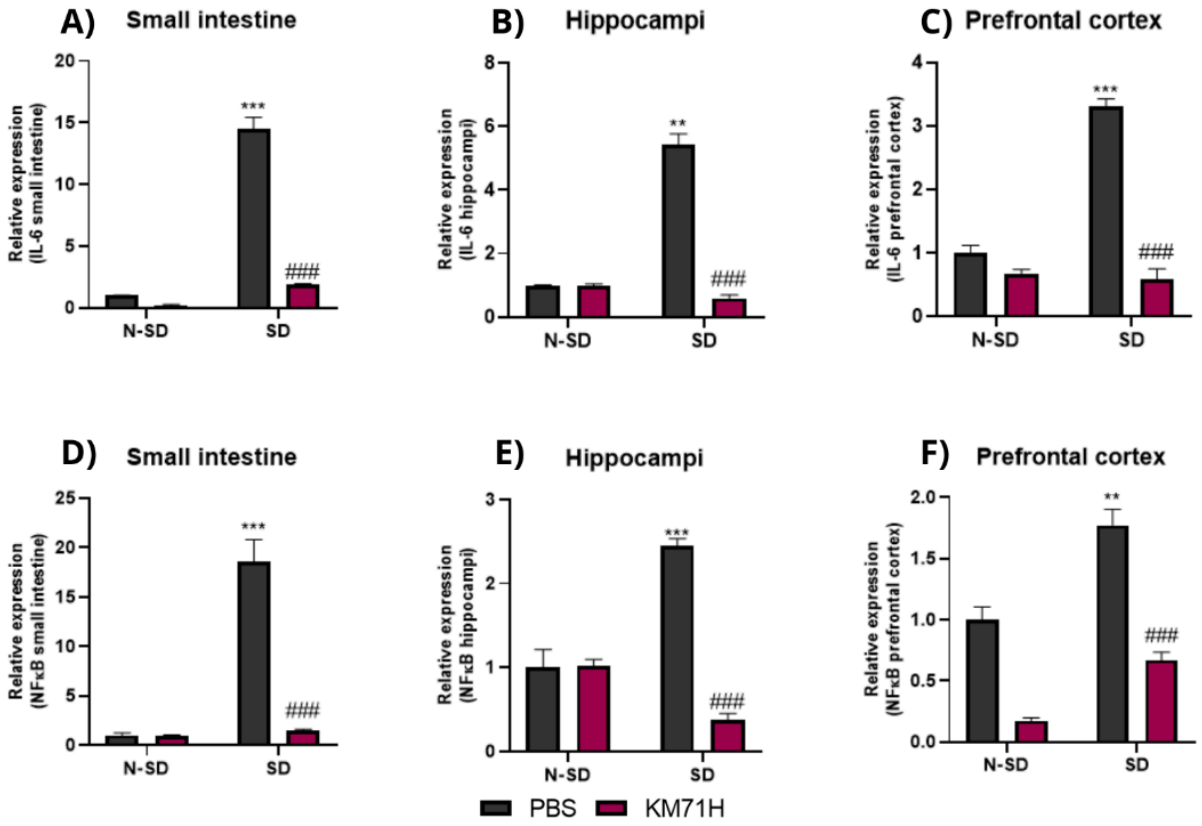


Figure 7

Relative mRNA levels of nuclear factor kappa B (NF-κB) and interleukin-6 (IL-6) in the small intestine (A,D), hippocampi (B,E), and prefrontal cortex (C,F). Data are expressed as mean ± SEM (n = 3). **p < 0.01, ***p < 0.001 compared with the control group, and ###p < 0,001 compared with the SD group. PBS: phosphate-buffered saline; N-SD: non sleep-deprived; SD: sleep deprivation. Statistical analyses were performed using two-way ANOVA followed by Tukey's post hoc test.

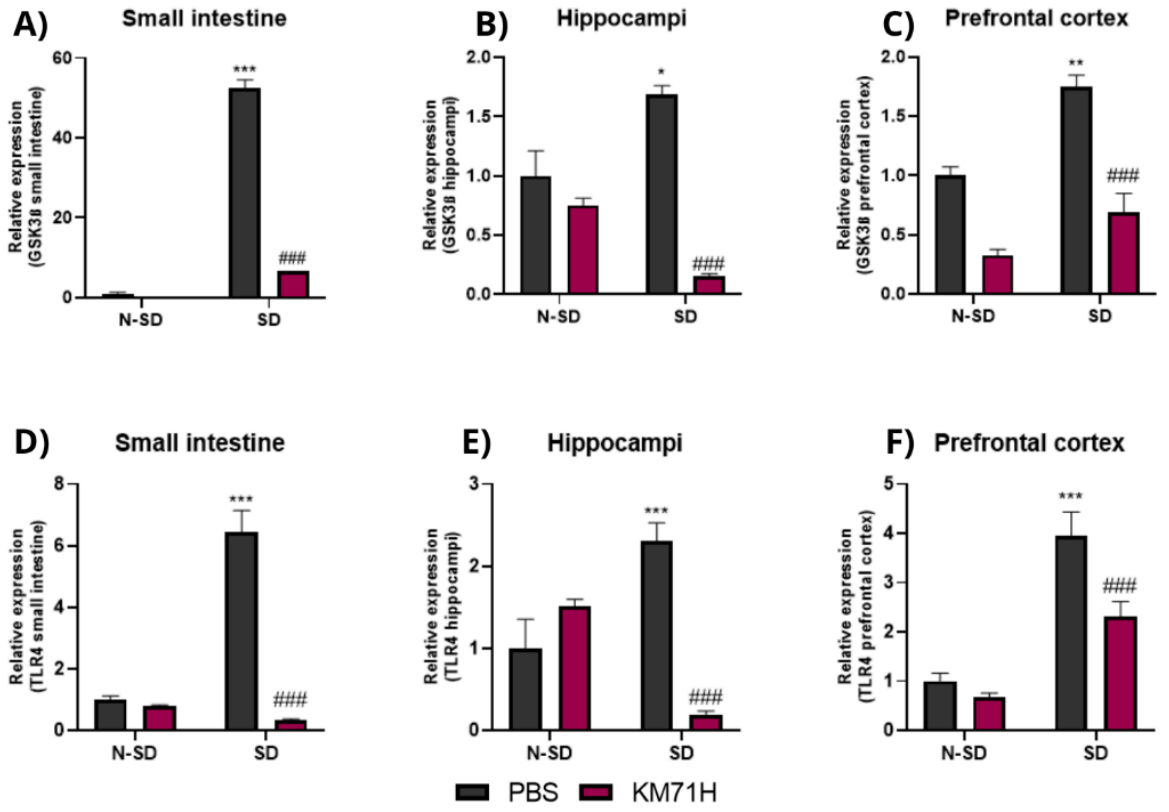


Figure 8

Relative expression of glycogen synthase kinase-3 beta (GSK3β) and toll-like receptor 4 (TLR4) in the hippocampi (A,B), prefrontal cortex (C,D), and intestine (E,F). Data are presented as mean ± SEM (n = 3). *p < 0.05, **p < 0.01, ***p < 0.001 compared with the control group, and $^{###}$p < 0,001 compared with the SD group. PBS: phosphate-buffered saline; N-SD: non sleep-deprived; SD: sleep deprivation. Statistical analyses were performed using two-way ANOVA followed by Tukey's post hoc test.

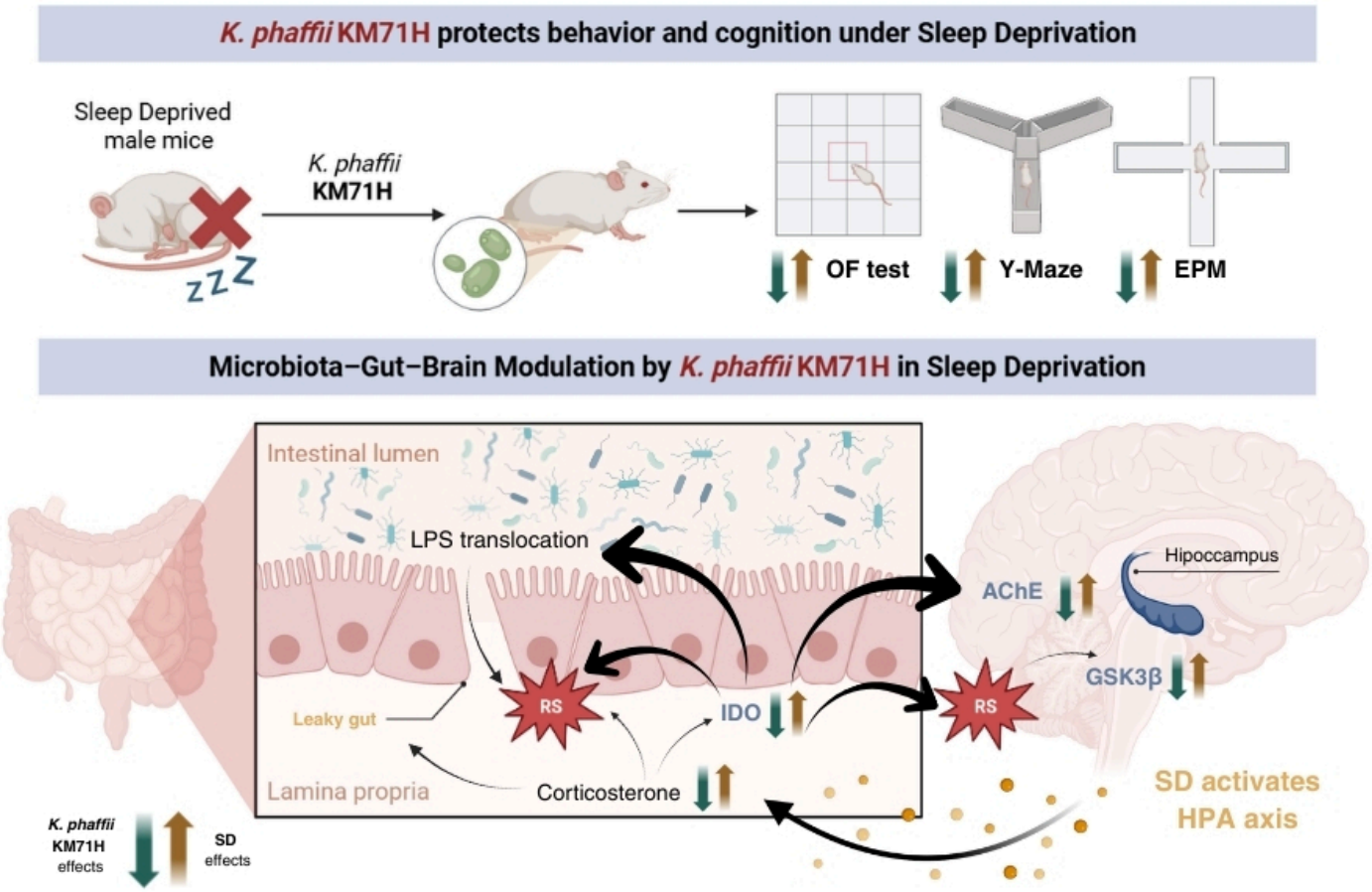


Figure 9

Graphical Abstract.