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Antidepressant and neuroprotective potential of stingless bee honey in a preclinical stress model

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ABSTRACT

Stingless bee honey (SBH), as a functional food supplement, is of particular interest in this context due to its potential to modulate neurobiological factors such as BDNF and neurotransmitter release, which are often disrupted in depressive disorders. SBH is a fermented honey rich in trehalulose, probiotics, organic acids including aliphatic acids, and various bioactive compounds. These properties mitigate neurodegenerative processes by reducing oxidative stress and inflammation, enhancing neuronal resilience, and supporting the maintenance of cognitive function and overall brain health. This preclinical study aims to investigate the antidepressant effects of SBH in the chronic restrained stress (CRS) depression model via behavioural, hormone secretions, and histological analyses. Forty-two Swiss Albino mice (8 weeks old) were divided into five groups, receiving SBH supplementation or paroxetine (positive control). CRS was induced for 2 h daily for 28 days. The evaluated effects include body weight, behavioural tests, neurotransmitter levels, hippocampal neuron integrity, and brain-derived neurotrophic factor (BDNF) expression. The results of the physicochemical analysis confirmed that the SBH complied with the Malaysian Standard (MS2683:2017). FESEM experiment indicates the presence of bacteria and yeast on the walls of cerumen pots. Behavioural assessments demonstrated a significant reduction in anxiety-like behaviours and immobility duration among SBH-treated groups, indicative of its anxiolytic and antidepressant-like effects. The biochemical evaluations via ELISA assay showed that SBH supplementation reduced corticosterone levels, maintained serotonin concentrations, and increased dopamine availability, whilst elevating the serum phenylalanine levels. Upregulated BDNF expression and downregulation of the pro-inflammatory cytokines were also observed. The increased level of BDNF has been closely linked with synaptic plasticity as well as regulating the monoaminergic neurotransmitter regulation. These findings underscore the potential of SBH as a neuroprotective supplement, likely mediated by monoamine modulation, neurogenesis, and anti-inflammatory effects, warranting further clinical investigations as a functional food with anti-depressant effects.

1. Introduction

Stingless bees, belonging to the Family Apidae and tribe Meliponini, differ from common Apis honeybees by lacking functional stingers. These highly eusocial species are vital pollinators that aid in maintaining the ecosystem balance, where they are native to tropical and subtropical regions, including Southeast Asia, Northern Australia, Africa, and parts of South America (Wayo, Sritongchuay, Chuttong, Attasopa, & Bumrungsri, 2020). Their honey commonly multi-floral, stored in cerumen-propolis pots, possesses a distinct sweet sourness flavor due to probiotic fermentation and contains several bioactive compounds, enhancing its antioxidant, antimicrobial, and anti-inflammatory properties (Biluca et al. 2020, Al-Hatamleh et al., 2020). It is also rich in free acidity which enhances therapeutic benefits including wound healing and immune-boosting qualities (Al-Hatamleh et al., 2020; Julika et al., 2020). Additionally, the presence of trehalulose, a rare low glycaemic

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index (GI) sugar, further contributes to the distinctive qualities of SBH, offering potential health benefits that have attracted growing research interest. Previous research showed that SBH supplementation improved memory and learning in mice via the BDNF signalling pathway. The BDNF signalling pathway plays a crucial role in promoting neuroplasticity and influencing memory formation, learning, and mood regulation, presenting SBH as a promising food supplement for preventing depression (Mustafa et al., 2019).

Subsequently, BDNF signalling also modulates the release of serotonin and dopamine in the mesolimbic dopamine system (Narita et al., 2003). Apart from BDNF, glucocorticoids also showed implications in neuronal function and depression. While the BDNF supports neuronal survival and plasticity, glucocorticoids which are often elevated during stress could modulate BDNF expression and impact neuroplastic outcomes as highlighted by past evidence (Al-Rahbi et al., 2014). Thus, the strong crossover between their functions is suggested as a mechanism of depression.

Depression is a prevalent mental illness marked by persistent sadness and a loss of interest in activities, also alters emotion, and behaviour to suicide (Stepanichev et al., 2014; World Health Organization, 2023). The World Health Organization (WHO) projects that by 2030, depression will become a leading global health burden, impacting approximately 280 million individuals across all age groups (World Health Organization, 2023).

Various pathological studies have identified causes of depression, with the monoamine hypothesis being the most relevant and well-accepted (Tiemeier, 2003). Conventional antidepressants targeting monoamines to treat depression often come with several drawbacks, including dizziness, sedation, sexual dysfunction, insomnia, anxiety, and high relapse rates, underscoring the need for alternative therapies (Torres et al., 2003).

Previous research highlights the role of inflammation relapses in depressive disorders. Chronic inflammation, marked by prolonged immune activation and elevated pro-inflammatory cytokines can affect brain function and behaviour. High levels of cytokines such as IL-1β, IL-6, and TNF-α are found in some individuals with depression, influencing neurotransmitter metabolism, reducing neurogenesis, and disrupting neural plasticity. Neuroinflammation can alter brain regions involved in mood regulation, such as the hippocampus and prefrontal cortex, impairing mood, cognition, and behaviour (Adeniyi et al., 2023; Audet & Anisman, 2013). Chronic stress exacerbates this immune response, negatively affecting the hypothalamic-pituitary-adrenal (HPA) axis and contributing to depressive symptoms (McEwen et al., 2016).

The relationship between monoamines and inflammation in depression is intricate and bidirectional. Monoamines, including serotonin (5-HT), norepinephrine, and dopamine (DA), are integral to the regulation of mood and behaviour (Jiang et al., 2022). Inflammation has the potential to disrupt the balance of these monoamines, thereby exacerbating depressive symptoms and perpetuating a reciprocal relationship between the two factors and thus should be the major target in treating depression. Inline, a higher antioxidant and anti-inflammatory profile of SBH may mitigate neuroinflammation. To date, no prior study has specifically evaluated the effects of SBH using the CRS model for depression, making this investigation the first to explore its potential antidepressant properties within a well-established preclinical framework of stress-induced mood disorders. This gap highlights the novelty and significance of the present study, aiming to investigate the potential of SBH as a functional food in alleviating depressive-like behaviour and exploring its potential for depression prevention.

2. Materials and methods

2.1. Source of stingless bee honey

The multiflora SBH was obtained from a local farm (Brainey Sdn Bhd) in Malaysia where this farm has been reported to comprise a consistent presence of phenylalanine. The SBH was harvested from *Heterotrigona itama* species (Hymenoptera: Apidae: Meliponini), with a good practice of harvesting. The moisture content of SBH was initially determined to be at 32 % and further dehydrated to up to 20 % using Honey Interlinked Dehydration and Dispenser Apparatus (HILDA) to comply with the moisture content limit set by the Codex Alimentarius for Honeys (Codex Alimentarius Commission, 2001) (Yong et al., 2022). Low moisture levels are essential for maintaining its stability whilst preventing overfermentation (Ahmed et al., 2016). The honey was further bottled at the Hazard Analysis and Critical Control Points (HACCP), HALAL, Good Manufacturing Practice (GMP), and International Organization for Standardization (ISO22000)-certified processing plants (Honeygold Enterprise Sdn Bhd) to ensure high-quality and hygienic honey handling.

2.2. Evaluation of stingless bee honey pot via field electron scanning electron microscopy

Cerumen propolis pot samples were freshly harvested, preserved with glutaraldehyde and osmium tetroxide, and dehydrated with gradually increasing ethanol concentrations. The samples were mounted on an aluminium stub using a conductive adhesive, coated with gold to prevent charging and viewed under the FESEM. High-resolution images at various magnifications were captured to specifically obtain the shapes and morphology of the probiotics (Erlandsen et al., 2002).

2.3. Drugs and chemicals

ELISA kits for corticosterone (ab108821), 5-hydroxytryptamine (ab133053), and phenylalanine (ab83376) were purchased from Abcam (Cambridge, UK). ELISA kits for BDNF (QY-E20219) and dopamine (QY-E20493) were obtained from Qayee (Shanghai, China). Paroxetine (ab120069), used as the control drug, was procured were ordered from Abcam (Cambridge, UK). Sodium acetate anhydrous was obtained from Merck (New Jersey, USA), and 2,4,6-Tris(2-pyridyl)-*s*-triazine was purchased from Sigma-Aldrich (St. Louis, MO, USA) for UPLC analysis. All chemicals used in this study were of analytical grade.

2.4. Physicochemical analysis of stingless bee honey

The SBH sample was analysed using ultra-performance liquid chromatography (UPLC) and standard methods following AOAC to determine the peak areas of the main components of sugar content, and hydroxymethylfurfural (HMF). Several other parameters including pH and moisture content were performed to evaluate its compliance with the MS2683:2017 standard (*Official methods of analysis of AOAC international*, 2005). Ultra performance liquid chromatography (UPLC) method was conducted as demonstrated in the Empower UPLC software. The sample was analysed in triplicate and reported on average.

2.5. Animals

Forty-two Swiss Albino mice (8 weeks old) were obtained from the Animal Research and Services Centre (ARASC), Universiti Sains Malaysia (USM). Swiss albino mice, a widely used outbred strain in biomedical research have shown consistent responses in experimental models of stress-related psychiatric disorders and suitability that is associated with depression studies (Marchette et al., 2018). All mice were acclimatized for 5 days with ad libitum access to water and standard pellet, maintained on a 12 h light/dark and controlled temperature (± 25 °C). All procedures were performed following the guidelines approved by the Animal Ethics Committee of Universiti Sains Malaysia (USM/Animal Ethics Approval/2018(112)(927)).

2.6. Experimental groups

All mice were caged individually after acclimatization and randomly divided into seven groups ($n = 42$); G1 (normal), G2 (stressed), G3 (paroxetine 10 mg/kg), G4 (stress treated with 200 mg/kg on day 15), G5 (stress treated with 200 mg/kg on day 1), G6 (stress treated with 2000 mg/kg on day 15), and G7 (stress treated with honey 2000 mg/kg on day 1) groups.

The dosage selection was calculated according to a 162 mg/kg honey dose extrapolated from a human into a mouse dose using the body surface area (BSA) formula as recommended by the FDA (Reagan-Shaw et al., 2008). This ensures the dose used in mice reflects a safe and relevant intake level comparable to human consumption. The formula is mentioned below (Nair & Jacob, 2016).

Human equivalence dose (HED), mg/kg =.

Animal dose, mg/kg x Correction factor (Km) ratio.

Km ratio for mice = 0.081.

The doses of 200 mg/kg and 2000 mg/kg of SBH are considered safe for mice based on prior studies. According to OECD guidelines, substances with no observed adverse effects at 2000 mg/kg in acute toxicity tests are classified as safe (OECD, 2001). Previous research also reported no signs of toxicity or mortality in rodents given SBH at these doses, supporting their safe use in animal studies (Fauzi et al., 2018). Meanwhile, G2 was exposed to stress only and the G3 group was given paroxetine drug (10 mg/kg) via intraperitoneal injection.

2.7. Induction of chronic restraint stress (CRS)

All groups underwent stress induction using the restraint stress model for studying psychogenic stress as described by (Kim et al., 2017). Stress was administered daily for 2 h, from day 1 to day 28, by placing the animals in specialized plastic restraint tubes (diameter: 6 cm × 10 cm × 9 cm), which completely restricted their movement. Body weight was monitored daily throughout the experiment. The CRS model was employed in conjunction with its ability to induce genetic and protein changes that resemble those observed in depression. Designed to mimic prolonged psychological stress, it offers a practical, cost-effective, and reliable method for modeling depression in rodents (Woo et al., 2018).

2.8. Behavioural and protein expression analyses

Behavioural analyses were conducted using the elevated plus-maze (EPM), forced swim test (FST), enzyme-linked immunosorbent assay (ELISA), and immunohistochemistry (IHC) to examine stress-induced responses, quantify targeted molecular biomarkers, and visualize neuroanatomical changes of protein expression in the brain tissues, respectively. These extensively validated tests are essential in preclinical studies for assessing emotional and psychological states in rodents, providing reliable behavioural endpoints that mirror human anxiety and depressive symptoms. Thereby enhancing the translational value of the findings in the context of neuroprotective and antidepressant research (Woo et al., 2018).

2.8.1. Elevated plus-maze (EPM)

The elevated plus-maze (EPM) was used to assess anxiety-like behaviour in rodents, following the method of (Sestakova et al., 2013). The maze was elevated 50 cm above the floor, and each mouse was placed in the center facing an open arm. The test was conducted on days 22 and 29. Their behaviour was videotaped for 5 min using ANY-maze software. The apparatus was cleaned with 70 % alcohol between each trial. The number of open-arm entries and the time spent in the open arms were recorded and presented in seconds (Mehta et al., 2017).

2.8.2. Force swimming test (FST)

The test was performed to assess depression-like behaviour and was conducted on days 22 and 29 following the method described by (Yan et al., 2015). A resting period was included to minimize the potential carryover effects from the previous test (McIlwain et al., 2001). Each mouse was placed in a glass cylindrical container (40 cm height, 16 cm diameter) with 30 cm of water maintained at 25 ± 2 °C. The mouse was videotaped for 6 min, and the total immobility time was measured in seconds. Immobility was defined as the mouse remaining motionless in the water, only moving to keep its head above the water surface, reflecting the despair status of the mouse.

2.9. CORT, 5-HT, DA, Phe, and BDNF measurement using ELISA

Enzyme-linked immunosorbent assay (ELISA) was conducted to determine the secretion levels of several hormones. The mice were anesthetized using a ketamine/xylazine cocktail (with the recommended dosage of 90 mg/kg and 5 mg/kg body weight) prior to sacrifice via intraperitoneal injection. Blood samples (1 mL, approximate volume) were collected and kept on ice and later centrifuged at 3000 rpm for 15 min at 4 °C to separate serum. The serum collected was evaluated for corticosterone (ab108821), serotonin (ab133053), dopamine (QY-E20493), and Phenylalanine (ab83376) following kit instructions. The left brain (hippocampus) tissue of mice was also collected following the anesthesia. The brain tissue was collected into RNA later and used to quantify BDNF protein levels (QY-E20219) using a commercially available sandwich ELISA kit. The serum and brain tissue samples were stored at −80 °C until further use (Monteiro et al., 2015).

2.10. Histology of hippocampus

After anesthetizing and decapitating the mouse, the brain was dissected, fixed in 10 % formalin, and stored at 4 °C before tissue processing. The brain was embedded in paraffin wax. Sections were trimmed to 5 μm, mounted on slides, air-dried, and stored in a slide box until the staining procedure. Nissl staining was performed to visualize neurons by highlighting bodies, which are rich in RNA and located in the rough endoplasmic reticulum. Nissl-positive cells in the hippocampus were counted across five microscopic fields as representative of the whole sample (Hasim et al., 2020). Only clearly visible neurons in a single focal plane were counted to prevent double-counting, ensuring only clear, distinct neurons were included in the analysis. Nissl-stained neurons were identified by their round shape, prominent nucleolus, and Nissl substance, while abnormal neurons showed reduced staining intensity and dark appearance. This method aids in neuronal structure, size, and presence of Nissl substances, which are commonly used to detect nerve cell damage (Kiernan, 2015).

2.11. Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM) in each experiment. The statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using GraphPad-Prism v.8.0.1 software (GraphPad, San Diego, CA). All tests were two-tailed and the significance level was set at $p < 0.05$.

3. Results

3.1. Physicochemical properties

Table 1 shows the physicochemical properties of SBH (dehydrated honey) in comparison to Malaysian Standard (2017) MS2683:2017 with moisture content, sugars, HMF, and pH values all within acceptable ranges. Findings showed that the collected SBH sample fulfilled the criteria of authentic honey according to the Malaysian standard. Notably, SBH contains a significant amount of trehalulose (17.08 ± 1.35 g/100 g), a rare disaccharide absent from standard guidelines but recognized for its unique properties of SBH as compared to other honey. The absence of sucrose and low levels of HMF further support the quality

Table 1
Physicochemical properties of stingless bee honey.

Physicochemical properties	Stingless bee honey	Malaysian standard (2017)
Moisture content (%)	20.00 ± 0.01	Max 35
Fructose (g/100 g)	28.85 ± 1.40	Max 85
Glucose (g/100 g)	35.18 ± 1.50	
Trehalulose (g/100 g)	17.08 ± 1.35	NA
Maltose	2.18 ± 1.15	Max 9.5
Sucrose	ND	Max 7.5
Hydroxylmethylfurfural (HMF)	8.50 ± 2.15	Max 30
pH	2.98 ± 0.13	2.5–3.8

NA; not available, ND; not detected.

and stability of SBH. Together, these highlighted the basic foundation of honey standardization.

3.2. Ultrastructural imaging of propolis using field emission scanning electron microscope

The surface morphology of the raw propolis of stingless bee *Heterotrigona itama* was examined via FESEM micrograph. Fig. 1A (under 5000× magnification) showed the presence of rod-shaped cells (bacilli) appearing in variety as singly and in pairs. Some presented to align end-to-end whilst comprised of smooth surfaces. Fig. 1B illustrated the ellipsoidal-shaped cells that formed in budding pairs with a slightly rough texture upon 10,000× magnification imaging. A distinct protuberance (indicates the connection between the mother and daughter cells) also clearly being observed in the budding cells. These further highlighted that the appearance of various microorganisms could be potentially identified as bacteria (Fig. 1A) and yeast (Fig. 1B), which were abundantly present on the smooth surface of propolis.

SBH is known to comprise beneficial microbes that may play a significant role in its bioactive properties in SBH, including its antioxidant and anti-inflammatory effects (Salomón et al., 2024). These microbial components, alongside its rich nutritional profile, support its potential as a neuroprotective agent. Growing evidence supports the involvement of the gut-brain axis in the regulation of mood and cognitive function, as the gut microbiota influence the neurochemical pathways and inflammatory responses linked to mental health. Through its probiotic-like activity, SBH potentially mitigates depression-related symptoms and supports brain health.

3.3. Body weight (%)

Fig. 2 represents the percentage of body weight changes in the mice model. It indicated that the changes in the body weight increased

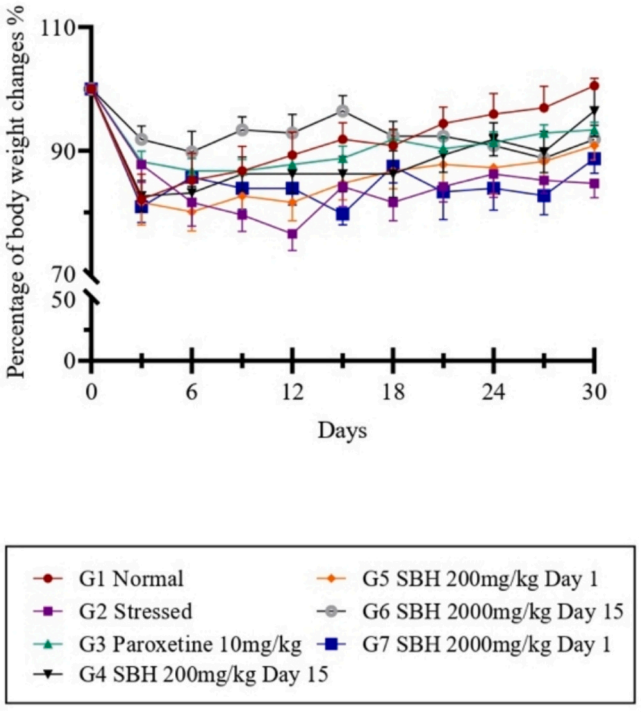

Fig. 2. The percentage of body weight changes over 30 days following stress induction and treatment. The graph showed that the food intake in all CRS-induced groups was not significant as compared to the G1 group. Data represented as means ± SEM. Data were analysed using one-way ANOVA.

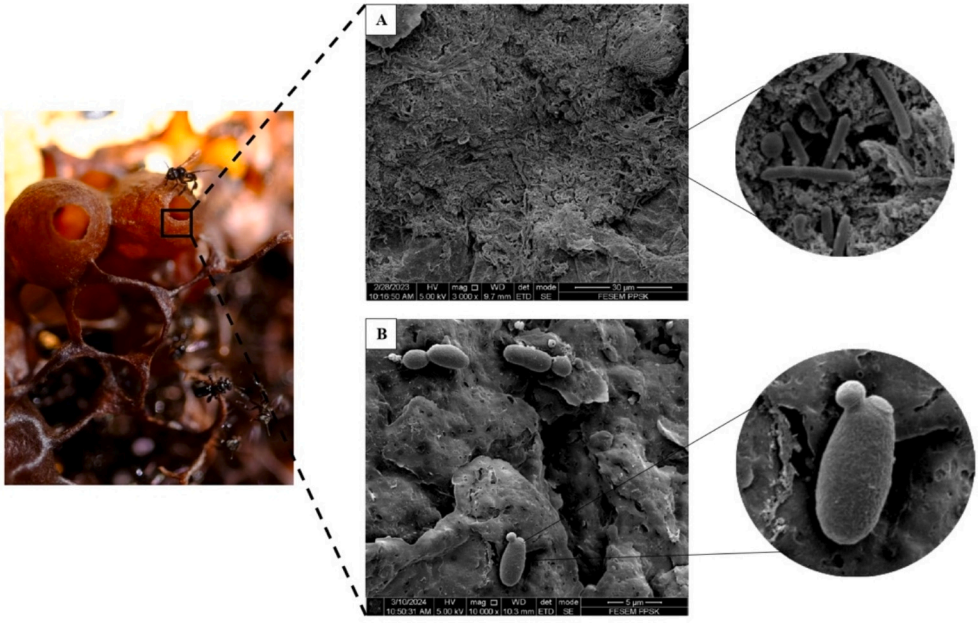

Fig. 1. Left image shows the gross view of SBH cerumen pots, entirely different from normal honeycombs. The presence of microorganisms observed on the surface of stingless bee propolis upon viewing under FESEM microscopy. (A) Bacteria; (B) yeast.

gradually, however, the body weight was more stable in the SBH-treated groups. Body weight in the untreated CRS (G2) showed significantly lower ($p < 0.05$) than in normal (G1) mice, highlighting that the chronic stress condition has been successfully developed that later suppressed the growth of body weight in G2. Whereas, observation on G3 and G4-G7 showed insignificant changes when compared to normal (G1), suggesting that SBH supplementation and the positive control helped to counteract the stress-induced weight loss. These findings highlight the hypothesis that SBH has protective effects against the physiological impacts of chronic stress.

3.4. Behavioural assessment

As shown in Fig. 3a and b, the 28-day CRS-induced anxiety-like behaviour has been evidenced by the decreased time spent and fewer entries into the open arms. The untreated CRS (G2) showed no movement into the open arms or time spent there, while G3 exhibited movement and time spent in the open arms. All SBH-treated groups (G4-G7) also showed movement into the open arms and increased time spent there, although no significant differences were observed compared to G2. One-way ANOVA analysis of the percentage of time spent in open arms and the number of open arm entries among all groups revealed no significant differences: $F_{(6,42)} = 0.92$, $p = 0.49$; $F_{(6,42)} = 1.48$, $p = 0.21$.

In the FST analysis (Fig. 3c), higher immobility time indicates a more severe depression state in CRS mice, indicating the stress induction has successfully developed the despair behaviour. Results showed that normal (G1) mice had significantly lower ($p < 0.001$) immobility time compared to untreated CRS mice (G2) mice. The treated groups (G3-G7) exhibited significant reduction ($p < 0.001$) in immobility times compared to G2, with the treated groups showing similar results to G1. This suggests that both paroxetine (10 mg/kg) and SBH treatment reduced immobility time in CRS mice. It is also observed that G5 (the group with low dose SBH treatment) which consumed from day 1 of stress induction exhibited a similar result upon compared to G3 (paroxetine). One-way ANOVA analysis confirmed the significance of immobility time differences, $F_{(6,42)} = 9.83$, $p < 0.001$ among groups.

3.5. Neurochemical analysis

Corticosterone (CORT) is a key stress hormone in rodents, analogous to cortisol in humans. Higher secretion levels have been closely associated with chronic stress and depressive-like states. The results of serum corticosterone levels in mice subjects are presented in Fig. 4a. In control groups, the normal (G1) and positive control (G3) mice groups showed lower corticosterone levels as compared to the untreated CRS (G2). In the SBH-treated groups, a significantly lower ($p < 0.05$) level of serum corticosterone was also observed in G4-G7 as compared to G2. Indeed, identical expression has been observed between SBH-treated and positive control groups. One-way ANOVA analysis showed a significant difference $F_{(6,42)} = 4.53$, $p = 0.0001$ among groups.

In rodents, serotonin plays a role in modulating the hypothalamic-pituitary-adrenal (HPA) axis, governing stress response. Reduced serotonin levels are closely linked to the development of depression. The effects of CRS on serotonin levels in mice are depicted in Fig. 4b. Groups G1, G3, and G4-G7 showed identical levels and significantly ($p < 0.001$; $p < 0.05$) higher serotonin levels compared to G2. In addition, identical levels between G1, G3, & G4-G7 primarily indicated that the serotonin was maintained at normal levels in all treated groups upon CRS induction. One-way ANOVA analysis showed a significant difference $F_{(6,42)} = 7.92$, $p < 0.0001$ among groups.

Dopamine plays an essential role in reward, motivation, and emotional regulation. Decreased dopamine levels are often associated with anhedonia (loss of pleasure) and reduced motivation, which are core symptoms of depression. Effects of dopamine levels within the 28

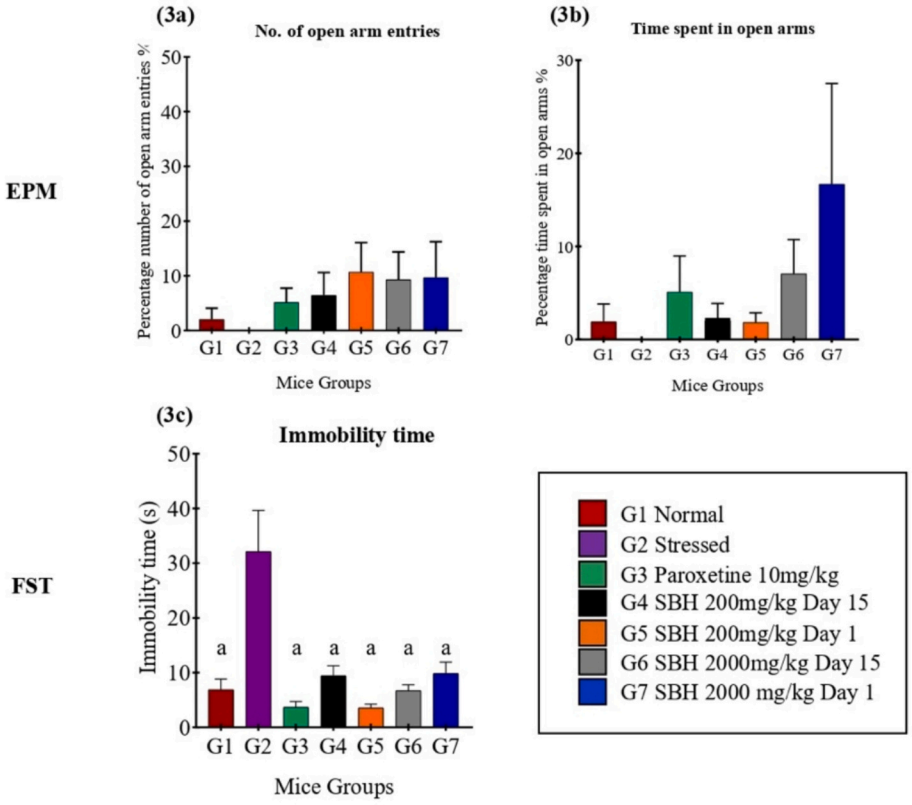

Fig. 3. (a)The percentage number of open arm entries (EPM) in mice; (b) the percentage of time spent in open arms (EPM) in mice, as indicators of anxiety-like behaviour. $p > 0.05$ vs. G2; (c) The immobility time of FST testing, as a benchmark for depressive-like behaviour in mice. $p < 0.001$ vs. G2. Administration of SBH significantly reduced the immobility time compared to the negative control group. Data represented as mean ± SEM.

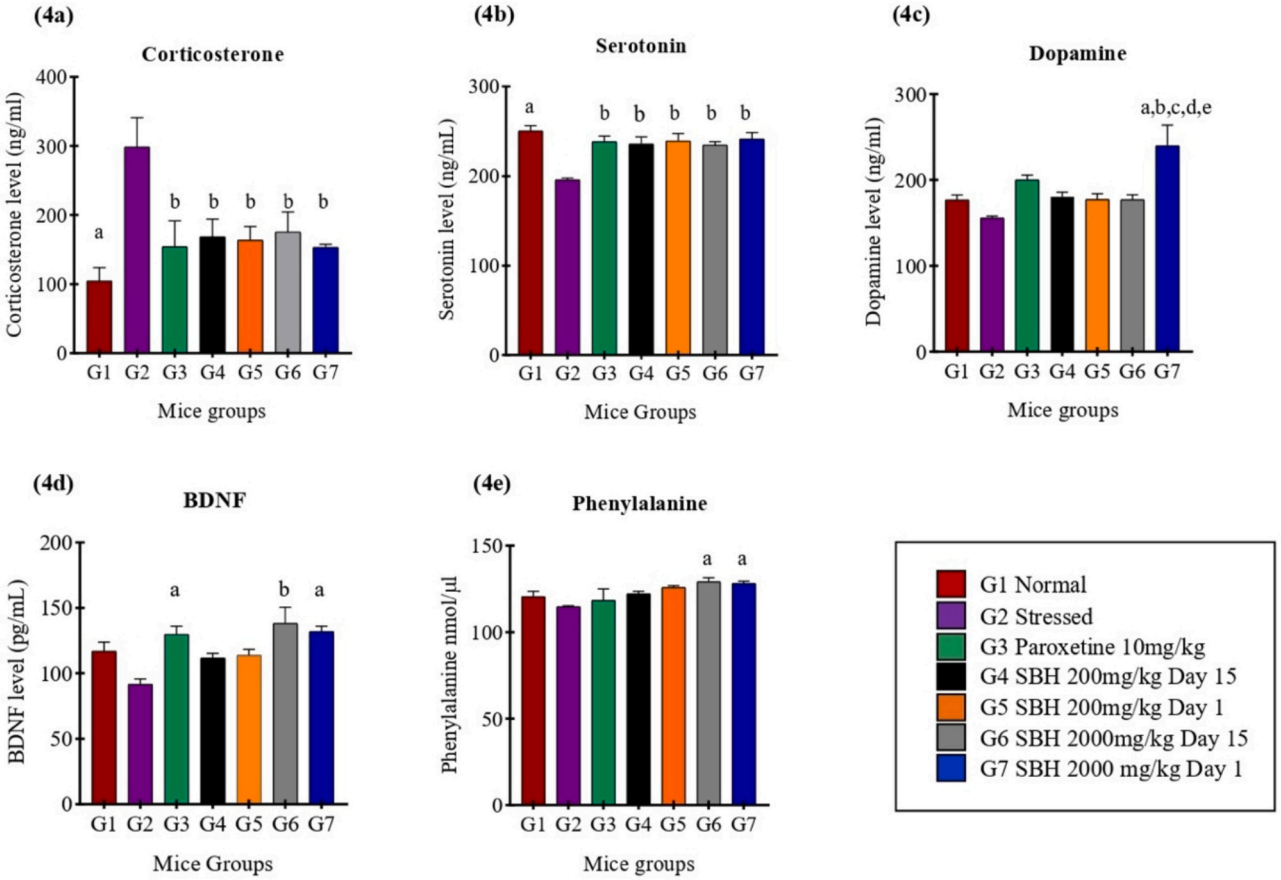

Fig. 4. (a) The serum corticosterone levels of mice after CRS for 28 days; (b) Effect of CRS on the serotonin concentration levels in mice; (c) Effect of CRS on the dopamine secretion levels in mice; (d) Effect of CRS on BDNF concentration levels in mice; (e) Analysis of Phe levels in serum blood of mice. Compared to the negative control group, SBH administration significantly decreased the level of corticosterone, whilst increasing dopamine, serotonin, BDNF, and phenylalanine expression. Statistical significance was determined by one-way ANOVA. $^{a}p < 0.01$; $^{b}p < 0.05$ vs. G2; $^{c}p < 0.05$ vs. G4; $^{d}p < 0.05$ vs. G5;^{e} $p < 0.05$ vs. G6. Data represented as mean ± SEM.

days of CRS-induced mice were presented in Fig. 4c. The G2 mice showed lower levels of serum dopamine as compared to G1 and G3, emphasizing that the CRS induction has primarily reduced the dopamine level. In the SBH-treated groups, a higher level of serum dopamine was observed. Strikingly, the high-dose SBH-treated group that began on day 1 (G7 mice) showed a prominent surge of dopamine ($p < 0.05$) compared to all other groups G1, G2, G4, G5, and G6. One-way ANOVA revealed a significant difference $F_{(6,42)} = 6.61$, $p < 0.0001$ among groups.

BDNF is an important protein that helps neurons grow, survive, and stay healthy, playing a key role in neuroplasticity, which allows the brain to adapt, form new connections, and recover from stress, all of which are crucial for learning and memory. The expressions of BDNF in the hippocampus of CRS mice were measured. As shown in Fig. 4d, the G2 mice showed decreased BDNF levels as compared to all groups. Whereas, G3 mice showed significantly higher ($p < 0.05$) as compared to G2. Nevertheless, only high-dose SBH-treated groups (G6 and G7) showed a significant increase ($p < 0.001$; $p < 0.05$) in comparison to G2. Expressions of BDNF in high-dose SBH treatment showed a comparable result with paroxetine, G3. Results indicated that the SBH supplementation possessed a direct effect to increase the BDNF level. One-way ANOVA revealed a significant difference $F_{(6,42)} = 5.76$, $p = 0.0002$ among groups.

Phenylalanine (Phe) levels can reflect changes in the body's biochemical processes, especially those related to neurotransmitter production. Higher phenylalanine levels in SBH-treated groups suggest that the treatment may help improve amino acid metabolism and support the production of neurochemicals that regulate mood. Fig. 4e shows the level of phenylalanine in the serum of experimental mice. The bar chart indicated that the G2 has a lower phenylalanine level compared to G1 and G3 mice. All SBH-treated groups (G4-G7) also showed the same pattern of increased phenylalanine as compared to G2. Nevertheless, only high-dose SBH treatments were significantly higher ($p < 0.05$) as compared to G2. One-way ANOVA revealed a significant difference $F_{(6,35)} = 3.13$, $p = 0.015$ among groups.

3.6. *Histopathological assessment of hippocampal neuronal structure and preservation*

The normal histology of the hippocampus region; CA3 and DG in G1, G3, G4, G5, G6, and G7, where abundant healthy intact neurons (black arrows) were observed. The normal architecture and arrangement of neurons were preserved as well as Nissl substances in the cytoplasm were visible as observed in Fig. 5A, C, D, E, F, G, and H, J, K, L, M, N. In contrast, the number of intact neurons in G2 was reduced in CA3 and DG. The damaged neurons were shrunken and sparse (red arrows), meanwhile, the intensity of cytoplasmic staining was reduced as illustrated in Fig. 5B and I. There was a significant neuronal loss with the existence of dark neurons which suggests neuronal damage.

Table 2 shows the number of intact neurons in the CA3 and DG areas of the hippocampus, respectively. In the CA3 area, there was a significant difference in the mean number of intact Nissl neurons across all groups ($F_{(6,42)} = 6.29$, $p < 0.0001$) (Table 2), with G1-G7 showing a significantly higher ($p < 0.05$) in Nissl intact neurons compared to G2.

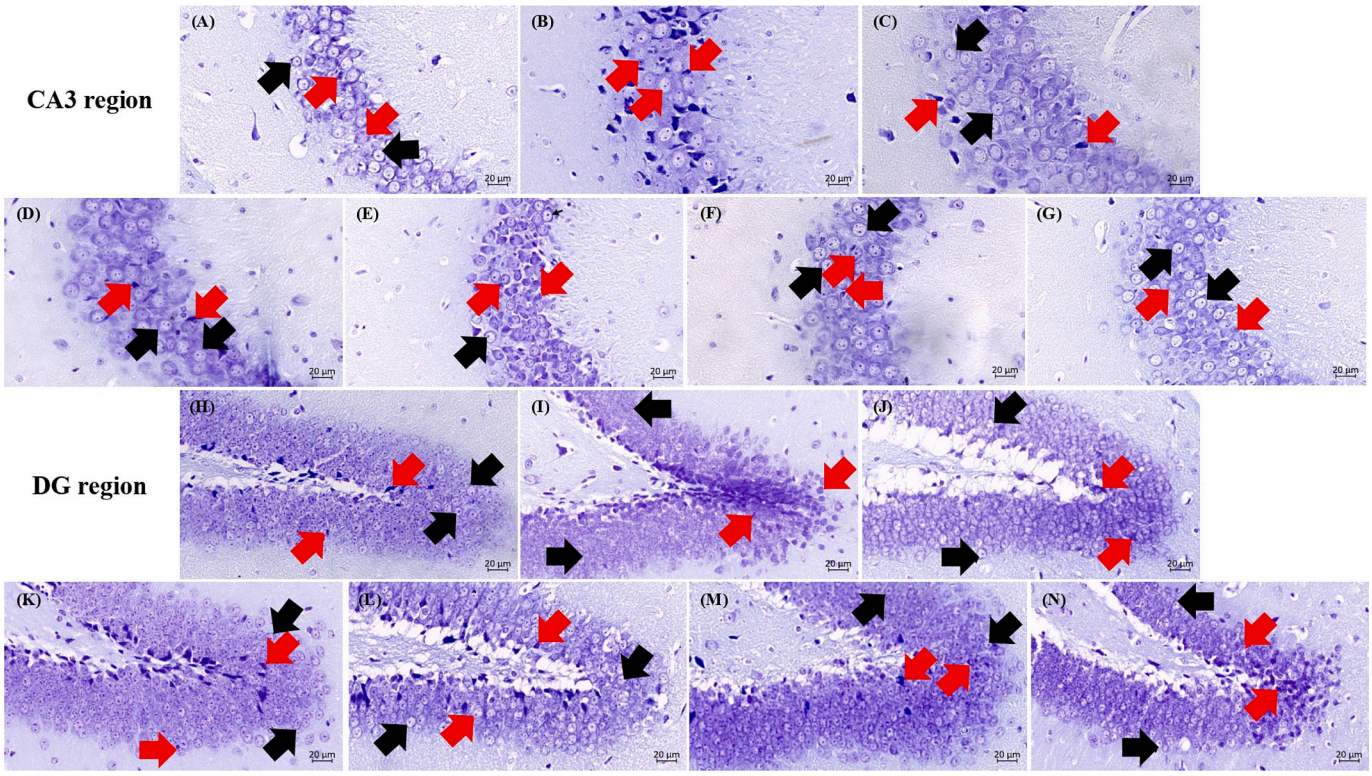

Fig. 5. Histology of intact neuron in hippocampus (**CA3**) region from (A) G1; normal, (B) G2; stressed, (C) G3; paroxetine, (D) G4; SBH 200 mg/kg day 15, (E) G5; SBH 200 mg/kg day 1, (F) G6; SBH 2000 mg/kg day 15 and (G) G7; SBH 2000 mg/kg day 1. (DG) region from (H) G1; normal, (I) G2; stressed, (J) G3; paroxetine, (K) G4; SBH 200 mg/kg day 15, (L) G5; SBH 200 mg/kg day 1, (M) G6; SBH 2000 mg/kg day 15 and (N) G7; SBH 2000 mg/kg day 1. The black arrows denote preserved neurons, while the red arrows indicate neuronal damage (Nissl staining, magnification of 20×, scale bar: 50 µm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Effect of treatment with SBH following exposure to CRS on morphometric measurement of the hippocampus in CA3 and DG area. One-way ANOVA between groups analysis was applied followed by post-hoc multiple comparison using Tukey's method. Data are expressed as mean ± SEM. $^{a}p < 0.01$ statistical comparison between treated and control groups; $^{b}p < 0.05$ statistical comparison between the treated and control group.

Groups	CA3 area (Mean ± SEM)	DG area (Mean ± SEM)	*p*-value
1	$13.71 \pm 1.40_{a}$	$11.91 \pm 0.72_{a}$	
2	7.06 ± 0.54	5.60 ± 0.69	
3	$14.74 \pm 1.50_{a}$	$12.63 \pm 1.03_{a}$	$^{a}p < 0.01$
4	$13.66 \pm 0.88_{b}$	$10.66 \pm 0.42_{b}$	
5	$13.37 \pm 0.90_{b}$	$10.77 \pm 1.27_{b}$	
6	$13.20 \pm 0.96_{b}$	$10.29 \pm 0.83_{b}$	$^{b}p < 0.05$
7	$14.34 \pm 0.80_{a}$	$10.37 \pm 0.96_{b}$	

Similarly, the DG area showed a significant difference ($F_{(6,42)} = 6.510$, $p < 0.001$) (Table 2), with G1-G7 showing a significantly higher ($p < 0.05$) in intact Nissl neurons compared to G2.

The intact neurons in CA3 and DG areas showed a significant increase of neuronal improvement in all treated-SBH groups (G4-G7) including the positive control group (G3) when compared to the untreated CRS mice (G2) group. This observation indicates reduced neuronal damage and enhanced neuroprotection, by which the hippocampal subregions are critically involved in memory formation, and emotional regulation, and are particularly sensitive to stress-induced neurodegeneration. The preservation of neuronal integrity in these areas further highlights the neuroprotective potential of SBH. The enhanced neuronal survival and regeneration observed in the SBH-treated groups further support its potential as a therapeutic agent to mitigate the effects of chronic stress and promote brain health.

3.7. Immunohistochemical localization of pro-inflammatory and anti-inflammatory cytokines

Fig. 6a and b showed the OD of expression in pro-inflammatory markers, IL-6, in the CA3 and DG areas of the hippocampus, respectively. Data were represented as mean ± SEM. In the CA3 area of the hippocampus, there were significant differences among groups [$F(6,14) = 3.76$, $p = 0.02$]. Analysis showed a significant decrease ($p < 0.005$) in the OD expression of IL-6 in CA3 in G7 as compared to the G2. Meanwhile, the DG area of the hippocampus also showed significant differences in all groups [$F(6, 14) = 5.29$, $p = 0.005$]. The G3, G6, and G7 exhibited a significant decrease ($p < 0.005$) in the OD expression in IL-6 in DG as compared to the G2.

Meanwhile, Fig. 6c and d showed the OD of expression in IL-1β in the CA3 and DG area of the hippocampus, respectively. Data were represented as mean ± SEM. In the CA3 area of the hippocampus, the G1 and G3-G7 showed a decreasing trend towards OD of expression IL-1β in CA3 as compared to the G2, however, there was no significant difference observed. Similarly, the OD of expression IL-1β in the DG area of the hippocampus showed no significant differences between all groups.

Fig. 7a illustrates immunohistochemical views of IL-6 expression in two distinctive areas, namely CA3 (red dotted) and DG (black dotted). In the analysed tissues of the CA3 region, the G2 group illustrated an intense brown DAB (3,3′-diaminobenzidine) staining indicating robust IL-6 immunoreactivity, particularly around the pyramidal neurons. A similar observation in which strong IL-6 signals were observed in the DG region, particularly within the granule cell layer. In contrast, the DAB (3,3′-diaminobenzidine) staining intensity visibly reduced within the inflamed cells of the hippocampus regions for SBH-treated groups (G7, G6, G5, G4, G4), paroxetine control (G3), and negative control (G1), indicating the suppression of stress-induced neuroinflammatory

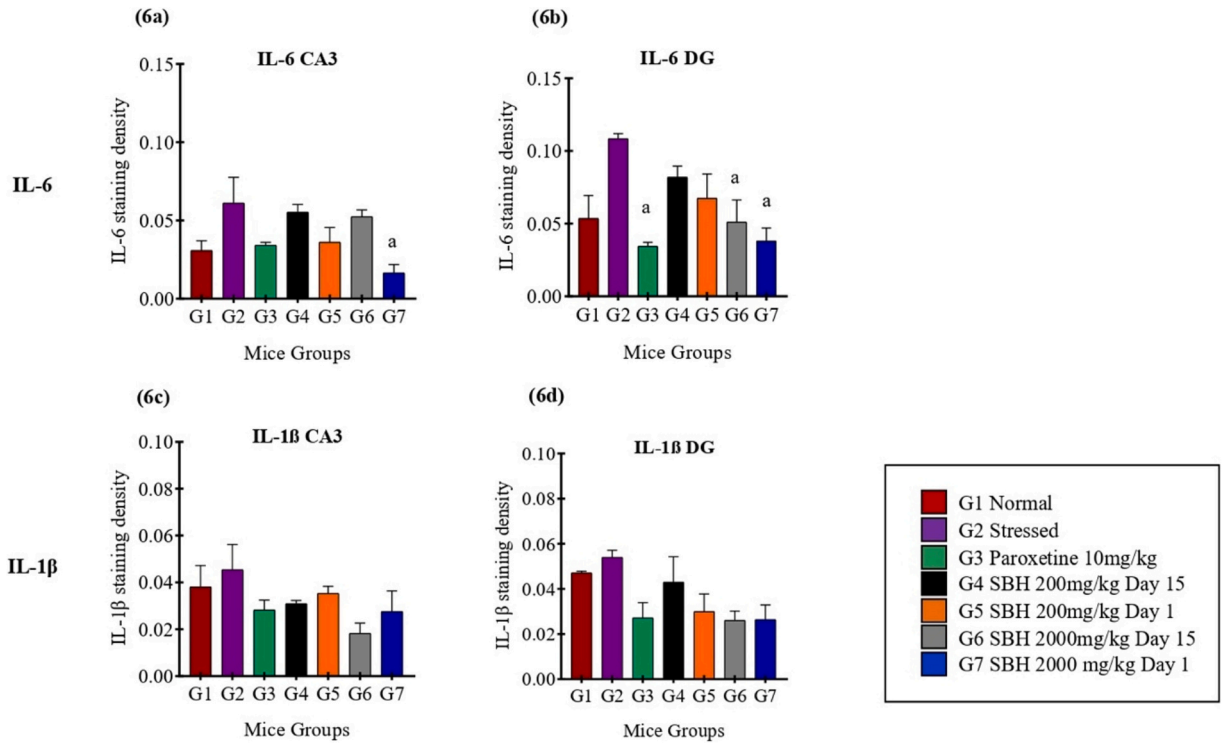

Fig. 6. (a) IL-6 expression in the CA3; (b) DG area of the hippocampus; (c) IL-1β expression in the CA3; (d) DG area of the hippocampus. SBH administration suppressed significantly the pro-inflammatory marker, IL-6 in both CA3 and DG regions compared to the negative control. $^{a}p < 0.005$ vs. *G2. Data represent the mean ± SEM.*

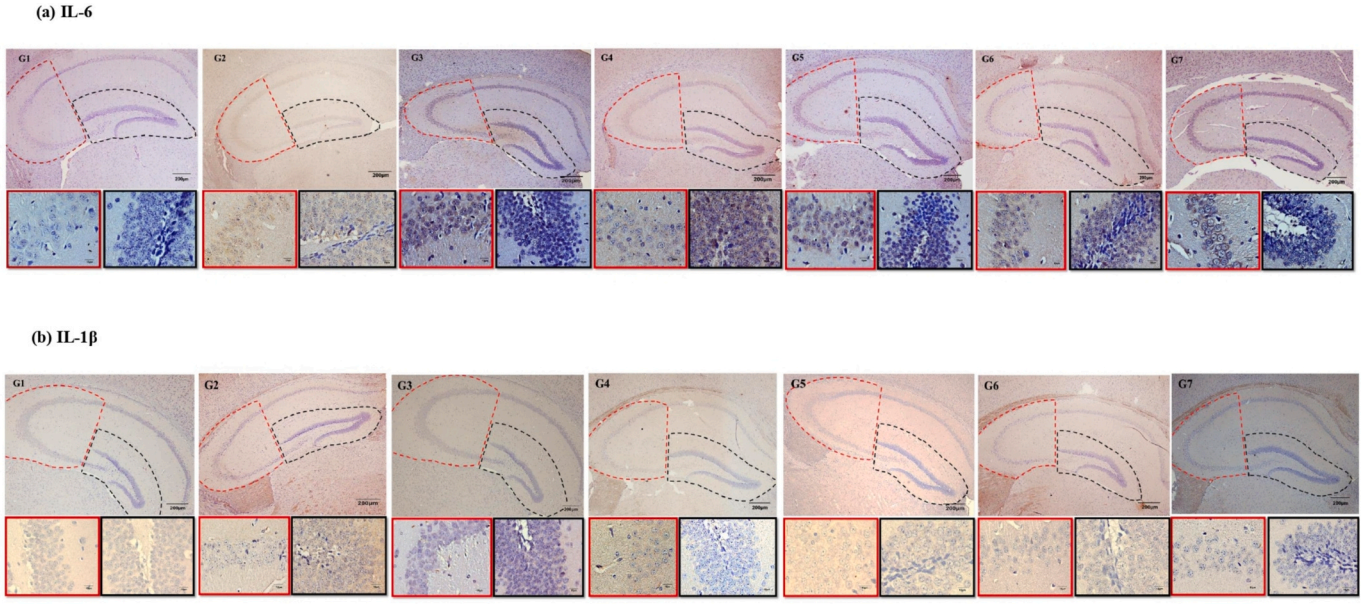

Fig. 7. Immunohistochemical analysis of IL-6 (red box) and IL-1β (black box) in CA3 and DG hippocampal regions. Both IL-6 and IL-1β expression are significantly reduced in high-dose SBH-treated groups (G6 and G7), supporting the anti-inflammatory role of SBH. *Images were viewed using 4× and 100× microscopic views.* (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

response, IL-6 protein.

As for the cytoplasmic staining of IL-1β in both CA3 and DG of the hippocampus region, only G7 and G3 evident intense purplish cytoplasmic staining (brown DAB counterstain with haematoxylin) upon comparison to G2, G4, G5, and G6 samples, where intense DAB staining has been observed (Fig. 7b). This further reflects their capabilities to downregulate the inflammatory response, leading to lower IL-1β expression.

4. Discussion

This present study highlighted that the stingless bee honey (SBH) used contains trehalulose sugar. Primary studies have found a predominant amount of trehalulose between 13 and 44 g per 100 g in SBH of

Heterotrigona itama sp. (Fletcher et al., 2020; Zawawi et al., 2022). Other disaccharides such as glucose, fructose, and maltose were also detected, with sucrose being absent in this SBH sample. This is closely related to the nectar source of the blossoms gathered by the bees, which later be conjugated with the enzyme invertase, catalyzing the breakdown of sucrose into glucose and fructose in which the fructose further converts into trehalulose via the formation of β-D-glucosyl enzyme intermediate (Olaitan et al., 2007; Zhang et al., 2022). Trehalulose is a unique sugar abundantly found in SBH that sets it apart from regular honey. It has been shown to have health benefits, including antioxidant, anti-inflammatory, and low glycaemic properties. In terms of brain health, trehalulose may help protect neurons and reduce oxidative stress, enhancing the overall neuroprotective effects of SBH (Fletcher et al., 2020; Zawawi et al., 2022). While trehalulose is still being studied, it shares similar biological properties in the modulation growth of gut microbiota with another structurally related sugar, trehalose, which provides a fermentable substrate for the growth and metabolic activity of beneficial gut microbiota, including Lactobacillus and Bifidobacterium sp. (Wang et al., 2018). This further suggests that trehalulose may possess a similar fermentable substrate although further studies are needed to directly assess its prebiotic effects.

Additionally, the moisture content of dehydrated SBH was recorded at 20 % along with its low hydroxymethylfurfural (HMF) content. Moreover, this view has been supported in that these criteria are crucial to be present in authentic SBH that is functionalized as an antioxidant-rich natural source (Mello dos Santos et al., n.d.). These properties highlighted the honey used meets the requirements outlined in the current Malaysian standard MS2683:2017. Processed honey is normally best limited to up to 22 % to preserve its beneficial contents whilst extending the shelf life (Shamsudin et al., 2019).

In parallel, SBH is rich in distinctive probiotics microbes, contributing to gut microbial balance, whereby this combination of prebiotic trehalulose and endogenous probiotics has the potential to enhance gut health and modulate the gut-brain axis, a bidirectional communication system that correlates the gastrointestinal tract with the central nervous system. Through this mechanism, SBH may influence neurochemical signalling, reduce systemic inflammation, and promote hippocampal integrity, particularly in regions susceptible to stress-related damage such as the CA3 and dentate gyrus. A previous clinical study using SBH showed the emerging neuroprotection potential with bioactive components that further supported brain plasticity, lowered the inflammation, and enhanced cognitive outcomes post-stroke, additionally (Sabarisah et al., 2022). These interactions may underlie the observed improvement in emotional and cognitive outcomes, reinforcing the potential of SBH as a natural, multifunctional neuroprotective agent.

Based on the morphology of diverse microbial observed on the surface of propolis via FESEM analysis, they potentially suggest the bacteria and yeast (Fig. 1) which indicate the presence of a probiotic population. They are primarily in line with the morphological features of *Saccharomyces cerevisiae* yeast and *Bacillus amyloliquefaciens* bacteria viewed under a scanning electron microscope (Kadaikunnan, Rejiniemon, Khaled, Alharbi, & Mothana, 2015; Molon et al., 2018). The high moisture content of SBH in this study at 30 % upon harvesting provides the optimum condition that favours their growth and allows for fermentation within the pot. This microbial activity is crucial for the fermentation process that contributes to the production of bioactive compounds with antioxidant properties. Bacillus species have accounted for the majority of the isolates discovered in stingless bee nest products (honey, propolis, and bee bread) alongside Firmicutes, Proteobacteria, and Actinobacteria, where these bacteria primarily secreted several enzymes to breakdown the complex biomolecules including phenylalanine amino acids into simpler, active compounds that benefit both gut health and brain function, including the production of dopamine neurotransmitters (Lani et al., 2017; Ngalimat et al., 2019). Another previous study supported current findings on the existence of yeasts, such as Saccharomyces spp., Mucor spp., Penicillium spp., and Schizosaccharomyces spp. where they are commonly detected in SBH that comprise moisture content beyond 21 %, highlighting their crucial roles in the fermentation process that eventually leads to the production of bioactive components with antioxidant effects (Salomón et al., 2024). The sugars in propolis are also catabolized into lactic acid and other organic compounds also support the growth of beneficial gut bacteria, reinforcing the probiotic benefits of SBH (Suyabatmaz et al., 2025; Deng et al., 2021). Decomposition of phenylalanine will directly breakdown into dopamine neurotransmitters (Lou, 1994). In the meantime, the production of various crucial metabolites including short-chain fatty acids (SCFAs) also occurs via indirect connection. The given further postulated that the presence of these beneficial microorganisms was an important characteristic that helped to increase the antioxidant properties of SBH, setting it apart from other honey. Hence, highlighting that the SBH has effective properties that play a crucial role in its probiotic benefits, largely attributed to the presence of beneficial bacteria and yeast within the hive environment itself. These microbial contributions make SBH a unique functional food that stands out for its potential to promote gut health, support the immune system, and enhance brain function.

Accumulating evidence suggested Lactobacillus as one of the most evaluated probiotics with the capability of alleviating depressive symptoms via in vivo and in vitro studies. For instance, decreased corticosterone and depression-related behaviour patterns upon administering *Lactobacillus rhamnosus* have been observed, indicating significant physiological effects of Lactobacillus on the central nervous system (Bravo et al., 2011). Other recent research indicated that administration of *Lactobacillus rhamnosus* and *Bifidobacterium lactis* exhibited increased secretion of dopamine neurotransmitters with significantly lowered TNF-α, IL-6, IL-18, and IL-1β levels in the serum, brain, and colon regions of chronic unpredictable mild stress (CUMS) group (Huang et al., 2022). Meanwhile, the BDNF and its receptor tyrosine kinase receptor B (TrkB), *N*-methyl-d-aspartic acid receptor 1 alongside the monoamine's dopamine, noradrenaline, and 5-hydroxytryptamine were reversely examined by *Lactobacillus casei* intervention (Gu et al., 2020). Although CUMS and CRS differ in their mechanism, both paradigms show similar outputs by activating the hypothalamic-pituitary-adrenal (HPA) axis including within the prolonged stressed condition, leading to the depletion of dopaminergic and serotonergic neurochemical markers alongside BDNF downregulation in the prefrontal cortex and hippocampus areas (Miao et al., 2020). In another study where the CRS model was induced with *Akkermansia muciniphila* probiotics, the abnormal changes in depressive molecular markers (dopamine, corticosterone, and BDNF) were reportedly upregulated (Ding et al., 2021). These observations imply that the probiotics could restore the imbalances disrupted by chronic stress by regulating neurotransmitters that directly enhance BDNF activation, and contribute to suppressing inflammation whilst promoting cognitive improvements.

CRS is a well-established and validated model for inducing depression-like behaviours in rodents, mimicking the physiological and behavioural symptoms observed in human depression (Chiba et al., 2012). The current study primarily evaluates the ability of SBH as an antidepressant in the CRS model and further compares it with paroxetine antidepressants and normal conditions. Past research that utilized peripheral biomarkers had demonstrated that CRS is the legitimate mouse model of depression through the shrinkage of hippocampal volume, abnormal brain functional connectivity, as well as identical gene expression and protein alterations possessed, resembling human depression (Seewoo et al., 2020).

It has been observed that SBH-treated and paroxetine groups are more susceptible to reducing stress-depressive behaviour, demonstrating lower anxiety levels and immobility time as observed in Fig. 3. Our finding is in line with previous studies that reported reduced time spent in despair using a high-dose paroxetine (10 mg/kg), serving the antidepressant effect (Kulikov et al., 2011). For the above reason, SBH is rich in microorganisms and various organic compounds and is capable of

producing a nearly similar effect as paroxetine (positive control), which is known to work as a selective serotonin reuptake inhibitor (SSRI) through serotonin reuptake transporter (SERT) inhibition which allows for persistent serotonin level in the treatment strategy of depression. When compared to paroxetine, SBH demonstrated comparable and in some measures potentially like antidepressant effects. Paroxetine treatment (G3) effectively elevated serotonin, dopamine, and BDNF levels, aligning with its known pharmacodynamic profile. However, G6 increased in BDNF and showed broader neurochemical restoration, including regulation of corticosterone levels and normalization of phenylalanine, suggesting a more comprehensive impact on both the central and peripheral markers of depression. Notably, G7 increased dopamine, suggesting a potential rapid-onset antidepressant effect, particularly on the reward-related pathways which is a significant advantage over SSRIs. In terms of tolerability and translational relevance, SBH presents several advantages. While paroxetine is associated with well-documented adverse effects, SBH, being a plant-derived food product, may offer a safer profile. Together, these findings indicate that SBH, particularly at a high dose, is not only equally effective as paroxetine in reversing depression-like neurochemical changes but may also be better tolerated, underscoring its promise as a translationally relevant alternative or adjunct to conventional antidepressants.

In general, stress leads to the lower expression of monoamine such as dopamine and serotonin along with the increase of corticosterone in the brain, inducing pro-inflammatory mediators that trigger depressive symptoms (Jiang et al., 2022). These biomarkers were specifically selected due to their central roles in stress response and monoaminergic neurotransmission, which are closely linked to neuropsychiatric and neurodegenerative conditions. Phenylalanine was chosen over other amino acids in conjunction with its natural presence in SBH and crucial role as a direct precursor in the biosynthesis of dopamine, norepinephrine, and epinephrine (Lou, 1994; Zulkifli et al., 2022). It has been studied that phenylalanine is an essential precursor for tyrosine contributes to the upregulation of BDNF genes that further enhances spatial memory performance in the treated mice (Moon et al., 2018; Mustafa et al., 2019). Therefore, one of the important points observed in the present study is that the high-dose SBH-treated group significantly increased the levels of dopamine and serotonin neurotransmitters, BDNF as well as phenylalanine expressions whilst reducing IL-6 cytokine significantly in the hippocampus tissue of CA3 and DG regions when compared to the CRS group. This could be further associated with the abundant presence of phenylalanine amino acids obtained in the high-dose SHB-treated group in comparison to the low-dose SBH-treated groups (G4 and G5), which influence the availability of L-tyrosine, key in dopamine synthesis (Daubner et al., 2011). On the other hand, decreased secretion of corticosterone was found to be significantly observed in all treated groups except for CRS mice. All findings of dopamine hormone, BDNF as well as inflammatory markers outlined that SBH synergistically targets both monoamines and inflammatory pathways. Taken together, these components operate in a complementary manner with antioxidant compounds such as flavonoids, phenolic acids, as well as polyphenols which are extensively detected in SBH (Santos et al., 2021). Therefore, enhances BDNF levels, modulates neurotransmitters, and lowers inflammation which improves cognitive function and mood regulation, especially in depression.

Phenylalanine has been known as an essential amino acid that is unable to be secreted by the body (Litwack, 2018). It has been investigated phenylalanine can produce phytochemical ferulic acid through the shikimate pathway, which could easily pass through the blood-brain barrier (BBB) and modulate neuro-immunomodulatory effect including the anti-depressant properties (Zheng et al., 2019). This strongly emphasizes that it is primarily ingested from the diet which in this study, direct elevation of phenylalanine in the serum was observed and has been directly acquired through SBH consumption. In a couple of studies, this dietary amino acid phenylalanine is regularly detected in SBH (Mustafa et al., 2019), where phenylalanine synthesizes and produces tyrosine, a BDNF receptor that is significantly associated with brain function (Slutsky & Etnier, 2019). Hence, this suggests that apart from the presence and direct mechanism of probiotics, other phytochemical compounds are postulated to be highly associated with elevating tyrosine receptor kinase B (TrkB) and BDNF that are crucial for neuroplasticity and neuronal survival, particularly in chronic stress conditions. However, future research is needed to understand this intriguing relationship.

Hippocampal inflammation particularly possesses an intimate relationship with gut microbes and brain disorders. The dentate gyrus (DG) has been the primary site of hippocampal neurogenesis with CA3 being the highly connected region to it upon exposure to chronic stress. Therefore, examination of intact neurons in high-dose SBH-treated through cresyl violet staining in the current study illustrated the presence of neurons characterized by their round shape, large nucleolus formation, and Nissl substances within the cytoplasm. A previous finding has reported that probiotic prophylaxis significantly decreases the number of neuronal deaths and apoptotic cells in the hippocampal CA1, CA3, and DG regions (Rahmati et al., 2019).

The overall evidence emphasizes that the aliphatic acids, amino acids, and probiotics in SBH are mainly functionalized as the key players that possess multiple biological properties including antioxidant and anti-inflammatory effects. This could be further explained by their capability to neutralize free radicals such as reactive oxygen stress (ROS), which are directly linked to oxidative stress and cellular damage, through the secretion of antioxidant enzymes (Zulkifli et al., 2022). By promoting cellular repair and lowering oxidative damage, the probiotics also undergo fermentation and produce several potential SCFAs that conjointly serve the antioxidant property. This study and its further details complement previous studies that highlighted raw honey exhibited nootropic effects, including memory enhancement and antidepressant neuropharmacological activities in supporting brain function (Mijanur Rahman et al., 2014). Hence, highlighting that SBH possesses a nootropic effect that further boosts cognitive function, although a deeper understanding of the mechanism action is still requisite.

The current findings of this study are the involvement of monoaminergic and anti-inflammatory pathways (Fig. 8), the precise mechanisms underlying these effects remain speculative. The lack of mechanistic blocking experiments, such as receptor-specific antagonists or genetic inhibition techniques, limits the ability to establish causal relationships and constitutes a key limitation of this study. Future studies incorporating such mechanistic approaches of the particular compounds presented in SBH and their capability to cross the blood-brain barrier (BBB) are necessary to validate the proposed pathways and elucidate their specific roles, along with the identification of specific strain of probiotics and identify the SCFAs or other organic compounds. Furthermore, translation into the clinical trial focusing on the assessment of major depressive disorder (MDD) patients via Beck Depression Inventory (BDI) scoring evaluation is crucial to objectively measure the anecdotal effects of SBH in treating MDD as well as reducing the side effects of conventional drugs.

The recognition of SBH as a functional food has garnered increasing scientific interest, especially related to its neuroprotective and psychotropic potential (Mustafa & Vit, 2024). Unlike synthetic antidepressants that commonly target a single neurotransmitter system and induce several side effects including sedation, weight gain, or sexual dysfunction, SBH promotes a multimodal mechanism of action with a favorable safety profile rooted in its long-standing dietary use. With its rich compositions of polyphenols, flavonoids, organic acids, and trehalulose sugars, they complement one another and are implicated in the pathophysiology of depression. These multifunctional properties align with the current understanding of depression as a multifactorial disorder engaging oxidative, inflammatory, and neurochemical imbalances which highlighted SBH as a promising candidate for alternative therapy. Most importantly, it does not possess known toxicity or adverse effects either at dietary or elevated dosages which is suitable to be translated

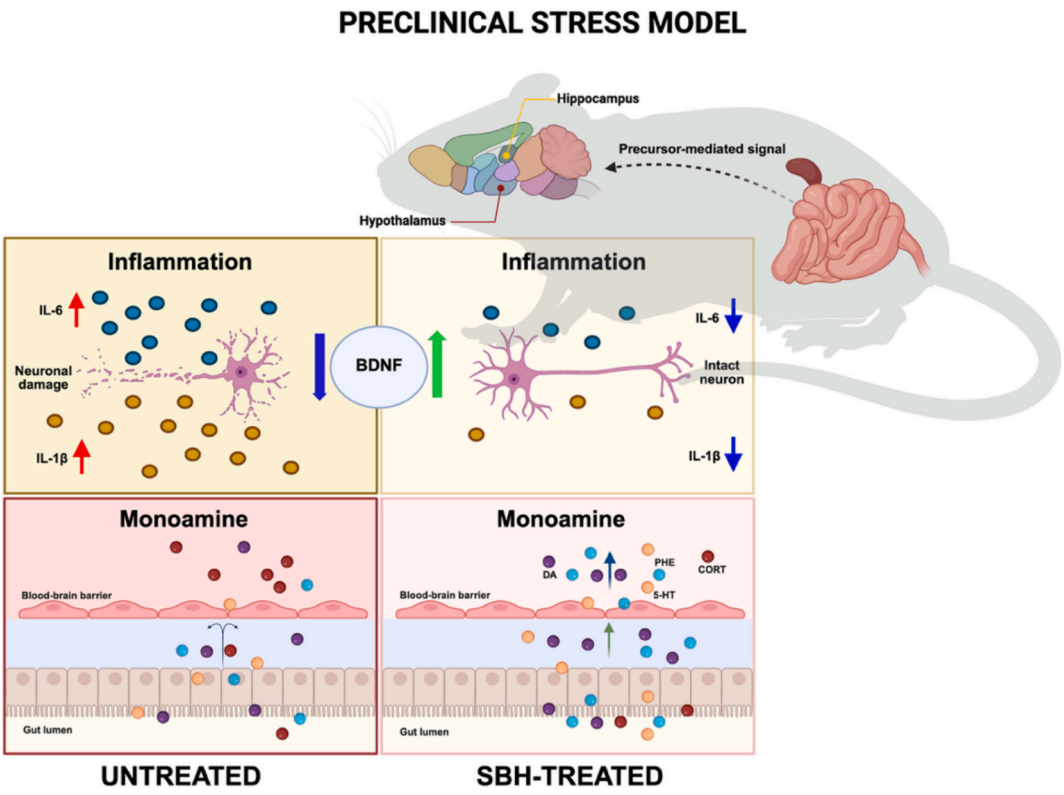

Fig. 8. Overall summary proposed for stingless bee honey (SBH) compared to untreated chronic restrained stress (CRS). SBH attenuates systemic and neuro-inflammation specifically in the hippocampus region while restoring central and peripheral monoaminergic balance and enhances BDNF-mediated neurogenesis, effectively reversing stress-induced immune, neurochemical, and neuronal disruption under CRS conditions.

into human clinical trials. Therefore, the results obtained provide a foundational framework that supports promising targets for further investigation.

5. Conclusions

Given the limitations of current pharmacotherapies and the growing interest in natural alternatives, it is crucial to explore novel, multi-functional agents such as SBH. Its unique composition, including trehalulose, antioxidants, good bacteria, and microbial metabolites, may target both monoaminergic systems and neuroinflammation. Therefore, this study investigates the antidepressant-like effects of SBH in a validated chronic stress mouse model, integrating behavioural, biochemical, and histological assessments.

Notable findings included increased dopamine levels, maintained secretion of serotonin, and prevention of corticosterone elevation, comparable to paroxetine. The dopamine increase may result from the interaction of bacteria and yeast probiotics in SBH as well as phenylalanine, enhancing monoamine production and the effect is likely dose-dependent. SBH also improved stress responses, body weight, and neuron preservation while reducing pro-inflammatory cytokines and preventing neuronal damage. Therefore, current findings indicate that SBH primarily possesses a direct effect on hormonal regulation whilst modulating the immune-inflammatory response. SBH has the potential to act as a functional food supplement and emphasizes its potential development as an adjuvant therapy for recurrent major depressive disorder (MDD) treatment, warranting further trial investigation.

Author contribution

Investigation, A.A.S.; Supervision, M.Z.M., W.A.N.W.A.; Formal Analysis, A.A.S., W.A.N.W.A., Writing-original draft, A.A.S.; Writing-review & editing, A.A.S, N.A.A.S., M.Z.M., W.A.N.W.A., S.H.S., J.M.A. Funding, M.Z.M. All authors contributed to the paper and approved the final draft of this manuscript.

CRediT authorship contribution statement

Anish Ameera Shaheran: Writing – original draft, Methodology, Investigation, Formal analysis. **Nurfatihah Azlyna Ahmad Suhaimi:** Writing – review & editing. **Wan Amir Nizam Wan Ahmad:** Software, Data curation. **Shazana Hilda Shamsuddin:** Writing – review & editing. **Jafri Malin Abdullah:** Visualization, Validation. **Mohd Zulkifli Mustafa:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Consent to participate

Not applicable.

Ethics statement

Ethical approval for the 'Antidepressant and Neuroprotective Effects of Stingless Bee Honey in Chronic Restraint Stress Mice Model' in this study was granted by University Research Ethics Committee, Reference Number [USM/IACUC/2018/(112)(927)], 12/27/2019.

I am pledged to acting with honesty, respect, and responsibility in all aspects of my work. In conducting animal studies, I am committed to ensuring the humane treatment of all animals involved. I will adhere to the principles of the 3Rs; Replacement, Reduction, and Refinement to minimize harm and the use of animals. I will ensure that animals are treated with respect and care, that studies are conducted with scientific rigor, and that any potential discomfort or distress is minimized. I will also comply with all relevant regulations and ethical guidelines to promote the welfare of animals in research.

Code availability

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data that has been used is confidential.

References

Adeniyi, I. A., Babalola, K. T., Adekoya, V. A., Oyebanjo, O., Ajayi, A. M., & Onasanwo, S. A. (2023). "Neuropharmacological effects of honey in lipopolysaccharide-induced neuroinflammation, cognitive impairment, anxiety and motor impairment," (in eng). *Nutritional Neuroscience, 26*(6), 511–524. https://doi.org/10.1080/1028415x.2022.2063578

Ahmed, M., et al. (2016). Physiochemical, biochemical, minerals content analysis, and antioxidant potential of national and international honeys in Pakistan. *Journal of Chemistry, 2016*(1), 8072305. https://doi.org/10.1155/2016/8072305

Al-Hatamleh, M. A. I., Boer, J. C., Wilson, K. L., Plebanski, M., Mohamud, R., & Mustafa, M. Z. (2020). Antioxidant-based medicinal properties of stingless bee products: Recent progress and future directions. *Biomolecules, 10, no. 6*. https://doi.org/10.3390/biom10060923 (in eng).

Al-Rahbi, B., Zakaria, R., Othman, Z., Hassan, A., & Ahmad, A. H. (2014). "enhancement of BDNF concentration and restoration of the hypothalamic-pituitary-adrenal axis accompany reduced depressive-like behaviour in stressed ovariectomised rats treated with either Tualang honey or estrogen," (in eng). *ScientificWorldJournal, 2014*, Article 310821. https://doi.org/10.1155/2014/310821

Audet, M. C., & Anisman, H. (2013). "interplay between pro-inflammatory cytokines and growth factors in depressive illnesses," (in eng). *Frontiers in Cellular Neuroscience, 7*, 68. https://doi.org/10.3389/fncel.2013.00068

Bravo, J. A., et al. (2011). "ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve," (in eng). *Proceedings of the National Academy of Sciences of the United States of America, 108* (38), 16050–16055. https://doi.org/10.1073/pnas.1102999108

Chiba, S., Numakawa, T., Ninomiya, M., Richards, M. C., Wakabayashi, C., & Kunugi, H. (2012). "chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex," (in eng). *Progress in Neuro-Psychopharmacology & Biological Psychiatry* (vol. 39). https://doi.org/10.1016/j.pnpbp.2012.05.018. no. 1, pp. 112–9, 1.

Daubner, S. C., Le, T., & Wang, S. (2011). "tyrosine hydroxylase and regulation of dopamine synthesis," (in eng). *Archives of Biochemistry and Biophysics, 508*(1), 1–12. Apr 1 https://doi.org/10.1016/j.abb.2010.12.017.

Deng, Z., Hou, K., Zhao, J., & Wang, H. (2021). "the probiotic properties of lactic acid Bacteria and their applications in animal husbandry," (in eng). *Current Microbiology, 79*(1), 22. https://doi.org/10.1007/s00284-021-02722-3

Ding, Y., et al. (2021). "a next-generation probiotic: Akkermansia muciniphila ameliorates chronic stress-induced depressive-like behavior in mice by regulating gut microbiota and metabolites," (in eng). *Applied Microbiology and Biotechnology, 105*(21–22), 8411–8426. https://doi.org/10.1007/s00253-021-11622-2

Erlandsen, S., Detry, J., Ottenwaelter, C., & Frethem, C. (2002). High resolution examination of biological samples using field emission scanning Electron microscopy. *Microscopy and Microanalysis, 8*(S02), 114–115. https://doi.org/10.1017/s1431927602101899

Fletcher, M. T., et al. (2020). Stingless bee honey, a novel source of trehalulose: A biologically active disaccharide with health benefits. *Scientific Reports, 10*(1), 12128. https://doi.org/10.1038/s41598-020-68940-0

Gu, F., Wu, Y., Liu, Y., Dou, M., Jiang, Y., & Liang, H. (2020). Lactobacillus casei improves depression-like behavior in chronic unpredictable mild stress-induced rats by the BDNF-TrkB signal pathway and the intestinal microbiota. *(in eng), Food Funct, 11*(7), 6148–6157. https://doi.org/10.1039/d0fo00373e

Hasim, H., Aziz, C., Qusyasyiah, S., Mohamed, M., Long, I., & Zakaria, R. (2020). Effects of Tualang honey on pain behaviour and oxidative stress in the thalamus of prenatally stressed rat offspring. *Malaysian Journal of Medicine and Health Sciences, 16*, 2636–9346.

Huang, G., Li, Y., Zhu, H., Feng, H., Shen, X., & Chen, Z. (2022). "emotional stimulation processing characteristics in depression: Meta-analysis of eye tracking findings," (in eng). *Frontiers in Psychology, 13*, 1089654. https://doi.org/10.3389/fpsyg.2022.1089654

Jiang, Y., Zou, D., Li, Y., Gu, S., Dong, J., Ma, X., … Huang, J. H. (2022). Monoamine Neurotransmitters Control Basic Emotions and Affect Major Depressive Disorders. *Pharmaceuticals, 15*(10). https://doi.org/10.3390/ph15101203

Julika, W., Ajit, T. D. A., Ismail, N., Aqilah, N., Naila, A., & Sulaiman Phd, I. T. A. Z. (2020). Sugar profile and enzymatic analysis of stingless bee honey collected from local market in Malaysia. *IOP Conference Series: Materials Science and Engineering, 736*, Article 062001. https://doi.org/10.1088/1757-899X/736/6/062001

Kadaikunnan, S., Rejiniemon, T. S., Khaled, J. M., Alharbi, N. S., & Mothana, R. (2015). In-vitro antibacterial, antifungal, antioxidant and functional properties of Bacillus amyloliquefaciens. *Annals of clinical microbiology and antimicrobials, 14*(1), 9. https://doi.org/10.1186/s12941-015-0069-1

Kim, Y., Lee, H. Y., & Cho, S. H. (2017). "antidepressant effects of Pharmacopuncture on behavior and brain-derived neurotrophic factor (BDNF) expression in chronic stress model of mice," (in eng). *Journal of Acupuncture and Meridian Studies, 10*(6), 402–408. https://doi.org/10.1016/j.jams.2017.08.007

Kulikov, A. V., Tikhonova, M. A., Osipova, D. V., Kulikov, V. A., & Popova, N. K. (2011). Association between tryptophan hydroxylase-2 genotype and the antidepressant effect of citalopram and paroxetine on immobility time in the forced swim test in mice. *Pharmacology Biochemistry and Behavior, 99*(4), 683–687. https://doi.org/10.1016/j.pbb.2011.06.020

Lani, M., Zainudin, A., Abd Razak, S. B., Razak, A., Mansor, A., & Hassan, Z. (2017). Microbiological quality and pH changes of honey produced by stingless bees, Heterotrigona itama and Geniotrigona thoracica stored at ambient temperature. *Malaysian Applied Biology, 46*, 89–96, 10/01.

Litwack, G. (2018). Chapter 13 - metabolism of amino acids. In G. Litwack (Ed.), *Human biochemistry* (pp. 359–394). Boston: Academic Press.

Lou, H. C. (1994). "dopamine precursors and brain function in phenylalanine hydroxylase deficiency," (in eng). *Acta Paediatrica. Supplement, 407*, 86–88. https://doi.org/10.1111/j.1651-2227.1994.tb13461.x

Marchette, R. C. N., Bicca, M. A., Santos, E. C. D. S., & de Lima, T. C. M. (2018). distinctive stress sensitivity and anxiety-like behavior in female mice: Strain differences matter. *Neurobiology of Stress, 9*, 55–63. https://doi.org/10.1016/j.ynstr.2018.08.002, 11/01/ 2018.

B. S. McEwen, C. Nasca, and J. D. Gray, "Stress effects on neuronal structure: Hippocampus, amygdala, and prefrontal cortex," Neuropsychopharmacology, vol. 41, no. 1, pp. 3–23, 2016/01/01 2016, doi: https://doi.org/10.1038/npp.2015.171.

McIlwain, K. L., Merriweather, M. Y., Yuva-Paylor, L. A., & Paylor, R. (2001). "the use of behavioral test batteries: Effects of training history," (in eng). *Physiology & Behavior, 73*(5), 705–717. https://doi.org/10.1016/s0031-9384(01)00528-5

Mehta, V., Parashar, A., & Udayabanu, M. (2017). "Quercetin prevents chronic unpredictable stress induced behavioral dysfunction in mice by alleviating hippocampal oxidative and inflammatory stress," (in eng). *Physiology & Behavior, 171*, 69–78. https://doi.org/10.1016/j.physbeh.2017.01.006

M. Mello dos Santos, N. Khan, L. Y. Lim, and C. Locher, "Antioxidant activity, physicochemical and sensory properties of stingless bee honey from Australia," *Foods*, vol. 13, no. 11, doi: https://doi.org/10.3390/foods13111657.

Miao, Z., Wang, Y., & Sun, Z. (2020). "the relationships between stress, mental disorders, and epigenetic regulation of BDNF," (in eng). *International Journal of Molecular Sciences, 21, no. 4*. https://doi.org/10.3390/ijms21041375

Mijanur Rahman, M., Gan, S. H., & Khalil, M. I. (2014). "neurological effects of honey: Current and future prospects," (in eng). *Evidence-based Complementary and Alternative Medicine, 2014*, 958721. https://doi.org/10.1155/2014/958721

Molon, M., Woznicka, O., & Zebrowski, J. (2018). "cell wall biosynthesis impairment affects the budding lifespan of the Saccharomyces cerevisiae yeast," (in eng). *Biogerontology, 19*(1), 67–79. https://doi.org/10.1007/s10522-017-9740-6

Monteiro, S., Roque, S., de Sá-Calçada, D., Sousa, N., Correia-Neves, M., & Cerqueira, J. J. (2015). "An efficient chronic unpredictable stress protocol to induce stress-related responses in C57BL/6 mice," (in eng). *Frontiers in Psychiatry, 6*, 6. https://doi.org/10.3389/fpsyt.2015.00006

Moon, J.-M., Thapliyal, N., Hussain, K. K., Goyal, R. N., & Shim, Y.-B. (2018). Conducting polymer-based electrochemical biosensors for neurotransmitters: A review. *Biosensors and Bioelectronics, 102*, 540–552. https://doi.org/10.1016/j.bios.2017.11.069

Mustafa, M. Z., & Vit, P. (2024). "Honeyomics and industrialisation of Madu Kelulut as a health supplement: Are we ready for scale-up?," (in eng). *Malays Journal Medical Science, 31*(5), 1–9. https://doi.org/10.21315/mjms2024.31.5.1

Mustafa, M. Z., et al. (2019). "stingless bee honey improves spatial memory in mice, probably associated with brain-derived neurotrophic factor (BDNF) and inositol 1,4,5-triphosphate receptor type 1 (Itpr1) genes," (in eng). *Evidence-based Complementary and Alternative Medicine, 2019*, 8258307. https://doi.org/10.1155/2019/8258307

Nair, A. B., & Jacob, S. (2016). "a simple practice guide for dose conversion between animals and human," (in eng). *Journal Basic Clinical Pharmacy, 7*(2), 27–31. https://doi.org/10.4103/0976-0105.177703

Narita, M., Aoki, K., Takagi, M., Yajima, Y., & Suzuki, T. (2003). "Implication of brain-derived neurotrophic factor in the release of dopamine and dopamine-related behaviors induced by methamphetamine," (in eng). *Neuroscience, 119*(3), 767–775. https://doi.org/10.1016/s0306-4522(03)00099-x

Ngalimat, M. S., Rahman, R. N. Z. R. A., Yusof, M. T., Syahir, A., & Sabri, S. (2019). Characterisation of bacteria isolated from the stingless bee, Heterotrigona itama, honey, bee bread and propolis. *(in eng), PeerJ, 7*, Article e7478. https://doi.org/10.7717/peerj.7478

Official methods of analysis of AOAC international. (2005). AOAC.

Olaitan, P. B., Adeleke, O. E., & Iyabo, O. O. (2007). Honey: A reservoir for microorganisms and an inhibitory agent for microbes. *African Health Sciences, 7*(3).

Rahmati, H., Momenabadi, S., Vafaei, A. A., Bandegi, A. R., Mazaheri, Z., & Vakili, A. (2019). "probiotic supplementation attenuates hippocampus injury and spatial learning and memory impairments in a cerebral hypoperfusion mouse model," (in eng). *Molecular Biology Reports, 46*(5), 4985–4995. https://doi.org/10.1007/s11033-019-04949-7

Sabarisah, H., et al. (2022). In S. Post-Stroke Rehabilitation, & Pratap (Eds.), *The cerebral plasticity Prospect of stingless bee honey-polyphenols supplementation in Rehabilitation of Post-Stroke vascular cognitive impairment.* Rijeka: IntechOpen. p. Ch. 8.

Salomón, V. M., Hero, J. S., Morales, A. H., Pisa, J. H., Maldonado, L. M., Vera, N., … Romero, C. M. (2024). Microbiological Diversity and Associated Enzymatic Activities in Honey and Pollen from Stingless Bees from Northern Argentina. *Microorganisms, 12*(4). https://doi.org/10.3390/microorganisms12040711

Santos, A. C. D., Biluca, F. C., Braghini, F., Gonzaga, L. V., Costa, A. C. O., & Fett, R. (2021). Phenolic composition and biological activities of stingless bee honey: An overview based on its aglycone and glycoside compounds. *Food Research International, 147*, Article 110553. https://doi.org/10.1016/j.foodres.2021.110553

Seewoo, B. J., Hennessy, L. A., Feindel, K. W., Etherington, S. J., Croarkin, P. E., & Rodger, J. (2020). Validation of chronic restraint stress model in young adult rats for the study of depression using longitudinal multimodal MR imaging. *eneuro, 7, no. 4*. https://doi.org/10.1523/ENEURO.0113-20.2020. pp. ENEURO.0113-20.2020.

Sestakova, N., Puzserova, A., Kluknavsky, M., & Bernatova, I. (2013). "determination of motor activity and anxiety-related behaviour in rodents: Methodological aspects and role of nitric oxide," (in eng). *Interdisciplinary Toxicology, 6*(3), 126–135. https://doi.org/10.2478/intox-2013-0020

Shamsudin, S., Selamat, J., Sanny, M., Jambari, R. S. A. N. N., & Khatib, A. (2019). "A comparative characterization of physicochemical and antioxidants properties of processed Heterotrigona itama honey from different origins and classification by chemometrics analysis," (in eng). *Molecules, 24, no. 21*. https://doi.org/10.3390/molecules24213898

A. B. Slutsky and J. L. Etnier, "Caloric restriction, physical activity, and cognitive performance: A review of evidence and a discussion of the potential mediators of BDNF and TrkB," International Journal of Sport and Exercise Psychology, vol. 17, no. 2, pp. 89–105, 2019/03/04 2019, doi: https://doi.org/10.1080/1612197X.2016.1223422.

Stepanichev, M., Dygalo, N. N., Grigoryan, G., Shishkina, G. T., & Gulyaeva, N. (2014). "rodent models of depression: Neurotrophic and neuroinflammatory biomarkers," (in eng). *BioMed Research International, 2014*, Article 932757. https://doi.org/10.1155/2014/932757

Suyabatmaz, Ş., Karaoğlu, Ş. A., Bozdeveci, A., & Akpınar, R. (2025). Honeybee-associated lactic acid bacteria and their probiotic potential for human use. *World Journal of Microbiology and Biotechnology*. https://doi.org/10.1007/s11274-022-03427-w. vol. 39, no. 1, p. 2.

Tiemeier, H. (2003). "biological risk factors for late life depression," (in eng). *European Journal of Epidemiology, 18*(8), 745–750. https://doi.org/10.1023/a:1025388203548

Torres, G. E., Gainetdinov, R. R., & Caron, M. G. (2003). Plasma membrane monoamine transporters: Structure, regulation and function. *Nature Reviews Neuroscience, 4*(1), 13–25. https://doi.org/10.1038/nrn1008. /01/01 2003.

Wang, R.-M., Li, N., Zheng, K., & Hao, J.-F. (2018). Enhancing acid tolerance of the probiotic bacterium Lactobacillus acidophilus NCFM with trehalose. *FEMS Microbiology Letters, 365, no. 19*. https://doi.org/10.1093/femsle/fny217. p. fny217.

Wayo, K., Sritongchuay, T., Chuttong, B., Attasopa, K., & Bumrungsri, S. (2020). Local and Landscape Compositions Influence Stingless Bee Communities and Pollination Networks in Tropical Mixed Fruit Orchards, Thailand. *Diversity, 12*(12). https://doi.org/10.3390/d12120482

Woo, H., Hong, C. J., Jung, S., Choe, S., & Yu, S. W. (2018). "Chronic restraint stress induces hippocampal memory deficits by impairing insulin signaling," (in eng), *Mol. Brain, 11*(1), 37. https://doi.org/10.1186/s13041-018-0381-8

Yan, S., et al. (2015). "antidepressant-like effects of Sanyuansan in the mouse forced swim test, tail suspension test, and chronic mild stress model," (in eng). *The Kaohsiung Journal of Medical Sciences, 31*(12), 605. https://doi.org/10.1016/j.kjms.2015.10.009

Yong, C. H., et al. (2022). "detecting adulteration of stingless bee honey using untargeted (1)H NMR metabolomics with chemometrics," (in eng). *Food Chemistry, 368*, Article 130808. https://doi.org/10.1016/j.foodchem.2021.130808

Zawawi, N., et al. (2022). Unique physicochemical properties and rare reducing sugar trehalulose mandate new international regulation for stingless bee honey. *Food Chemistry, 373*. https://doi.org/10.1016/j.foodchem.2021.131566, 131566. /03/30/ 2022.

Zhang, J., Hungerford, N. L., Yates, H. S. A., Smith, T. J., & Fletcher, M. T. (2022). How is Trehalulose formed by Australian stingless bees? - an intermolecular displacement of nectar sucrose. *Journal of Agricultural and Food Chemistry, 70*(21), 6530–6539. https://doi.org/10.1021/acs.jafc.2c01732. /06/01 2022.

Zheng, X., Cheng, Y., Chen, Y., Yue, Y., Li, Y., Xia, S., … Cao, Y. (2019). Ferulic Acid Improves Depressive-Like Behavior in Prenatally-Stressed Offspring Rats via Anti-Inflammatory Activity and HPA Axis.*International. Journal of Molecular Sciences, 20* (3). https://doi.org/10.3390/ijms20030493

Zulkifli, N. A., et al. (2022). "the potential neuroprotective effects of stingless bee honey," (in eng). *Frontiers in Aging Neuroscience, 14*, 1048028. https://doi.org/10.3389/fnagi.2022.1048028

World Health Organization, W. (2023). Depressive disorder (depression). Retrieved from Retrieved from https://www.who.int/news-room/fact-sheets/detail/depression.