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Dietary Supplementation with Whole Propolis Differentially Modulates Cognitive and Anxiety-like Behaviors in Male and Female Mice

Abdulbasit Amin[1a*], Stephen Eneape[2b], Yaaqub Abiodun Uthman[1c], Mary Oluwatobi Dada[1d], Wahab Imam Abdulmajeed[1e], Abdulmusawwir Alli-Oluwafuyi[3f], Imam Aminu[2g], Aboyeji Lukuman Oyewole[1h]

[1]Department of Physiology, [2]Department of Anatomy, Faculty of Basic Medical Sciences, [3]Department of Pharmacology & Therapeutics, Faculty of Basic Clinical Sciences, University of Ilorin, Ilorin, Nigeria.

ORCIDs: 0000-0001-5460-9581[a], 0009-0007-9970-3177 [b], 0000-0002-8888-5696[c], 0009-0008-1648-6507[d], 0000-0002-0944-2353 [e], 0000-0002-1769-1747[f], 0000-0003-2371-3065 [g], (0000-0001-6777-4201[h].

***Corresponding Author:** Email: amin.a@unilorin.edu.ng, Tel: +2348024018750

ABSTRACT

Propolis is a complex bee-derived natural product with reported neuroprotective properties, largely based on studies using solvent extracts or isolated bioactive constituents. However, the neurobehavioral effects of consuming whole propolis as a dietary component remain poorly understood. In this study, we investigated the impact of dietary supplementation with whole propolis on cognitive and anxiety-like behaviors in male and female mice. Adult mice (8-10 week old) received standard chow or chow supplemented with 5% whole propolis for 14 days, after which behavioral assessments were conducted using the Novel Object Recognition test, Y-maze, and the zero plus maze. Whole propolis supplementation enhanced recognition memory in both male and female mice, as indicated by increased exploration of the novel object. In contrast, spatial working memory assessed by spontaneous alternation in the Y-maze was impaired in male mice but unchanged in females. Anxiety-like behavior, evaluated using the zero plus maze, was increased in both sexes, as reflected by reduced open-arm exploration. These findings demonstrate that whole propolis consumption differentially modulates neurobehavioral outcomes in a sex-dependent manner. The study highlights the importance of evaluating natural products in their unrefined dietary form and underscores the need to consider sex as a biological variable when assessing the neurobehavioral impact of dietary bioactive compounds.

Keywords: propolis, memory, anxiety, locomotion, mice

INTRODUCTION

Propolis is a resinous natural product collected by honeybees from plant buds and exudates and subsequently modified with beeswax and salivary enzymes such as β-glucosidase, α-amylase, glucose oxidase, and many more. Chemically, propolis is highly complex, comprising flavonoids, phenolic acids, terpenoids, aromatic aldehydes, waxes, and trace minerals, with its precise composition varying according to botanical source and geographic origin[1]. Traditionally, propolis has been used for its antimicrobial[2,3] and anti-inflammatory properties[4], and increasing scientific interest has focused on its potential effects on the nervous system[5].

A growing body of experimental evidence suggests that propolis possesses neuroactive properties. Several studies have demonstrated that propolis-derived compounds exert antioxidant and anti-inflammatory effects within the brain, mechanisms that are relevant to cognitive function and emotional regulation[6]. In rodent models, propolis has been reported to improve learning and memory, attenuate oxidative stress in neural tissue, and protect against neurotoxicity induced by chemical insults or ageing-related processes. These effects have been linked to reduced lipid peroxidation, enhanced endogenous antioxidant defense systems, and modulation of neurotransmitter pathways[7–9].

However, the majority of studies investigating the neurobehavioral effects of propolis have relied on solvent extracts, such as ethanolic or aqueous extracts, or on isolated bioactive constituents including caffeic acid phenethyl ester (CAPE), chrysin, and pinocembrin. For example, ethanolic propolis extracts have been shown to improve memory performance in models of neurodegeneration and cerebral ischemia, while CAPE has been reported to exert neuroprotective and anxiolytic effects through its antioxidant and anti-inflammatory actions[5,10]. Although these studies have provided valuable mechanistic insight, extraction procedures fundamentally alter the chemical profile of propolis

and may selectively enrich certain compounds while excluding others that are naturally co-consumed.

Importantly, humans and animals consuming propolis as a dietary supplement are typically exposed to whole propolis, rather than purified extracts or isolated molecules[11]. The biological effects of whole propolis may therefore reflect the combined and potentially synergistic actions of multiple constituents acting simultaneously[12]. Despite this, there is a notable paucity of studies examining the behavioral consequences of whole propolis consumption, particularly when administered as part of the diet rather than as a gavage-delivered extract.

In addition, sex differences in neurobehavioral responses to dietary bioactive compounds remain underexplored. Male and female rodents differ in cognitive strategies, anxiety-like behavior, and susceptibility to neuroactive substances, partly due to differences in hormonal regulation and neural circuitry[13,14]. Many previous propolis studies have either focused exclusively on male animals or have not analyzed sex as an independent biological variable, limiting the generalizability of their findings.

Behavioral paradigms, such as the Novel Object Recognition test, Y-maze, and Zero plus Maze, provide robust and anatomically relevant measures of recognition memory, spatial working memory, and anxiety-like behavior, respectively. These behaviors are mediated by distinct but overlapping neural substrates, including the hippocampus, prefrontal cortex, and limbic structures, making them suitable tools for assessing the neurobehavioral impact of dietary interventions[15–17].

In the present study, we investigated the effects of dietary supplementation with 5% whole propolis incorporated directly into standard feed on cognitive and anxiety-related behaviors in both male and female mice. By avoiding extraction or purification procedures, this study aims to reflect the physiological relevance of propolis consumption better and to determine whether whole propolis differentially modulates neurobehavioral outcomes in a sex-dependent manner.

MATERIALS AND METHODS

Animals and experimental design

Adult male and female mice (8-10 weeks old) were used in this study. Animals were housed at the animal holding facility of the Faculty of Basic Medical Sciences, University of Ilorin, and the Bioresearch Hub Laboratory, Ilorin, under standard laboratory conditions with free access to food and water. Mice were randomly assigned to either a control group (7 male and 8 female mice) receiving standard chow or a treatment group (8 male and 8 female mice) receiving chow supplemented with 5% whole propolis for 2 weeks. Both male and female cohorts were studied independently to assess sex-specific effects.

Whole propolis (1 kg) was purchased from Fowa Naturals, Lagos, Nigeria, thoroughly mixed, and pelleted with standard feed to achieve a final concentration of 5%. The experimental diet was administered for the duration of the study. All experimental procedures were conducted in accordance with institutional guidelines, which are in line with the international guidelines for the care and use of laboratory animals. The Ethical approval was granted by the University of Ilorin ethical review committee (UERC) (UIL/UERC/21/68LD001).

Food intake, body mass assessment, and glycemia

Food intake was monitored daily throughout the experimental period. The amount of chow provided to each cage was weighed, and the remaining food was measured after 24 hours. Daily food consumption was calculated as the difference between the amount of food offered and the amount remaining and was expressed as grams per mouse per day by dividing the total food consumed per cage by the number of mice housed in that cage. Body mass was measured daily for each mouse using a calibrated digital balance. Measurements were recorded in grams and were obtained at the same time each day to minimize variability related to circadian influences. Blood glucose levels were assessed at the end of the experimental period. Mice were gently restrained, and a small drop of blood was obtained via tail puncture. Glycaemia was measured immediately using a OneTouch handheld glucometer in accordance with the manufacturer's instructions, and glucose values were recorded in mmol/L.

Behavioral assessments

Behavioral testing was conducted following dietary exposure and was performed during the light phase under consistent environmental conditions. Animals were acclimatized to the testing room 2 hours before assessment.

Novel object recognition test

The Novel Object Recognition (NOR) test was used to assess recognition memory based on the natural tendency of rodents to explore novel stimuli on day 12 of propolis administration. The NOR was conducted according to a standard protocol as previously described[18], with minor modifications. Briefly, mice were first habituated to the empty open-field arena for 5 minutes on the day preceding the test to reduce novelty-induced anxiety. During the familiarization (training) phase, mice were placed in the arena containing two identical objects and allowed to explore for 5 minutes. Following a 1-hour retention interval, one of the familiar objects was replaced with a novel object, and mice were reintroduced into the arena for a 5-minute test phase.

The apparatus consisted of a square open-field arena with opaque walls and a non-reflective floor. Behavior was recorded, and exploration time directed toward the familiar and novel objects was quantified.

Exploration was defined as sniffing or touching the object while oriented towards it, excluding climbing or sitting on the object. Recognition memory was assessed based on the relative time spent exploring the novel versus the familiar object.

Y-maze test

Spatial working memory was evaluated using the Y-maze apparatus, consisting of three arms positioned at 120° angles. Each mouse was placed at the end of one arm and allowed to freely explore the maze for 5 minutes (Day 13 post propolis administration). Total arm entries were recorded as a measure of locomotor and exploratory activity. Spontaneous alternation behavior was calculated as the proportion of successive entries into all three arms without repetition, serving as an index of spatial working memory.

Zero plus maze test

Anxiety-like behavior was assessed using the zero plus maze, comprising two open arms and two closed arms arranged in a circular configuration elevated above the floor. Each mouse was placed in the maze and allowed to explore for 5 minutes (Day 14 post propolis administration). Time spent in the open arms and closed arms was recorded manually from the video recordings of the mice. Reduced open-arm exploration was interpreted as increased anxiety-like behavior.

Statistical analysis

propolis-treated groups were performed separately for male and female mice using the unpaired Student's t-test. Statistical analyses were conducted using appropriate statistical software, and differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of whole propolis on body mass, feeding behavior and glycaemia

Body mass, food intake, and blood glucose levels were monitored to determine whether dietary supplementation with whole propolis altered general metabolic parameters in male and female mice (Figure 1). In male mice, body mass increased progressively over the 14-day experimental period in both control and propolis-treated groups, with no marked differences between groups at any time point (Figure 1A). Daily food intake remained stable throughout the study and was comparable between control and propolis-fed males, indicating that propolis supplementation did not affect feeding behavior (Figure 1B). Blood glucose levels measured at the end of the experiment showed no significant difference between control and propolis-treated male mice (Figure 1C).

Similarly, female mice exhibited a gradual increase in body mass over time in both control and propolis-treated groups, with no apparent differences between treatments across the experimental period (Figure 1D). Daily food intake in female mice remained consistent and did not differ between groups (Figure 1E). Terminal blood glucose measurements also revealed no significant differences between control and propolis-treated females (Figure 1F). Collectively, these findings indicate that dietary supplementation with 5% whole propolis does not significantly alter body mass gain, food consumption, or basal glycemic status in either male or female mice over the duration of the study.

Whole propolis enhances recognition memory in male and female mice

Recognition memory was assessed using the Novel Object Recognition test (Figure 2). In male mice, dietary supplementation with whole propolis significantly reduced the time spent exploring the familiar object while significantly increasing exploration of the novel object compared to controls. Female mice exhibited a similar pattern, with propolis-fed animals spending less time on the familiar object and significantly more time exploring the novel object. These findings indicate that consumption of whole propolis enhances recognition memory in both sexes, as reflected by increased novelty preference.

Sex-specific effects of propolis on spatial working memory

Spatial working memory was evaluated using the Y-maze test (Figure 3). In male mice, propolis supplementation resulted in a significant reduction in total arm entries and a significant decrease in spontaneous alternation behavior compared to controls, indicating impaired spatial working memory. In contrast, female mice showed no significant differences in either total arm entries or spontaneous alternation following propolis consumption, suggesting a sex-dependent effect of whole propolis on spatial working memory.

Whole propolis increases anxiety-like behavior in both sexes

Anxiety-related behavior was assessed using the zero plus maze (Figure 4). Male mice receiving propolis spent less time in the open arms compared to controls, while time spent in the closed arms was not markedly altered. Similarly, female mice fed propolis also exhibited reduced open-arm exploration with no significant change in closed-arm duration. These results suggest that whole propolis consumption is associated with increased anxiety-like behavior in both male and female mice.

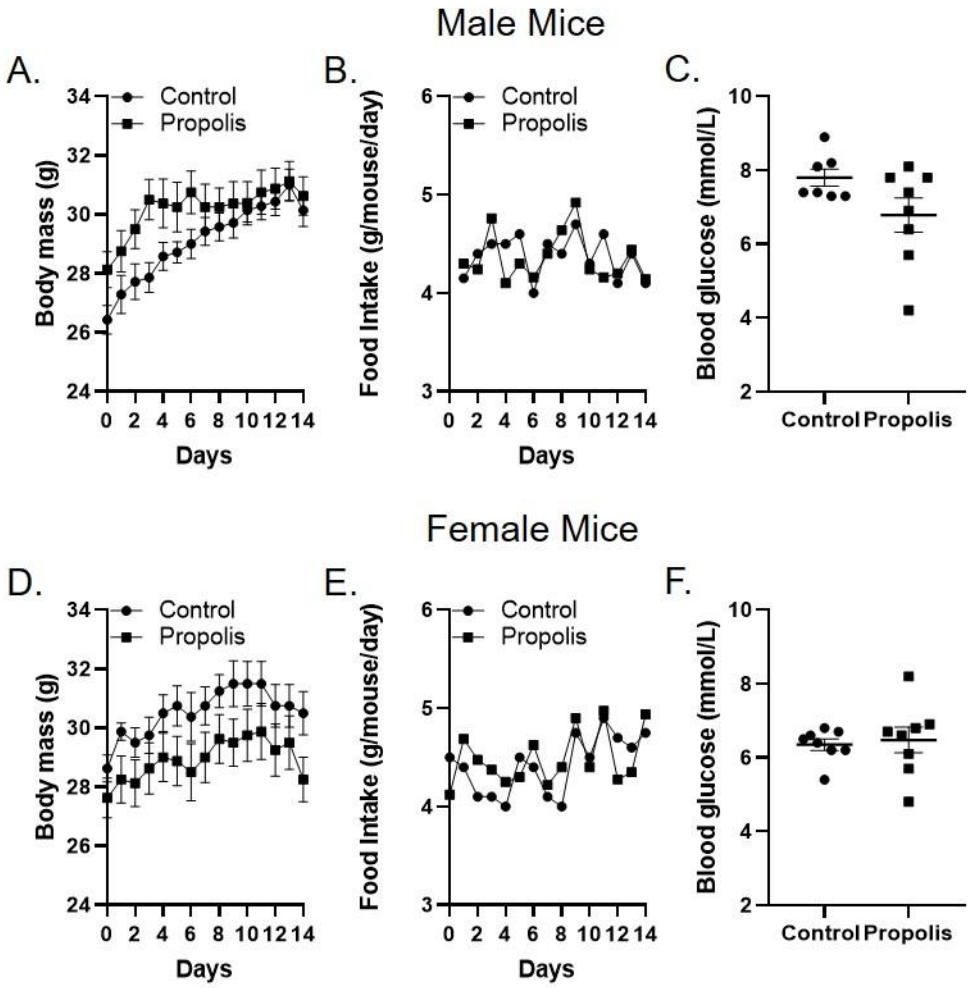

Figure 1. Effect of whole propolis on body mass, daily food intake, and terminal blood glucose levels**.** Panels A–C show data from male mice, while panels D–F show data from female mice. Body mass changes over time are shown in panels A (males) and D (females). Daily food intake, expressed as grams per mouse per day, is shown in panels B (males) and E (females). Blood glucose levels measured at the end of the experiment are shown in panels C (males) and F (females). Data are presented as mean ± SEM, with individual data points shown for blood glucose measurements. No significant differences were observed between the control and propolis-treated groups.

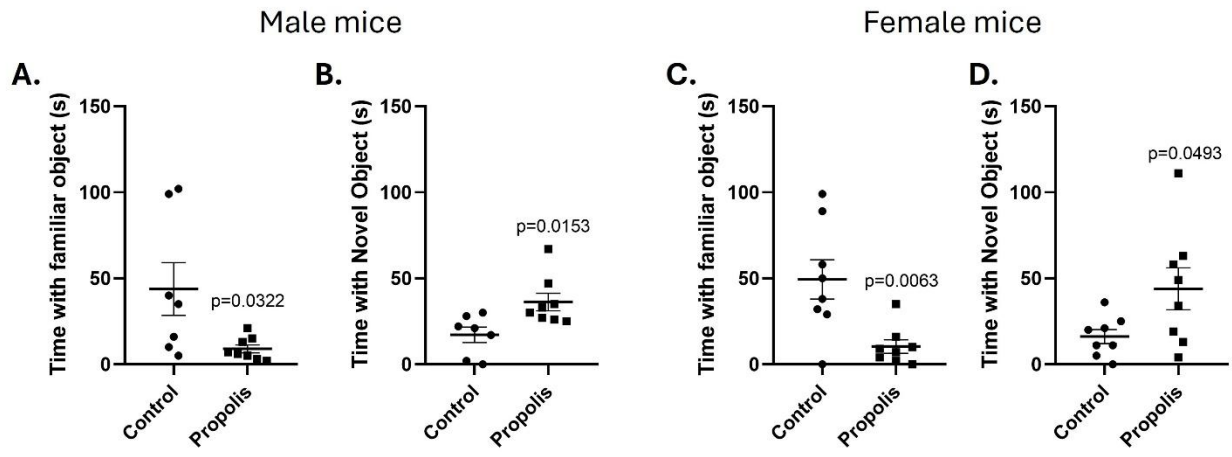

Figure 2. Effect of dietary whole propolis on recognition memory in male and female mice. Novel Object Recognition (NOR) test showing time spent exploring the familiar and novel objects in control and propolis-fed mice. Panels A and B represent male mice, showing time spent with the familiar object (A) and novel object (B). Panels C and D represent female mice, showing time spent with the familiar object (C) and novel object (D). Mice receiving 5% whole propolis in their diet exhibited reduced exploration of the familiar object and increased exploration of the novel object compared to controls in both sexes, indicating enhanced recognition memory. Data are presented as mean ± SEM, with individual data points shown. Statistical significance is indicated by the corresponding p-values.

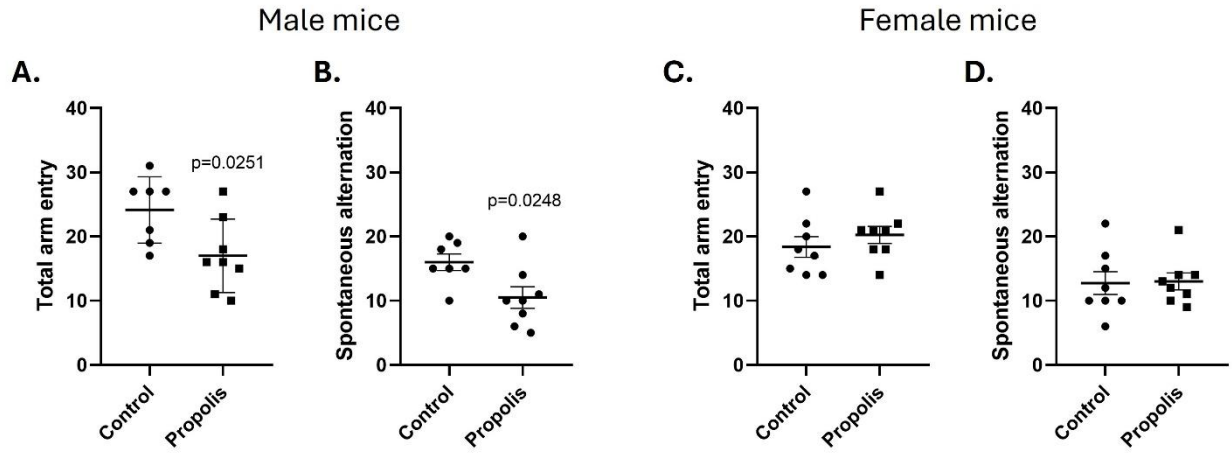

Figure 3. Effect of dietary whole propolis on spatial working memory in male and female mice. Y-maze performance showing total arm entries and spontaneous alternation behavior in control and propolis-fed mice. Panels A and B represent male mice, showing total arm entries (A) and spontaneous alternation (B). Panels C and D represent female mice, showing total arm entries (C) and spontaneous alternation (D). Male mice receiving propolis exhibited reduced locomotor activity and decreased spontaneous alternation, indicating impaired spatial working memory, whereas female mice showed no significant differences compared to controls. Data are presented as mean ± SEM with individual values displayed.

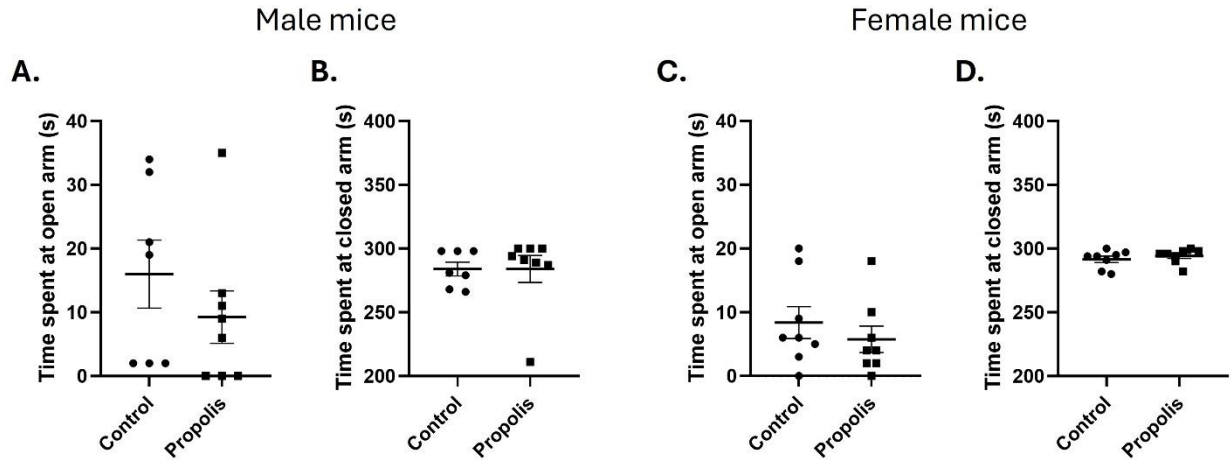

Figure 4. Effect of dietary whole propolis on anxiety-like behavior in male and female mice. Zero plus maze assessment of anxiety-like behavior in control and propolis-fed mice. Panels A and B represent male mice, showing time spent in the open arms (A) and closed arms (B). Panels C and D represent female mice, showing time spent in the open arms (C) and closed arms (D). Propolis-fed mice of both sexes spent less time in the open arms compared to controls, suggesting increased anxiety-like behavior, while time spent in the closed arms remained largely unchanged. Data are presented as mean ± SEM with individual data points shown.

DISCUSSION

In the present study, dietary supplementation with whole propolis incorporated directly into standard feed produced distinct effects on cognitive and anxiety-like behaviors in male and female mice. Whole propolis enhanced recognition memory in both sexes, impaired spatial working memory selectively in males, and increased anxiety-like behavior in both male and female mice. Importantly, these behavioral outcomes were observed without the use of solvent extracts or isolated bioactive compounds, thereby providing novel insight into the neurobehavioral impact of propolis consumption in its natural, unrefined form. Notably, these effects occurred in the absence of significant changes in body mass, food intake, or basal glycaemia, indicating that the observed behavioral alterations are unlikely to be secondary to gross metabolic disturbances.

The improvement in recognition memory observed in both male and female mice suggests that whole propolis positively influences neural circuits involved in object recognition and memory retention. Recognition memory depends largely on hippocampal–perirhinal cortex interactions and is sensitive to oxidative stress and inflammatory processes within these regions. Previous studies using ethanolic or aqueous propolis extracts have reported enhanced memory performance in rodent models of neurodegeneration, ageing, and chemically induced cognitive impairment, often attributing these effects to antioxidant and anti-inflammatory mechanisms[4,4,6]. For instance, ethanolic Brazilian green propolis extract has been shown to ameliorate memory deficits in Alzheimer's disease–like mouse models while reducing oxidative stress and neuroinflammation[9]. Similarly, water-soluble propolis derivatives have been reported to reverse scopolamine-induced memory impairment in mice[7]. The present findings extend this literature by demonstrating that whole propolis consumed as part of the diet is sufficient to enhance recognition memory, suggesting that the combined and potentially synergistic actions of multiple propolis constituents can support memory processes without chemical extraction. The absence of alterations in food consumption or glycemic status further supports a direct neuromodulatory influence rather than an indirect effect mediated by changes in energy balance or metabolic state.

In contrast to the improvement observed in recognition memory, whole propolis supplementation impaired spatial working memory in male mice, as indicated by reduced spontaneous alternation in the Y-

maze, while female mice were unaffected. Spatial working memory relies on coordinated activity between the hippocampus and medial prefrontal cortex and is particularly sensitive to changes in exploratory drive, motivational state, and neural excitability. Previous work examining propolis-derived compounds has often reported neuroprotective or memory-enhancing effects; however, these studies have predominantly focused on isolated constituents. For example, pinocembrin, a major flavonoid found in propolis, has been shown to protect against ischemia-induced cognitive impairment and neuronal damage through antioxidant and anti-inflammatory pathways[19]. The discrepancy between these extract- or compound-based findings and the present results suggests that whole propolis may exert more complex effects on neural circuits underlying spatial memory, particularly in males. Importantly, the male-specific impairment occurred despite stable body mass trajectories and unaltered food intake, suggesting that reduced spontaneous alternation was not driven by differences in nutritional status or generalized sickness behavior. The observed sex-specific impairment may reflect differences in hippocampal–prefrontal network sensitivity, hormonal modulation, or behavioral strategies rather than a uniform cognitive deficit.

Whole propolis supplementation also increased anxiety-like behavior in both sexes, as evidenced by reduced open-arm exploration in the zero plus maze. Anxiety-related behavior in this paradigm reflects activity within limbic structures, including the hippocampus and amygdala, and is influenced by neurochemical balance and stress responsiveness. Interestingly, previous studies using propolis extracts have reported anxiolytic-like effects, particularly in stress-exposed rodents. For example, propolis oil and ethanolic extracts have been shown to reduce anxiety-like behavior in elevated maze paradigms[20]. In addition, isolated propolis constituents such as chrysin and caffeic acid phenethyl ester (CAPE) have demonstrated anxiolytic and neuroprotective effects via antioxidant and anti-inflammatory mechanisms[21]. The anxiogenic-like effect observed in the present study, therefore, highlights a potential divergence between whole-propolis consumption and extract-based administration, possibly due to the presence of waxes, resins, and multiple interacting compounds that may modulate neural excitability or behavioral inhibition. The lack of accompanying changes in glycaemia or feeding behavior suggests that the anxiogenic phenotype is unlikely to reflect metabolic stress, but rather a direct effect on anxiety-regulating neural circuits.

Most experimental studies investigating the neurobehavioral effects of propolis have relied on solvent extracts or isolated bioactive molecules, approaches that selectively enrich specific compounds while excluding others that are naturally co-consumed. Reviews of propolis in neurological disorders consistently emphasize extract-based methodologies and note the relative lack of studies examining whole propolis consumption[22]. In real-world contexts, humans and animals consuming propolis as a dietary supplement are exposed to the entire chemical matrix rather than purified fractions. By demonstrating that whole propolis alters behavior without affecting core metabolic parameters, the present findings underscore the importance of evaluating natural products in forms that more closely reflect dietary exposure. The present findings suggest that whole propolis may produce behavioral effects that differ qualitatively from those reported for extracts, underscoring the importance of studying natural products in forms that more closely reflect dietary exposure.

The sex-dependent effects observed in this study further reinforce the importance of including both male and female animals in behavioral research. Male and female rodents differ in cognitive strategies, baseline anxiety-like behavior, and hormonal regulation of neural circuits, particularly within the hippocampus and limbic system. Reviews of behavioral neuroscience have consistently documented sex differences in anxiety and cognitive outcomes across commonly used paradigms[13]. Many previous propolis studies have focused exclusively on male animals or have not analyzed sex as an independent biological variable, limiting the generalizability of their findings. The present results demonstrate that dietary bioactive compounds such as propolis can exert divergent behavioral effects depending on sex, highlighting the necessity of sex-stratified analyses in nutritional and behavioral neuroscience.

In conclusion, this study demonstrates that dietary supplementation with whole propolis enhances recognition memory, differentially affects spatial working memory in males, and increases anxiety-like behavior in both sexes. These behavioral effects occur independently of changes in body mass, food intake, or basal glycemia, highlighting a direct and sex-dependent neurobehavioral influence of whole propolis consumption. These findings highlight the complex, sex-dependent neurobehavioral effects of whole propolis and underscore the importance of evaluating natural products in their unrefined, dietary form. Future studies integrating behavioral outcomes with neurochemical and anatomical analyzes will be

essential for elucidating the mechanisms underlying these effects.

Conflict of interests: The authors have no conflict of interest to declare.

Authors' contributions: AA conceptualized, designed the experiment, and drafted the manuscript. SE, YAU, & MOD designed, carried out the experiment, and analyzed the data. WIA, AAO, IA, & ALO analyzed the data and revised the manuscript.

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