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RESEARCH

Mad Honey Modulates Reproductive Hormones and Sexual Behavior in Male Rats

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Abstract

Mad honey has been traditionally used in the Black Sea region of Türkiye in relation to male sexual function, yet its underlying physiological mechanisms remain unclear. In this study, we explored how acute and chronic mad honey exposure affects sexual behavior and androgen-related endocrine parameters in adult male rats. Thirty-six sexually experienced rats were assigned to acute (single-dose) or chronic (30-day) treatment protocols and received saline, pine honey, or mad honey. Serum and testicular levels of testosterone (T), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and 5-alpha-reductase (5αR) were measured, and sexual behavior was assessed using established experimental paradigms. While testicular hormone levels remained unchanged, mad honey administration led to significant increases in serum T and 5αR without altering FSH or LH. Behaviorally, acute mad honey exposure prolonged ejaculation latency, whereas chronic treatment shortened the post-ejaculatory interval. These findings suggest that mad honey modulates circulating androgen-related parameters and sexual behavior through time-dependent mechanisms, although the functional significance of the total testosterone increase requires further investigation.

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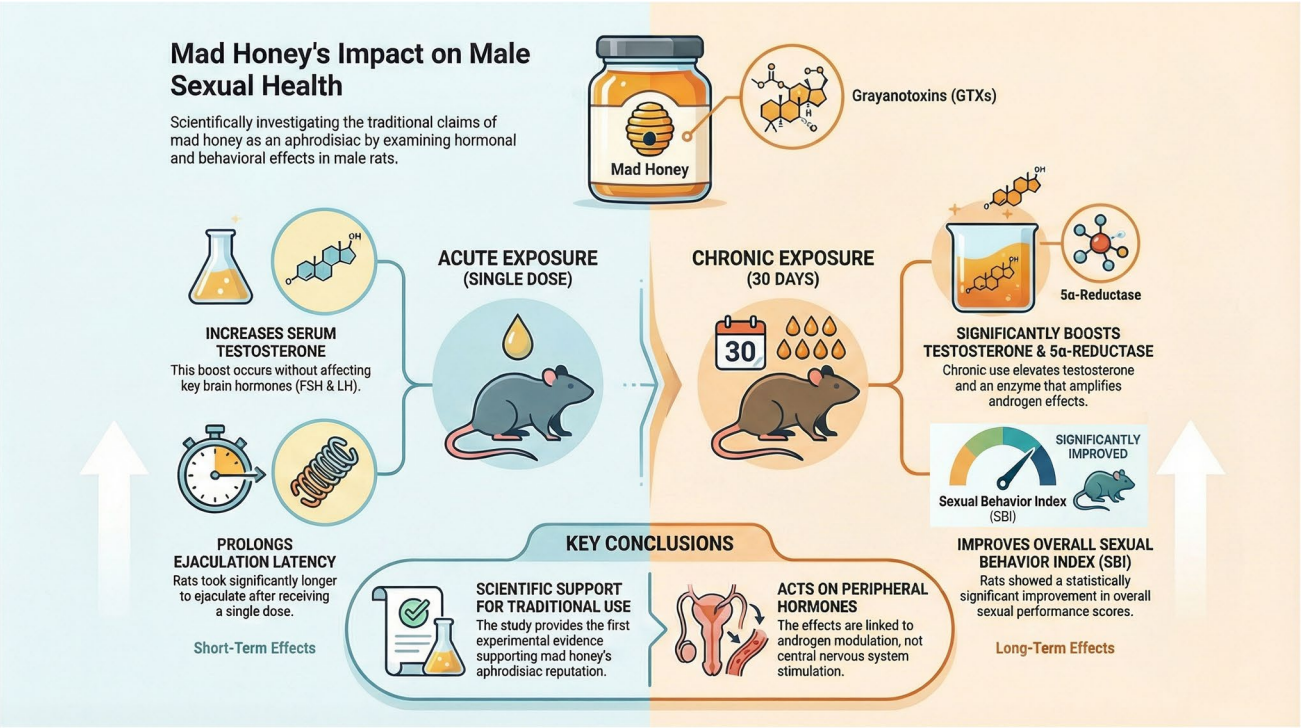
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Graphical abstract



Keywords Grayanotoxin · Mad honey · Male reproductive function · Neuroendocrine hormones · Sexual behavioral tests

1 Introduction

Mad honey (MH) poisoning typically occurs unintentionally during routine dietary consumption, but it may also arise from its deliberate use in traditional or alternative medicinal practices [1, 2]. Grayanotoxins (GTXs) are plant-derived neurotoxins present in MH that act on voltage-gated sodium channels and may enter the human food chain through honey produced from the nectar of *Rhododendron* species of the *Ericaceae* family (for grayanotoxin-I molecular structure model: Supplementary Figure S1) [3]. Although relatively uncommon, GTX/MH intoxication is clinically well characterized and is known to cause potentially life-threatening bradycardia, hypotension, and alterations in mental status [1, 4]. Intoxication most commonly results from ingestion of MH produced in the Black Sea region, where MH has long been used in traditional medicine for wound healing, arthritis, gastrointestinal complaints, intestinal disorders, and hypertension, and is traditionally regarded as enhancing male sexual performance [5, 6].

Experimental studies have demonstrated that low doses of grayanotoxins (GTXs) stimulate pancreatic insulin release through parasympathetic activation, leading to hypoglycemia, whereas consumption of honey containing higher GTX concentrations triggers adrenal medullary catecholamine secretion and consequently elevates blood glucose levels [7, 8]. Research on various types of honey has further indicated modulatory effects on reproductive hormones—including testosterone (T), luteinizing hormone (LH), and follicle-stimulating hormone (FSH)—and has suggested an increase in T production and sperm count in male rats [9, 10]. In addition, MH has been reported to reduce cellular damage and apoptosis during testicular ischemia-reperfusion injury, indicating potential protective effects on reproductive function [11].

Given that MH has been used for centuries in the Black Sea region as a traditional remedy for enhancing sexual vigor, this study aimed to examine, for the first time, the effects of GTX-containing MH on both male reproductive hormone profiles and validated sexual behavior parameters, and to determine whether scientific evidence examine these longstanding cultural claims in an experimental framework.

2 Materials and Methods

2.1 Experimental Animals

This study was conducted in full compliance with the ARRIVE guidelines and all relevant national and international regulations governing the care and use of laboratory animals. Ethical approval was obtained from Karadeniz Technical University Animal Experiments Local Ethics Committee (Protocol No. 2020/12) and from Fırat University Animal Experiments Local Ethics Committee (Decision No. 01, dated 15 January 2020). All procedures were reviewed and deemed ethically appropriate by both committees.

The study utilized 36 adult male Sprague–Dawley rats (4–5 months old; body weight 250–300 g) with confirmed normal sexual activity. Animals were obtained from an accredited institutional animal facility and housed in standard polycarbonate cages (3–4 animals per cage) under controlled environmental conditions (temperature: 22 ± 2 °C; relative humidity: 50–60%) with a 12:12 h light–dark cycle (lights on at 07:00 h). Animals had free access to standard laboratory chow and water *ad libitum*. All animals were allowed to acclimatize to the laboratory environment for at least one week prior to the experiments. Efforts were made to minimize stress and environmental variability throughout the study. All experimental procedures were conducted during the same period of the day to minimize circadian variability.

Animals were randomly assigned into six groups ($n = 6$ per group), and all treatments were administered via an orogastric gavage tube at a dose of 80 mg/kg. The experimental groups were organized as follows (Fig. 1):

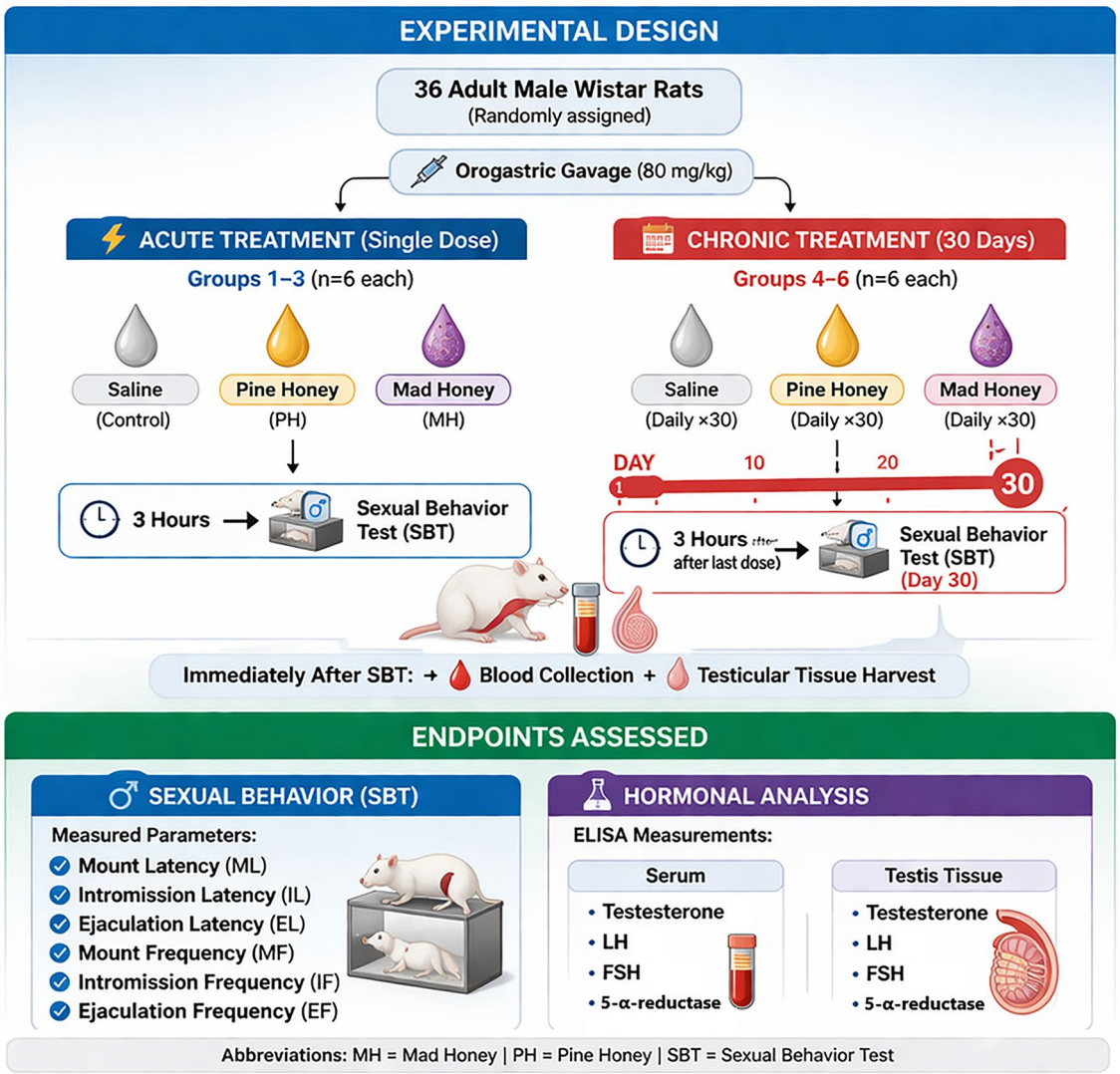


Fig. 1 Schematic overview of the experimental design

Group 1: Acute control – single dose of physiological saline solution (PSS).

Group 2: Acute PH – single dose of pine honey (PH).

Group 3: Acute MH – single dose of MH.

Group 4: Chronic control – PSS administered daily for 30 days.

Group 5: Chronic PH – PH administered daily for 30 days.

Group 6: Chronic MH – MH administered daily for 30 days.

The measurement of GTX in the MH used for experiments was performed at the Rize Food Control Laboratory using the LC-MS/MS method. The GTX level in the honey was measured as 12 mg/kg. In addition, based on the administered dose of mad honey (80 mg/kg body weight) and its quantified GTX content (12 mg/kg), the estimated grayanotoxin exposure corresponded to approximately 0.96 mg/kg body weight.

For the sexual behavior tests (SBT), sexually mature female Sprague–Dawley rats (2–3 months old) were included exclusively as stimulus animals. To prevent pregnancy, bilateral oophorectomy was performed before the experiments. Estrus was induced through subcutaneous injections of estradiol (10 µg) administered 48 h prior to SBT and progesterone (500 µg) administered 4 h prior, each dissolved in 0.1 mL of sesame oil. These hormonally primed females were used solely to facilitate SBT procedures, and no data obtained from them were included in the study.

The study consisted of acute (single-dose) and chronic (30-day) treatment paradigms. In the acute groups, animals received a single administration of saline, pine honey (PH), or mad honey (MH), followed by sexual behavior testing (SBT) 3 h later. In the chronic groups, animals received daily administrations for 30 days, and SBT was performed on Day 30, 3 h after the final dose. Blood and testicular tissue samples were collected immediately after behavioral assessment.

2.2 Sexual Behavior Tests (SBT)

Rats exhibit optimal mating activity during the dark phase of the light–dark cycle. Accordingly, all sexual behavior tests (SBTs) were performed during the dark phase under dim red illumination between 5:00 and 6:00 PM, following an adjusted light–dark schedule in the behavioral laboratory. All sessions were recorded using the Limelight video-tracking system and subsequently analyzed by an impartial observer who was blinded to group assignment.

Prior to any saline (SF), PH, or MH administration, all male rats were sexually experienced through two standardized SBT sessions, each lasting 30 min, with a 3–4-day rest interval between sessions. Following the single-dose administration in Groups 1–3, and on Day 30—the final day of chronic administration—in Groups 4–6, a 30-minute SBT was conducted 3 h after the last treatment.

During each 30-minute SBT, the following behavioral parameters were assessed: mount latency, intromission latency, ejaculation latency (defined as 30 min for males failing to ejaculate within the test period), mount frequency, intromission frequency, ejaculation frequency, post-ejaculatory interval, intromission ratio, and copulation ratio. These variables were used to quantify mounting, intromission, and ejaculation patterns, and selected parameters were subsequently integrated to generate the sexual behavior index (SBI) [12]. The SBI was calculated using the following formula: SBI = [(number of mounts + number of intromissions + number of ejaculations)/(mount latency + intromission latency + ejaculation latency + post-ejaculatory interval)] × 100. Higher SBI values were interpreted as reflecting a greater overall sexual behavior performance index within the observation period. Behavioral definitions were based on standard methodologies used in rodent sexual behavior studies [13–15] (Table 1).

2.3 Preparation of Serum and Tissue Samples

All animals were euthanized by rapid decapitation immediately after completion of the SBT, without prior anesthesia,

Table 1 Definitions of sexual behavior parameters used in the study

Parameter	Definition
Mount latency (ML)	Time interval between the introduction of the female and the first mount
Intromission latency (IL)	Time from female introduction to the first intromission
Ejaculation latency (EL)	Time from the first intromission to ejaculation
Mount frequency (MF)	Total number of mounts without intromission during the observation period
Intromission frequency (IF)	Total number of intromissions during the test session
Ejaculation frequency (EF)	Total number of ejaculations during the test session
Post-ejaculatory interval (PEI)	Time interval between ejaculation and the next mount (or resumption of copulatory activity)
Intromission ratio (IR)	Ratio of intromissions to total sexual attempts (intromissions + mounts)
Copulation ratio (CR)	Proportion of copulatory attempts resulting in ejaculation
Sexual behavior index (SBI)	Composite index calculated from selected behavioral parameters

in accordance with the approved ethical protocols. This procedure was selected to avoid potential confounding effects of anesthetic agents on neuroendocrine and reproductive hormone measurements, which were primary outcomes of the present study. Previous studies have demonstrated that anesthetic agents may significantly influence hypothalamic–pituitary–gonadal axis activity and circulating hormone levels, including suppression of LH surge and modulation of hypothalamic neurotransmission [16, 17]. Therefore, rapid decapitation was applied as a scientifically justified approach to preserve the validity of neuroendocrine endpoints while minimizing procedural distress.

Blood samples were collected into serum separator tubes, allowed to clot at room temperature for 30 min, and centrifuged at 1,800 × g for 10 min. The resulting serum and the testicular tissues harvested post-decapitation were aliquoted into microtubes and stored at − 80 °C until analysis. Before biochemical assessment, samples were thawed at room temperature and gently vortexed.

Approximately 30 mg of testicular tissue was excised from each animal and homogenized in 1.5 mL phosphate-buffered saline (PBS; pH 7.4) using a homogenizer (IKA Ultra-Turrax T-25, Staufen im Breisgau, Germany) at 9,500 rpm. Homogenates were then centrifuged at 1,800 × g for 10 min, and biochemical parameters were measured in the resulting supernatants. (One animal in Group 6 died during the treatment period; therefore, SBT procedures and subsequent biochemical analyses for this group were conducted on five rats.)

2.4 Measurement of Serum and Tissue Hormone Levels

Serum and testicular tissue concentrations of FSH, LH, T, and 5-alpha-reductase (5αR) were measured in the Biochemistry Department using validated ELISA kits from Bioassay Technology Laboratory, Shanghai, China. The specific catalog numbers used were as follows: FSH (Catalogue No: E0182Ra), LH (Catalogue No: EA0013Ra), T (Catalogue No: E0259Ra), and 5αR (Catalogue No: E1585Ra). Total protein content in the tissue homogenates was determined using the Thermo Scientific Pierce BCA Protein Assay Kit (Catalogue No: 23227), with results expressed in mg/mL. To ensure standardization across samples, all tissue hormone concentrations measured by ELISA were normalized to total protein content and reported as hormone level per mg of protein.

All assays were performed according to the manufacturer's instructions. The detection ranges and assay sensitivities were as follows:

FSH: 0.05–20 mIU/mL; sensitivity: 0.022 mIU/mL.

LH: 0.5–100 mIU/mL; sensitivity: 0.31 mIU/mL.

T: 0.1–40 ng/mL; sensitivity: 0.043 ng/mL.

5α-reductase (5αR): 0.05–30 ng/mL; sensitivity 0.024 ng/mL.

All samples were measured in duplicate, and standard curves were generated for each assay. Serum hormone concentrations were reported in ng/mL for T and 5αR and in mIU/mL for FSH and LH, whereas testicular tissue hormone concentrations were normalized to total protein content and reported as ng/mg protein or mIU/mg protein, respectively.

2.5 Statistical Analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 22.0; IBM Corp., Armonk, NY, USA). Data were expressed as mean ± standard deviation (SD) and median values. Comparisons among multiple independent groups were conducted using the Kruskal-Wallis test, as the data did not meet normal distribution assumptions (Normality of data distribution was assessed using the Shapiro–Wilk test). When a significant overall difference was detected, post-hoc pairwise comparisons were performed using Bonferroni - adjusted tests. A p-value of <0.05 was considered statistically significant. In addition to the primary between-group comparisons within the acute and chronic treatment arms, exploratory post-hoc comparisons were performed between the acute MH (Group 3) and chronic MH (Group 6) groups to further evaluate time-dependent effects of mad honey exposure. These comparisons were not pre-specified in the initial study design and were interpreted as exploratory analyses.

3 Results

Quantitative data were summarized as mean ± SD (median). No significant differences were observed in mount latency, intromission latency, ejaculation latency, mount frequency, intromission frequency, ejaculation frequency, post-ejaculatory interval, intromission ratio, copulation ratio, or sexual behavior index (SBI) between Groups 1 and 2, representing the expected acute effects. However, Group 3 exhibited a longer ejaculation latency compared with Group 2 ($p = 0.003$; $p_{2–3} = 0.002$), while the difference between Groups 1 and 3 was not statistically significant (Table 2).

Among the chronic groups, post-ejaculatory interval was higher in Group 4 compared with Group 5 ($p = 0.003$; $p_{4–5} = 0.002$), whereas the difference between Groups 5 and 6 was not statistically significant. No additional SBT parameter differed significantly across chronic groups (Table 3).

For the SBI parameter, Group 4 displayed significantly lower scores compared with Group 6 (SBI: $p = 0.036$; $p_{4–6}$

Table 2 The SBT parameters [mean±SD, (median)] of rats in the acute administration groups

Parameter (SBT)	Group 1 (SF) (n=6)	Group 2 (PH) (n=6)	Group 3 (MH) (n=6)	p values
Mount latency	7.2±2.1 (8)	7.5±1.4 (8)	5.8±1.3 (6)	0.221
Intromission latency	7.2±1.7 (7.5)	6.3±1.6 (6.5)	6.8±1.5 (7)	0.615
Ejaculation latency	504.5±76.7 (507)	381.2±54.9 (387)	572.7±54.7 (592.5)	**0.003** **p^{2-3}=0.002**
Mount frequency	3.8±2.5 (4)	2.5±1.0 (2.5)	3.5±1.4 (3)	0.427
Intromission frequency	31.2±4.8 (31)	31.5±4.9 (31)	29.8±5.6 (30)	0.848
Ejaculation frequency	2.2±0.8 (2)	1.8±0.4 (2)	1.7±0.8 (1.5)	0.427
Post-ejaculatory interval	405.0±79.5 (388)	427.0±54.7 (454.5)	468.1±80.1 (487.5)	0.331
Intromission ratio	0.8±0.1 (0.76)	0.8±0.2 (0.9)	0.9±0.04 (0.9)	0.504
Copulation ratio	0.07±0.01 (0.07)	0.06±0.02 (0.06)	0.05±0.02 (0.05)	0.211
Sexual behavior index	12.2±1.04 (12.1)	12.2±1.4 (12.1)	10.9±0.8 (10.7)	0.084

Bold values indicate statistically significant differences, $p < 0.05$

Table 3 The SBT parameters [mean±SD, (median)] of rats in the chronic administration groups

Parameter (SBT)	Group 4 (SF) (n=6)	Group 5 (PH) (n=6)	Group 6 (MH) (n=5)	p values
Mount latency	13.5±2.6 (13.5)	17.2±8.8 (14.5)	18.8±8.7 (16)	0.596
Intromission latency	37.5±20.4 (36.5)	40.8±22.9 (38)	41.2±13.1 (41)	0.857
Ejaculation latency	1459.7±286.8 (1457.5)	1416.0±541.6 (1714.5)	1385.8±569.4 (1800)	0.917
Mount frequency	1.7±0.8 (1.5)	3.3±2.0 (4)	2.4±1.5 (2)	0.299
Intromission frequency	26.5±10.1 (30)	24.3±4.7 (23)	26.4±5.1 (27)	0.687
Ejaculation frequency	1.0±0.6 (1)	0.7±0.8 (0.5)	0.8±1.1 (0)	0.711
Post-ejaculatory interval	461.8±29.7 (462)	399.0±7.4 (399)	432.8±21.9 (432)	**0.003** **p^{4-5}=0.002**
Intromission ratio	0.9±0.1 (0.9)	0.8±1.9 (0.8)	0.9±0.2 (1.0)	0.132
Copulation ratio	0.02±0.01 (0.02)	0.03±0.02 (0.03)	0.05±0.03 (0.05)	0.383
Sexual behavior index	8.1±2.2 (8.45)	9.6±1.5 (9.4)	10.5±0.2 (10.5)	**0.036** **p^{4-6}=0.031**

Bold values indicate statistically significant differences, $p < 0.05$)

= 0.031), whereas the difference between Groups 4 and 5 did not reach statistical significance. No other behavioral parameters exhibited significant variation among groups (Table 3).

In the acute treatment groups, serum T levels were higher in Group 3 compared with Group 1 (T: p=0.028; p_{1-3} = 0.022), while no statistically significant difference was observed in Group 2 (Fig. 2; Table 4). Serum LH, FSH and 5αR levels did not differ significantly among groups (Figs. 3, 4 and 5; Tables 4 and 5). No significant differences were detected in testicular tissue hormone levels among the acute groups (p>0.05, Figs. 2, 3, 4 and 5; Tables 4 and 5).

In the chronic treatment groups, serum FSH and LH levels did not differ significantly among Groups 4, 5, and 6. However, both T and 5αR levels were higher in Group 6 than in Groups 4 and 5 (T*: p=0.007; p_{4-6} = 0.014; p_{5-6} = 0.019; 5αR*: p=0.006; p_{4-6} = 0.007; p_{5-6} = 0.037, Figs. 2 and 3; Table 5). Testicular tissue hormone levels indicated no significant differences within the chronic arm (p>0.05, Figs. 2, 3, 4 and 5; Table 5).

In an exploratory post-hoc comparison between acute (Group 3) and chronic (Group 6) MH-treated rats, serum T and 5αR concentrations were higher in the chronic group (p=0.004, Figs. 2 and 3; Tables 4 and 5).

In a direct comparison of the key SBT parameters between acute (Group 3) and chronic (Group 6) MH-treated rats, the chronic treatment group exhibited significantly higher mount latency, intromission latency, and ejaculation latency values (p=0.004), with no significant differences in the remaining behavioral measures (Tables 2 and 3).

4 Discussion

This study investigated the effects of MH containing GTXs on sexual behavior and reproductive hormone profiles in male rats under acute and chronic exposure conditions. These findings demonstrate a complex and dose-duration-dependent pattern of neuroendocrine and behavioral modulation and contribute to the growing body of literature examining natural compounds affecting male reproductive physiology [1, 2, 6, 10, 18–20].

The significant elevation in serum T levels observed in both acute (p = 0.022) and chronic (p = 0.014) MH groups

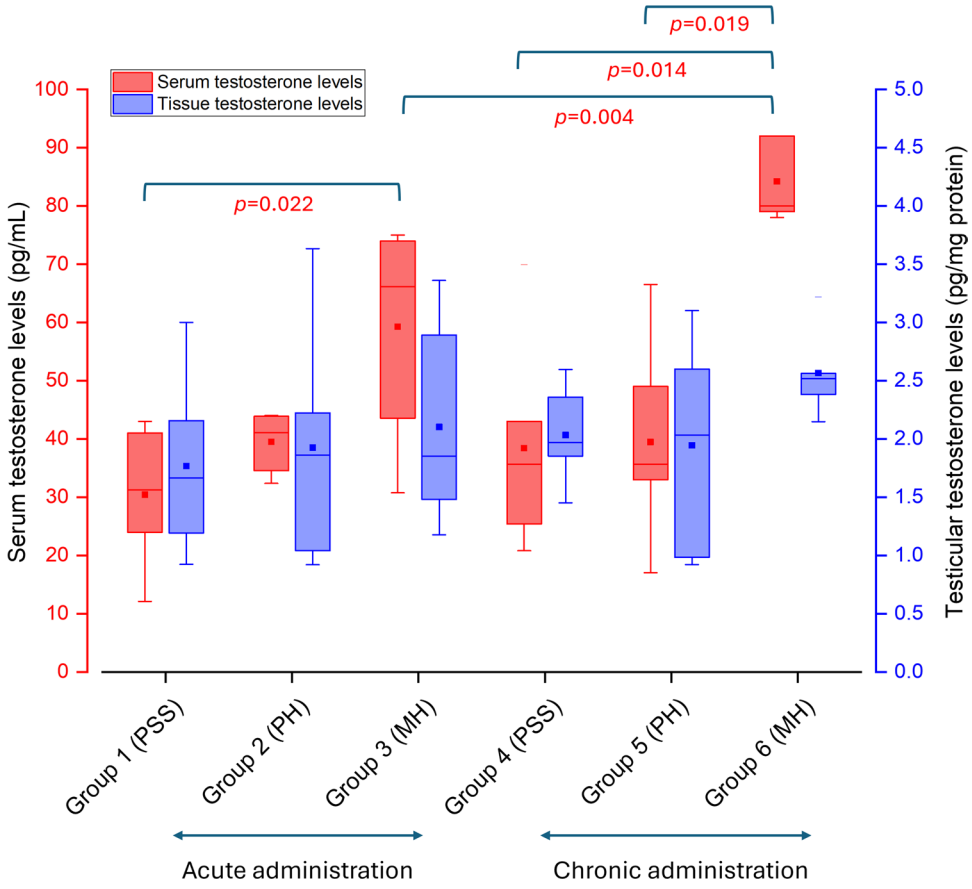


Fig. 2 Serum and testicular testosterone levels of rats in the acute and chronic administration groups

Table 4 Serum and testicular hormone parameters [mean ± SD, (median)] of rats in the acute administration groups

Parameter		Group 1 (SF) ($n=6$)	Group 2 (PH) ($n=6$)	Group 3 (MH) ($n=6$)	p values
FSH	Serum (mIU/mL)	4.31 ± 1.68 (4.34)	3.50 ± 1.39 (3.12)	3.98 ± 1.07 (4.20)	0.548
	Tissue (mIU/mg protein)	0.05 ± 0.03 (0.04)	0.07 ± 0.02 (0.07)	0.06 ± 0.04 (0.06)	0.528
LH	Serum (mIU/mL)	1.84 ± 0.21 (1.81)	1.81 ± 0.58 (1.88)	2.20 ± 0.55 (2.10)	0.325
	Tissue (mIU/mg protein)	0.03 ± 0.01 (0.02)	0.03 ± 0.01 (0.02)	0.03 ± 0.00 (0.02)	0.972
T	Serum (ng/mL)	30.4 ± 11.6 (31.2)	39.5 ± 5.13 (41.1)	59.3 ± 18.00 (66.1)	**0.028** p^{1-3}**=0.022**
	Tissue (ng/mg protein)	1.77 ± 0.75 (1.67)	1.92 ± 0.99 (1.86)	2.10 ± 0.86 (1.85)	0.884
5αR	Serum (ng/mL)	0.38 ± 0.10 (0.32)	0.41 ± 0.06 (0.43)	0.50 ± 0.10 (0.49)	0.109
	Tissue (ng/mg protein)	0.02 ± 0.00 (0.02)	0.03 ± 0.01 (0.03)	0.03 ± 0.01 (0.03)	0.747

Bold values indicate statistically significant differences, $p < 0.05$

Values are presented as mean ± SD (median). Serum hormone levels are reported as ng/mL for T and 5αR, and mIU/mL for FSH and LH. Testicular tissue hormone levels are normalized to total protein content and reported as ng/mg protein for T and 5αR, and mIU/mg protein for FSH and LH

compared to controls represents a key finding of this investigation. This increase in circulating total T was particularly pronounced in the chronic treatment group, which also indicated significantly higher levels compared to PH controls (p = 0.019). These results align with previous studies demonstrating the T-boosting properties of honey and its bioactive compounds [9, 11, 21].

The hypothalamic–pituitary–gonadal (HPG) axis is the primary regulatory system controlling male reproductive hormone production. In this axis, GnRH secretion from the hypothalamus stimulates the release of LH and FSH from the pituitary gland, which in turn regulate testicular steroidogenesis and spermatogenesis. In the present study, circulating T levels increased without accompanying changes in LH or FSH levels. Under physiological conditions, activation of the HPG axis would typically be reflected by elevations in gonadotropins. Therefore, although a contribution of central neuroendocrine pathways cannot be completely excluded,

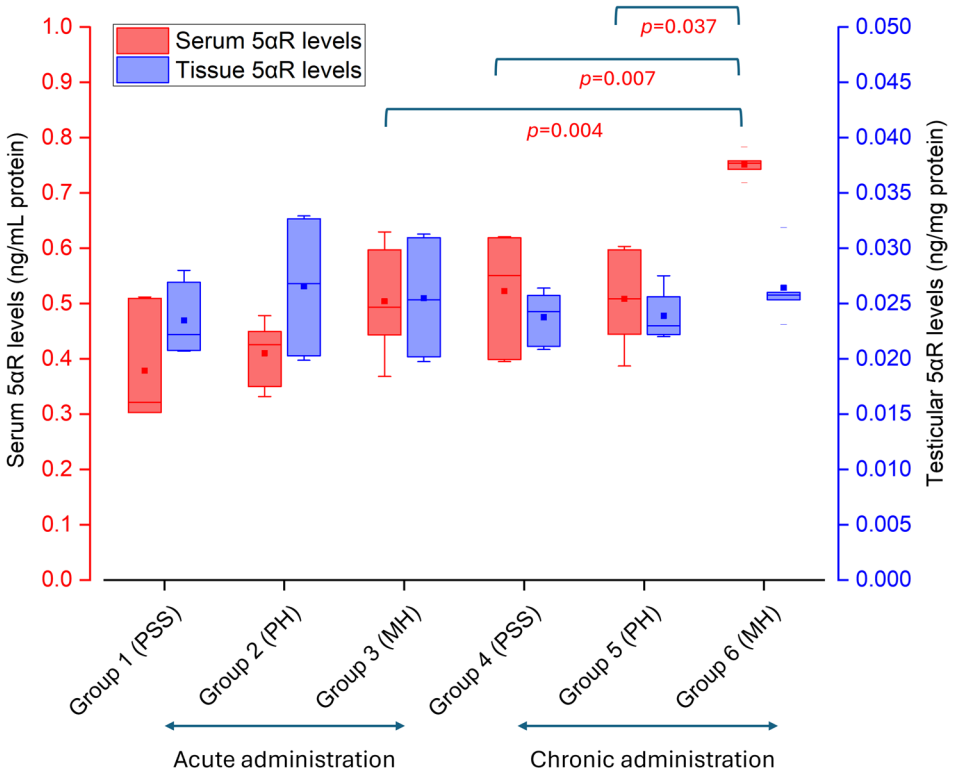


Fig. 3 Serum and testicular 5-α reductase levels of rats in the acute and chronic administration groups

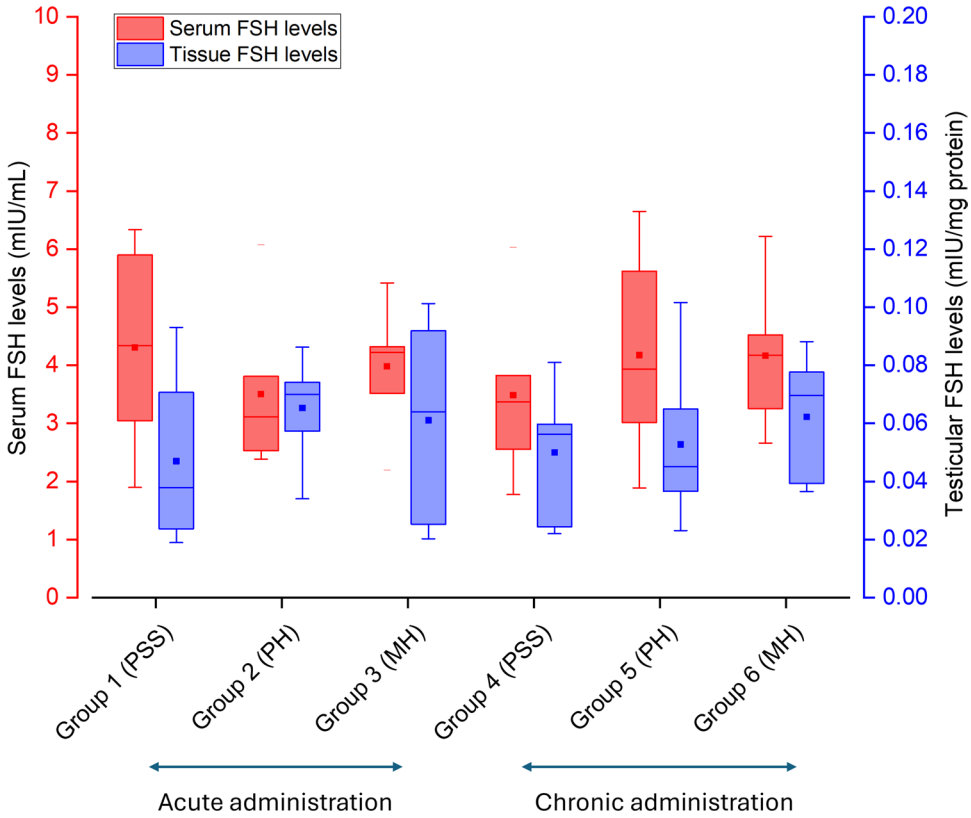


Fig. 4 Serum and testicular FSH levels of rats in the acute and chronic administration groups

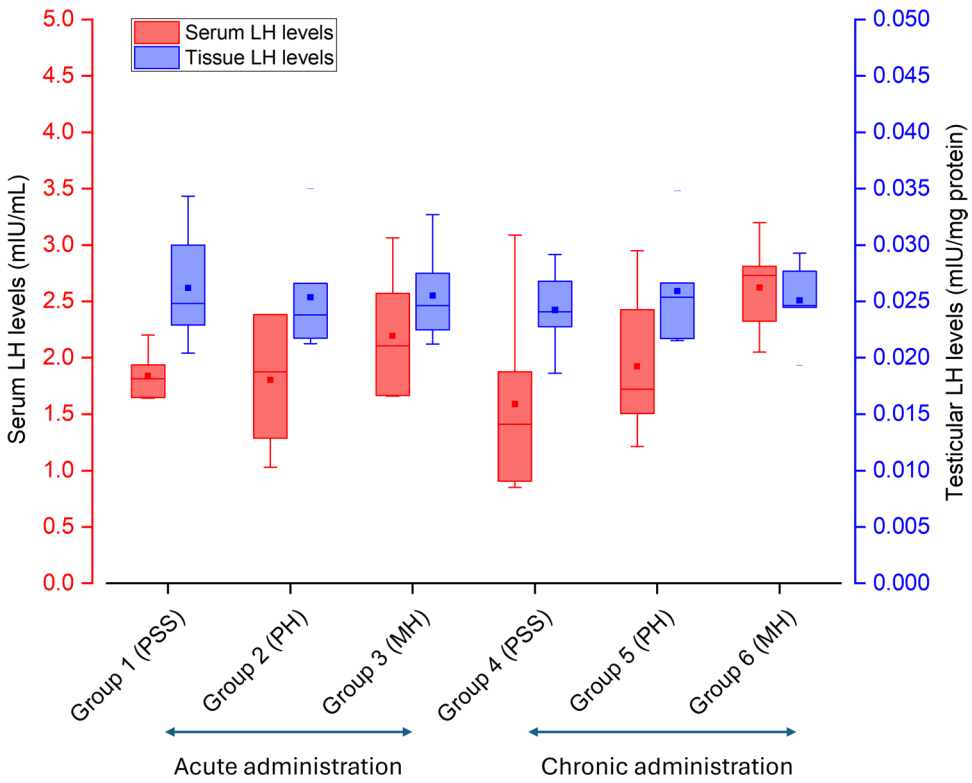


Fig. 5 Serum and testicular LH levels of rats in the acute and chronic administration groups

Table 5 Serum and testicular hormone parameters [mean ± SD, (median)] of rats in the chronic administration groups

Parameter		Group 4 (SF) ($n=6$)	Group 5 (PH) ($n=6$)	Group 6 (MH) ($n=5$)	p values
FSH	Serum (mIU/mL)	3.49 ± 1.45 (3.40)	4.17 ± 1.75 (3.90)	4.17 ± 1.36 (4.20)	0.594
	Tissue (mIU/mg protein)	0.05 ± 0.02 (0.06)	0.05 ± 0.03 (0.05)	0.06 ± 0.02 (0.07)	0.696
LH	Serum (mIU/mL)	1.59 ± 0.84 (1.48)	1.92 ± 0.65 (1.78)	2.62 ± 0.45 (2.71)	0.079
	Tissue (mIU/mg protein)	0.02 ± 0.00 (0.02)	0.03 ± 0.00 (0.03)	0.03 ± 0.00 (0.03)	0.869
T	Serum (ng/mL)	38.4 ± 17.8 (35.75)	39.5 ± 16.79 (35.78)	84.2 ± 7.15 (80.04)	**0.007** **p^{4-6}=0.014** **p^{5-6}=0.019**
	Tissue (ng/mg protein)	2.03 ± 0.41 (1.97)	1.95 ± 0.87 (2.03)	2.57 ± 0.40 (2.51)	0.193
5αR	Serum (ng/mL)	0.52 ± 0.11 (0.55)	0.51 ± 0.09 (0.51)	0.75 ± 0.02 (0.75)	**0.006** **p^{4-6}=0.007** **p^{5-6}=0.037**
	Tissue (ng/mg protein)	0.02 ± 0.00 (0.02)	0.02 ± 0.00 (0.02)	0.03 ± 0.00 (0.03)	0.315

Bold values indicate statistically significant differences, $p < 0.05$

Values are presented as mean ± SD (median). Serum hormone levels are reported as ng/mL for T and 5αR, and mIU/mL for FSH and LH. Testicular tissue hormone levels are normalized to total protein content and reported as ng/mg protein for T and 5αR, and mIU/mg protein for FSH and LH

the absence of significant changes in LH and FSH suggests that the observed hormonal effects are more likely mediated through peripheral mechanisms rather than direct activation of the HPG axis. Building on this framework, the hormonal profile observed in this study provides several important mechanistic insights into how GTX-containing MH modulates androgen physiology. Of particular interest, serum T levels increased significantly in both acute and chronic MH groups, while intratesticular T concentrations remained unchanged. This dissociation suggests that MH may alter circulating total T levels without overt activation of the HPG axis; however, whether this reflects increased bioactive androgen availability remains uncertain and requires further validation with comprehensive androgen profiling. Although not statistically significant, LH, FSH, and 5αR levels indicated a tendency to increase in MH-treated animals. Instead, MH may facilitate increased release of pre-existing T stores from the testes or reduce hepatic or peripheral metabolic clearance, both of which would elevate systemic concentrations without altering testicular content.

However, this interpretation warrants cautious consideration. Because only total T levels were measured, it remains unclear whether the observed increase reflects a true rise in bioactive free T or is influenced by changes in sex hormone–binding globulin (SHBG). Alterations in SHBG concentrations could modify the balance between bound and free fractions without necessarily reflecting increased androgenic activity. In addition, although 5αR levels were elevated, direct measurement of dihydrotestosterone (DHT) was not performed, limiting our ability to fully characterize downstream androgen metabolism. Furthermore, changes in hepatic clearance or peripheral distribution of T may also contribute to elevated circulating levels. Therefore, multiple complementary mechanisms—including altered binding dynamics, enzymatic conversion, and metabolic clearance—may underlie the observed hormonal profile.

Grayanotoxins, the primary bioactive components of mad honey, are known to bind to voltage-gated sodium channels and maintain them in a persistently activated state, thereby increasing neuronal excitability [1]. This effect may influence autonomic and central nervous system activity, including pathways involved in neuroendocrine regulation [5]. Alterations in neuronal signaling could, in turn, modulate hypothalamic or peripheral mechanisms controlling reproductive hormone balance. In addition, enhanced neuronal excitability may indirectly affect testicular steroidogenesis or peripheral androgen metabolism through autonomic or local regulatory pathways. Given that circulating T levels increased without significant changes in LH and FSH, these findings are more consistent with peripheral modulation rather than direct activation of the HPG axis [10]. However, the precise pathways linking grayanotoxin-induced neuronal effects to endocrine outcomes remain to be elucidated.

Honey contains a variety of biologically active components, including flavonoids (such as quercetin and chrysin), phenolic acids (such as caffeic acid), enzymes, and trace elements (including zinc), which may contribute to antioxidant activity and modulation of steroidogenic and endocrine pathways rather than exerting direct aphrodisiac effects [21]. On the other hand, the present findings may also be considered in the context of plant-derived aphrodisiac compounds known to influence reproductive function through modulation of the HPG axis. For example, *Tribulus terrestris* has been associated with increased LH secretion and subsequent stimulation of testicular steroidogenesis, while *Panax ginseng* has been shown to enhance sexual behavior and T production through both central and peripheral mechanisms, including modulation of hypothalamic signaling and nitric oxide pathways [22, 23]. In addition, bioactive phytochemicals such as flavonoids and saponins have been reported to support Leydig cell function and regulate steroidogenic enzyme activity, thereby contributing to increased androgen production.

However, in contrast to these compounds, which often act through direct endocrine modulation of the HPG axis, the effects observed in the present study appear to occur independently of gonadotropin activation. This distinction further supports the hypothesis that grayanotoxin-containing mad honey exerts its androgen-modulating effects primarily through peripheral or neurophysiological mechanisms rather than classical endocrine pathways.

An additional key finding is the significant increase in serum 5αR levels, particularly in the chronic MH group, indicating an upregulation of the enzymatic pathway responsible for converting T to its more potent metabolite, DHT. This pattern suggests that chronic MH exposure may influence androgen-related pathways; however, without free T, DHT, SHBG, or direct steroidogenic pathway measurements, it cannot be concluded that androgenic signaling was functionally enhanced. Therefore, the increase in serum 5αR should be interpreted as a biochemical association rather than direct evidence of increased DHT production or functional androgenic activity. The combination of elevated T and 5αR—occurring independently of gonadotropin changes—aligns with a peripheral, HPG-independent mechanism of androgen modulation. Such a mechanism may help explain the observed modulation of sexual behavior parameters, although these changes do not necessarily reflect improved performance, as both T and DHT are critical regulators of male sexual behavior. To our knowledge, this study is among the first to suggest that MH may modulate serum 5αR levels, highlighting a potential novel pathway through which this traditional substance influences androgen metabolism and sexual function. Further studies are warranted to elucidate how GTX compounds interact with Leydig cell signaling, steroidogenic enzyme expression, and androgen clearance pathways.

Our behavioral assessments indicated limited but specific changes in sexual behavior parameters following MH administration, with statistically significant differences observed primarily in selected measures such as PEI and SBI; however, these changes were not consistently aligned with classical indices of improved sexual performance. The chronic treatment group did not demonstrate consistent changes across most behavioral parameters; however, selected indices such as SBI differed significantly compared to controls. These behavioral findings do not uniformly indicate improved sexual performance; rather, they suggest a complex and time-dependent modulation of sexual behavior. In established rodent sexual behavior paradigms, increased latency measures and prolonged post-ejaculatory interval are generally interpreted as indicators of delayed or altered performance rather than enhancement. Accordingly,

the present findings should be interpreted with caution and do not provide consistent support for a global aphrodisiac effect. The relationship between T levels and male sexual behavior is well-established, with androgens playing crucial roles in the expression of sexual motivation and copulatory behavior [24].

The comparison between MH and PH groups provides important context for our findings. PH, used as an active control, indicated minimal effects on hormonal parameters and sexual behavior, suggesting that the observed endocrine and behavioral effects are more closely related to MH's unique composition than to general honey properties. This specificity likely relates to the presence of grayanotoxins and other rhododendron-derived compounds in MH, which are absent in PH [25].

Furthermore, the findings reveal distinct neuroendocrine and behavioral profiles between acute and chronic MH administration. While acute MH exposure significantly increased serum T and prolonged ejaculation latency—potentially through grayanotoxin-mediated voltage-gated sodium channel activation and enhanced sympathetic activity [26–28]—chronic administration produced sustained elevations in both serum T and 5αR levels, accompanied by significantly prolonged post-ejaculatory intervals. However, these acute–chronic comparisons should be interpreted with caution, as they were based on exploratory post-hoc analyses rather than pre-specified statistical contrasts. Notably, despite elevated circulating total T, sexual activation parameters were impaired rather than enhanced in chronically treated animals, suggesting a dissociation between total androgen levels and functional sexual performance. This behavioral–hormonal mismatch may indicate that increased circulating total T does not necessarily translate into enhanced bioactive androgen signaling or improved copulatory function. Several explanations may account for this dissociation, including altered androgen receptor sensitivity, changes in androgen binding or metabolism, and central neurophysiological effects of grayanotoxins. Given that grayanotoxins act on voltage-gated sodium channels and may alter neuronal excitability, chronic exposure could affect hypothalamic or autonomic circuits involved in sexual motivation, arousal, and copulatory recovery. Therefore, the observed behavioral profile may reflect a complex interaction between peripheral endocrine modulation and central neurophysiological effects rather than a simple androgen-driven enhancement of sexual behavior.

In addition, the flavonoid and phenolic content of MH may modulate aromatase and 5αR activity [29–31], altering the critical balance between T, DHT, and estradiol that regulates sexual motivation and performance [32–34]; second, elevated total T may reflect increased SHBG levels [21], thereby reducing bioavailable free T despite higher total concentrations; and third, chronic grayanotoxin exposure may disrupt glutamatergic and GABAergic signaling within hypothalamic sexual circuits [32, 33], impairing arousal and copulatory recovery independent of peripheral androgen status. The absence of significant FSH and LH changes further suggests that central neuroendocrine modulation, rather than pituitary axis suppression, underlies these behavioral effects [24, 35]. Collectively, these results indicate that MH exerts dose- and duration-dependent effects on male reproductive physiology, which may account for the variable traditional claims regarding its aphrodisiac properties.

Despite the observed androgen-modulating effects, the translational relevance of mad honey is limited by the well-established toxicity of grayanotoxins. Mad honey intoxication is known to cause significant cardiovascular effects, including bradycardia, hypotension, and conduction disturbances, which may be life-threatening at higher doses. Importantly, these effects are strongly dose-dependent, and even small variations in grayanotoxin exposure may lead to markedly different physiological outcomes. Therefore, careful dose standardization and controlled administration are essential when evaluating the biological effects of mad honey. Accordingly, the present findings should not be interpreted as supporting the direct clinical use of mad honey for sexual dysfunction.

Recent clinical evidence further underscores the variable and dose-dependent toxicological profile of mad honey. In a recent case series from Nepal, Koirala et al. reported that wild (mad) honey ingestion produced a broad clinical spectrum ranging from mild pruritus and dizziness to syncope, hypotension, bradycardia, and even airway compromise, with some patients requiring intensive monitoring and supportive treatment [36]. These findings are consistent with other recent clinical reports from Nepal showing that even relatively small, ingested amounts may precipitate marked cardiovascular manifestations, including hypotension and bradycardia. Collectively, these observations highlight the narrow margin between potential pharmacological and toxic effects of grayanotoxin-containing honey and reinforce the importance of precise dose standardization, controlled administration, and careful safety evaluation in any future translational or pharmacological investigation of mad honey-derived compounds [19, 36, 37].

Instead, our results should be considered within a mechanistic and hypothesis-generating framework. The observed modulation of T and 5αR suggests that grayanotoxin-containing compounds may influence androgen physiology through peripheral pathways. This raises the possibility that specific bioactive components, once identified and pharmacologically refined, could serve as templates for the development of safer therapeutic agents. Future studies should therefore focus on dose–response relationships, long-term

safety profiling, and the isolation of active compounds responsible for the observed endocrine effects. In addition, investigation of molecular pathways involved in androgen regulation and neuroendocrine signaling would provide deeper mechanistic insight into the effects of grayanotoxin-containing compounds. Furthermore, structure–activity relationship studies and the development of less toxic analogues may help to translate these findings into clinically relevant applications [21]. The dose used in this study (80 mg/kg) was selected based on previous experimental studies investigating honey-derived bioactive compounds and was intended to represent a sub-toxic exposure level capable of eliciting measurable physiological effects without inducing overt toxicity. This dosing approach allows separation of pharmacological modulation from toxicological effects. The estimated GTX exposure in this study (approximately 0.96 mg/kg) provides a reference point for future dose–response investigations and facilitates comparison with toxicological and pharmacological studies.

4.1 Limitations

Several limitations should be considered when interpreting these findings. First, the study was conducted in healthy, sexually active rats, and the effects in animals with sexual dysfunction or hypogonadism remain unknown. In addition, the translation of effective doses from rats to humans requires careful consideration of pharmacokinetic differences between species.

A key limitation of the present study is that androgen assessment was limited to total T levels. Free T, DHT, and SHBG were not measured, preventing a comprehensive evaluation of androgen bioavailability and metabolism. Previous work by Tatlı O et al. has indicated that MH increases both free and total T, suggesting that unmeasured androgen fractions may also have been affected [10]. Because the biological activity of androgens depends largely on their free fraction, future studies should include these parameters to better characterize the hormonal milieu. Consequently, the mechanisms underlying the observed increase in circulating T may not be fully resolved, and alternative explanations such as altered binding capacity, enzymatic conversion, or metabolic clearance should be considered. Furthermore, no molecular or histological analyses of reproductive tissues were performed, which limits mechanistic interpretation at the cellular level.

Second, the evaluation of FSH and LH in testicular homogenates may not accurately reflect their dynamic, pulsatile secretion patterns; serum measurements at multiple time points would provide a more precise assessment.

Third, variations in sexual experience and age among animals may influence behavioral performance; although animals were standardized, subtle differences in baseline motivation could not be fully controlled.

A further limitation of the present study is the relatively small sample size ($n=6$ per group), which was determined in accordance with commonly used group sizes in similar experimental models and ethical considerations to minimize animal use. Although several statistically significant differences were detected, the limited sample size may reduce sensitivity for identifying more subtle behavioral and hormonal effects. In addition, the loss of one animal in the chronic MH group (final $n=5$) may have influenced group comparisons. Therefore, the findings should be interpreted with caution, and future studies with larger cohorts and formal power calculations are warranted.

Another important limitation is the inherent toxicity of mad honey due to grayanotoxin content, which restricts its direct translational applicability. Although the total grayanotoxin (GTX) level in the honey was measured, detailed characterization of individual GTX isoforms was not performed, which may limit precise interpretation of toxin-specific effects. The cardiovascular risks associated with grayanotoxin exposure should be carefully considered, and thus, the present findings are better interpreted as mechanistic insights rather than evidence supporting therapeutic use.

Finally, although no overt treatment-related adverse effects were systematically observed during the 30-day treatment period, the optimal dosing regimen and long-term safety profile of MH remain to be established, particularly given the known toxicity of grayanotoxins at higher concentrations. In addition, the loss of one animal in the chronic MH group warrants cautious interpretation in terms of safety.

5 Conclusions

The present study shows that MH administration was associated with increased circulating total T levels and selective, parameter-specific changes in sexual behavior in male rats; however, these behavioral findings do not support a consistent improvement in sexual performance, and the biological significance of the hormonal changes remains uncertain. These findings provide partial mechanistic context for the traditional association of MH with male sexual function and may help guide future mechanistic studies on androgen-related and neurobehavioral pathways. However, further research is needed to fully elucidate the mechanisms of action, optimize dosing regimens, establish safety profiles prior to clinical application, and clarify the long-term neuroendocrine and behavioral consequences of MH use.

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Author contributions KFG and AG conceptualized and designed the study. ZS, OB, NUE and SD performed the experiments and collected the data. KFG, TG, NUE, and SP contributed to data analysis and interpretation. OT contributed to methodological support and experimental procedures. AG supervised the study and AKS contributed to the interpretation of the findings, prepared all figures and tables. KFG, AKS and AG drafted the manuscript. All authors critically revised the manuscript and approved the final version.

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Data Availability The data that support the findings of this study are available from the corresponding author, AKS, upon reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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