ORIGINAL ARTICLE

# Protective Effects of Tualang Honey on the Testes of Rats Subjected to Chronic Combined Stress

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## ABSTRACT

**Introduction:** Persistent exposure to stress can lead to a wide range of pathological effects, including reproductive dysfunctions. Tualang honey, renowned for its antioxidant properties has been utilised in traditional and modern medicine. This study aims to investigate the protective effects of Tualang honey against stress-induced testicular damage in male rats. **Method:** Twenty-four male rats were divided into control group, stress-exposed group, Tualang-honey-supplemented group and stress-exposed with Tualang-honey-supplemented group. Restraint stress test (RST) and Forced swimming test (FST) were imposed on the rats for 21 days. Serum testosterone and corticosterone concentration were measured using ELISA. Testes were harvested, weighed, processed and stained for spermatid counts and measuring seminiferous epithelial height and diameter. One-way analysis of variance (ANOVA) was used to compare the numerical data between groups. **Results:** The testosterone concentration, spermatid count, seminiferous epithelial height and diameter in stress group were significantly lower compared to control group ($p < 0.05$). Corticosterone concentration was significantly greater in the stress group than in the control group. The spermatid count and seminiferous epithelial height and diameter of honey-treated groups were significantly higher than the stress-only group ($p < 0.05$). The corticosterone concentration of honey-treated groups was significantly higher compared to the stress-only group. Meanwhile, the testosterone concentration of honey-treated groups was higher than the stress-only group although the results were insignificant. **Conclusion:** Tualang honey has the potential to ameliorate corticosterone concentration and induce morphological alterations by increasing spermatid counts and seminiferous epithelial thickness and diameter in rat testes exposed to prolonged stress.
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**Keywords:** Chronic stress; Stress; Tualang honey; Reproductive; Neuroendocrine

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## INTRODUCTION

Stress, a normal component of human life, can be defined as a non-specific response of the body to any demand that threatens haemostasis which requires and necessitates an adjustment or response to maintain equilibrium. Stress can influence any system in the human body, including the nervous system, musculoskeletal, respiratory, cardiovascular, gastrointestinal, reproductive, endocrine, metabolic, and immune systems (1). Chronic stress is recognised to be a risk factor for reproductive function and a contributing factor to the rising rate of infertility in the population. Infertility has been recognized as a public health problem in the past decades, with approximately ten to fifteen per cent of couples worldwide facing fertility problems and more than half of the cases involved male infertility (2).

In a male, stress can alter specific hormones at different levels in both hypothalamic-pituitary-gonadal (HPG) and hypothalamic-pituitary-adrenal (HPA) axes, especially the glucocorticoids (GC) and gonadal hormones. Stress-induced suppression of the HPG axis results in reduced testosterone production, which may consequently cause significant changes in testicular morphology and functions (3). Studies have shown that chronic stress causes structural and functional changes in the testes (4). Chronic stress exposure has been documented to impair sperm production, sperm quality and decreased serum testosterone levels in rats. In addition, stress-exposed rats have been shown to experience a reduction in testicular weight and several histological alterations. Studies also have reported that a high corticosterone level and low serum testosterone levels can lead to impaired spermatogenesis, arrestment of germ cells lineage and decrease in spermatid count, sperm count and motility

of spermatozoa in stress-induced rat models (5-7).
Tualang honey, one of the most popular nature-based products in Malaysia, is renowned for its medicinal properties and therapeutic range. It is produced by the Apis dorsata species of Asian rock bees, whose colonies are mostly located in the tropical jungle of northern Peninsular Malaysia. Kishore et al. (2011) reported that Tualang honey possessed a greater antioxidant content and superior radical scavenging action than other local honey (8). Tualang honey has been demonstrated to lower oxidative stress markers due to its high phenolic acid and flavonoid content. Other researchers have also shown that honey supplementation is capable of minimising the effects of chronic stress on the reproductive system and its implications on male fertility (9).

A previous study found that Tualang honey enhanced blood testosterone levels in male rats by reducing oxidative damage to Leydig cells and increasing their capacity to produce testosterone (10). According to a study conducted by Mohamed et al. (2011), a daily intake of 1.2 g/kg of Tualang honey increases the number of Leydig cells, the diameter of seminiferous tubules and epithelial heights in male rats' testes (11). The effect of Tualang honey is reported to be localised at the testicular level, as evidenced by reduced serum testosterone concentration without affecting the pituitary hormones such as the Luteinising hormone (LH) and Follicular-stimulating hormone (FSH) (11). Honey supplementation during prenatal restraint stress exposure has significantly increased testis and epididymis weights and improved the percentages of abnormal spermatozoa and sperm motility in male rat offspring (12). Another effect of honey is proven to increase the survivability and quality of semen for preservation (9). We are conducting this study to investigate the potential protective effects of Tualang honey supplementation on testes following chronic stress exposure in male rat models. It is hoped that the findings attained from this research will extend the knowledge of the consequences of stress and how Tualang honey may benefit and protect the male reproductive system.

## MATERIALS AND METHODS

### Animals

This study was authorised by the Animal Ethics Committee of Health Campus, Universiti Sains Malaysia (AECUSM) [USM/Animal Ethics Approval/2017/ (105) (846)]. This study's experimental methods were conducted in accordance with the committee approval and the Animal Research: Reporting in Vivo Experiment (ARRIVE) guidelines for reporting animal research. Appropriate decisions were taken to minimise the number of animals utilised. This was crucial for producing trustworthy data and reducing animal suffering.

Six- to seven-week-old male Sprague-Dawley rats were obtained from the Animal Research and Service Centre (ARASC) at Universiti Sains Malaysia. The animals were housed in pairs in cages made of polypropylene with bedding. They were kept in a typical animal room temperature (20 – 24 °C) with adequate ventilation and a twelve-hour light/dark photoperiod. Except for during the experimental period, the animals were provided with free access to standard food pellets and water. Before the experiment was commenced, the rats were acclimatised to their housing space and the researchers for a week. Their body weights were monitored, and the cage hygiene were maintained every other day to ensure their health.

### Experimental design

Twenty-four (N = 24) male Sprague-Dawley rats were randomly divided into four groups. Control group (C) – The rats were not subjected to any stress procedures or honey supplementation. They remained in their cages with minimum daily handling to minimise indirect stress exposure. Honey-supplemented group (H) – The rats received 1.0 g/kg body weight of Tualang honey twice daily for twenty-one days without being subjected to any stressful treatments. Stress-exposed group (S) – The rats were exposed to stress procedures for twenty-one days without honey supplementation. Stress-exposed with honey-supplementation group (HS) – The rats were given 1.0 g/kg body weight of Tualang honey twice daily and subjected to stress procedures for twenty-one days.

The rats in H and HS groups were given Tualang honey by oral gavage twice daily. The first gavaging process was performed at 8 a.m. before commencing the stress procedure. The subsequent gavaging process was performed at 3 p.m., after completing the stress protocol. Each gavaging session took about 30 to 45 minutes to complete. The rats in the C and S groups were given an equal volume of normal saline to ensure both groups experienced a comparable amount of stress during feeding. Tualang honey (AgroMas) was obtained from the Federal Agricultural Marketing Authority (FAMA), Kelantan, Malaysia. The manufacturer subjected the honey to several procedures, including filtration, concentration, evaporation, irradiation, and sterilisation. The honey's final concentration was 1.3 g/mL.

### Stress procedure

In this research, a combinatory stress model consisting of the restraint stress test (RST) and the forced swimming test (FST) was applied based on previous studies (7, 13). All animals in the S and HS groups were subjected to the combination of the two stress models in a random sequence daily to prevent habituation (14, 15). In the RST procedure, the animals were immobilised for five hours daily for twenty-one days in an open-ended, flexible plastic mesh with metal clippers at both ends (5). The rats were placed in pairs in clean cages with bedding. The restrainer was modified to fit the animals without jeopardizing their respiration or causing them

discomfort. For the FST procedure, animals were forced to swim in a plastic container filled with water at room temperature for fifteen minutes daily for twenty-one days under continual observation (14). The water level was kept to a depth surpassing their body length to ensure that neither their feet nor tails could reach the bottom of the container. During the post-stress test, they were dried immediately and returned to their cages.

**Tissue preparation**

Decapitation was performed the following day after the end of treatment (Day 22). Blood was taken in 20ml polypropylene centrifuge tubes devoid of anticoagulant. The samples were allowed to coagulate at ambient temperature (37 °C). The obtained sera were aliquoted and frozen at -80 °C until assayed for biochemical study. Bilateral testicles were dissected and separated from the epididymis. Next, the adhering connective tissues were removed, and the testes were individually weighed. Then, after the testes were transversely sectioned at the rete testis, they were fixed in a ten percent formalin solution (16).

**Assay of corticosterone and testosterone level**

The serum corticosterone and testosterone concentrations were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience Biotechnology Co., Ltd., Houston, Texas, USA) in accordance with the manufacturer's instructions. The concentrations of corticosterone and testosterone were expressed as ng/mL.

**Histological assessment of testis**

The collected testes were submerged in a ten percent formalin fixative solution. Each testis was cut, trimmed and put into a cassette, followed by dehydration and embedded in paraffin. Testicular tissue was serially sectioned at 4µm thickness using a Leica auto-cut multipurpose microtome. Adopting the approach of systematic uniform random sampling (SURS), one segment was sampled at 40µm intervals (every ten sections) with a random beginning between section one and section ten (17). Each selected segment was maintained at a constant interval. The specimens were pre-cut in a transverse segment nearest to the rete testes to produce the optimal tissue orientation with a large number of rounded seminiferous tubules (16, 18). The sections were then stained with hematoxylin and eosin (H&E) staining. The seminiferous epithelium was studied on the slides to determine the height and spermatid (elongated) distributions.

For the determination of the spermatid count, the grid-superimposed technique was conducted by using Cellsens image analyser software (Olympus, Japan). Three areas measuring 100µm x 100µm were taken from the grid by systematic random selection from each slide. The grids containing late-type seminiferous tubules with at least one visible spermatid were filled with more epithelial tissue than empty spaces and randomly selected under 40x magnification. Then, the spermatids were counted manually in each selected grid using the image analyser software. The height of the seminiferous epithelium was measured at the intersections of each quadrant (90°, 180°, 270° and 360°) from the tubular basement membrane to the most apical portion of epithelium visible near the lumen by drawing two temporary perpendicular lines crossing the centre of the seminiferous tubule (19). The diameter, on the other hand, was selectively measured at the most rounded tube to ensure a constant measurement.

**Statistical analysis**

The data were gathered and then analysed using version twenty-six of Statistical Package for the Social Sciences (SPSS) (IBM Corp., Armonk. NY, USA). The data obtained were subjected to normality and homogeneity of variance analysis using Levene's test. One-way ANOVA was used to analyse descriptive data with a normal distribution and equal variance, followed by Tukey's post-hoc test, to determine the mean differences in all parameters between the study groups. The data was reported as means ± standard error of the means (SEM). A p-value $< 0.05$ was regarded as statistically significant for all analyses.

## RESULTS

**Body weight changes**

Figure 1 shows the data on body weight changes in the rats. The result revealed that the body weights of the rats in the S group (125.0 ± 5.5 g) were significantly lower than those of the rats in the C group (174.6 ± 7.0 g). The measurement of the rats' body weight in the S group demonstrated that sufficient stress was successfully applied throughout the duration of the experiment to induce weight changes. The results also showed that the body weights of the rats in the HS group (152.7 ± 7.3 g) were significantly higher than those of the rats in the S group.

**Testicular weight changes**

Figure 2 shows the data on testicular weight changes in the rats. Statistical analysis revealed that the testicular weights of the rats in the S group (1.17 ± 0.07 g) were significantly lighter than those of the rats in the C group (1.41 ± 0.05 g). This result was further evidence that the stress level administered to the rats during the experiment was highly successful in producing desired stress effects on the rats. The testicular weights of the rats in the HS group (1.31 ± 0.06 g) were heavier than the rats in the S group; however, the difference was insignificant.

**Serum corticosterone concentration**

Figure 3 illustrates the data on corticosterone concentrations. Statistical analysis revealed that the serum corticosterone levels of the rats in the S group (35.21 ± 1.51 ng/ml) were significantly higher than those

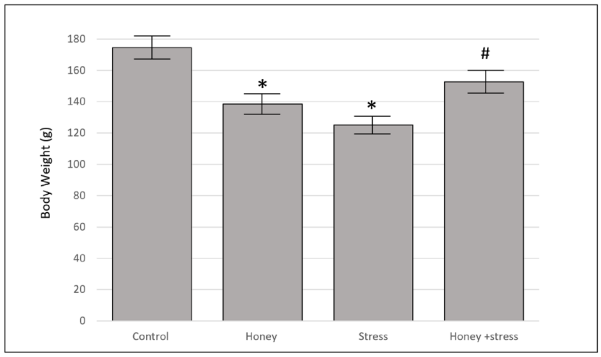


**Figure 1: Total body weight (g) of the rats in control (C), honey supplemented control (H), stress (S) and honey supplemented stress (HS) groups.** Data are presented as means ± standard error of the means. Significant differences determined by one-way ANOVA followed by Tukey's test with $p < 0.05$. * $p < 0.05$ in comparison with C group. # $p < 0.05$ compared to the S group.

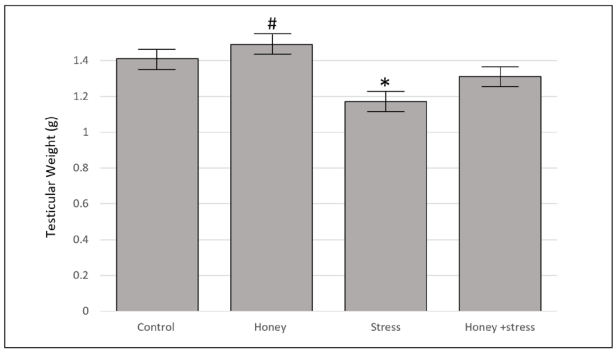


**Figure 2: Testicular weight (g) of the rats in control (C), honey supplemented control (H), stress (S) and honey supplemented stress (HS) groups.** Data are presented as means ± standard error of the means. Significant differences determined by one-way ANOVA followed by post-hoc Tukey's test with $p < 0.05$. * $p < 0.05$ in comparison with C group. # $p < 0.05$ compared to the S group.

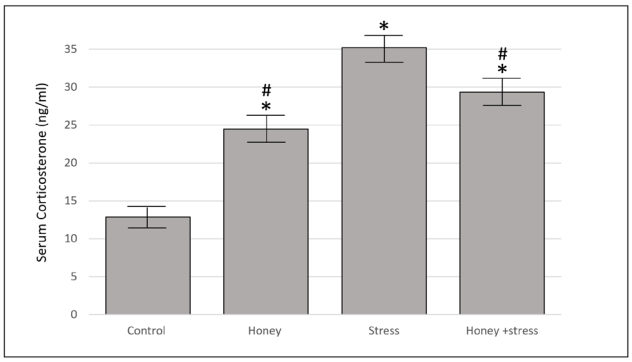


**Figure 3: Serum corticosterone concentration (ng/ml) in control (C), honey supplemented (H), stress (S) and honey supplemented stress (HS) groups.** Data are displayed as means ± standard error of the means (n = 6 per group). Data were analysed by one-way ANOVA followed by post-hoc Tukey's test. * $p < 0.05$ in comparison with control group. # $p < 0.05$ compared to the S group.

in the C group (12.86 ± 1.05 ng/ml). This discrepancy reflects that the intensity of stress applied was effective in inducing the changes in serum corticosterone levels. The serum corticosterone concentrations were profoundly lower in the rats in the HS group (29.33 ± 1.54 ng/ml) and H group (24.48 ± 1.69 ng/ml) compared to the rats in the S group.

**Serum testosterone concentration**

Figure 4 illustrates the data on testosterone concentrations. The results revealed that the blood testosterone levels of the rats in the S group (4.8 ± 1.7 ng/ml) were significantly lower than those in the C group (13.2 ± 1.9 ng/ml). These results imply that the rats were subjected to an adequate quantity of stress throughout the duration of the trial. The testosterone concentrations in the HS group (6.2 ± 1.4 ng/ml) were higher than the S group. However, the difference was insignificant.

**Quantitative analysis of spermatid counts**

Figure 5 represents the data on spermatid counts in the seminiferous tubules. The result demonstrated that the spermatid counts in the rats in the S group (39.6 ± 1.12 µm) were significantly lower compared to those in the C group (45.3 ± 1.08 µm). The results also showed that the spermatid counts in the H (47.3 ± 1.64 µm) and HS groups (44.2 ± 1.07 µm) were significantly higher than those in the S group with a p-values of < 0.05.

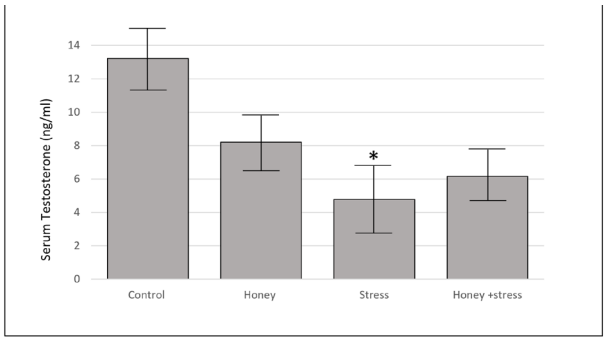


**Figure 4: Serum testosterone concentration (ng/ml) in control (C), honey supplemented (H), stress (S) and honey supplemented stress (HS) groups.** Data are displayed as means ± standard error of the means (n = 6 per group). Data were analysed by one-way ANOVA followed by post-hoc Tukey's test. * $p < 0.05$ in comparison with control group.

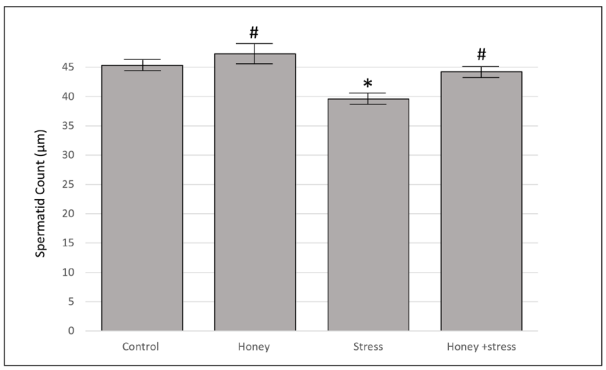


**Figure 5: Spermatid counts (µm) of rat seminiferous tubules in control (C), honey supplemented (H), stress (S) and honey supplemented stress (HS) groups.** Data are presented as means ± standard error of the means (n = 6 per group). Significant differences determined by one-way ANOVA followed by post-hoc Tukey's test with $p < 0.05$. * $p < 0.05$ in comparison with C group. # $p < 0.05$ compared to the S group.

**Quantitative analysis of seminiferous epithelial height**

Figure 6 shows the data on the seminiferous epithelial height. The result revealed that the epithelial heights in the S group (40.98 ± 0.52 µm) were significantly lower than the C group (46.82 ± 2.03 µm). The results also showed that the epithelial heights of seminiferous tubules in the H group (49.50 ± 1.21 µm) and HS group (46.52 ± 1.31 µm) were significantly higher than those in the S group.

**Quantitative analysis of seminiferous tubules diameter**

Figure 7 shows the data on the diameters of the seminiferous tubules. Statistical analysis revealed that the diameters in the S group (200.55 ± 7.35 µm) were significantly narrower than those in the C group (230.92 ± 3.64 µm). The results also showed that the diameters of the seminiferous tubules in the HS group (247.51 ± 5.10 µm) were significantly wider than those of the rats in the S group.

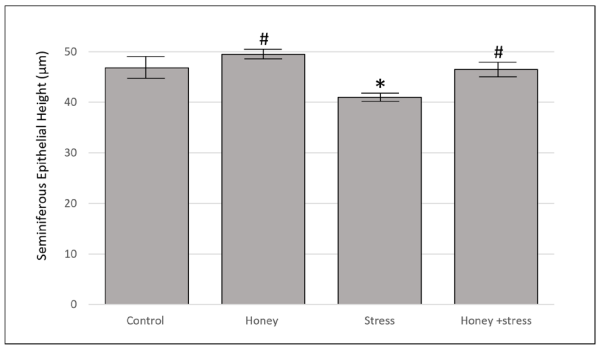


**Figure 6: Epithelial heights (µm) of the rats' seminiferous tubules in control (C), honey supplemented control (H), stress (S) and honey supplemented stress (HS) groups.** Data are presented as means ± standard error of the means. Significant differences determined by one-way ANOVA followed by post-hoc Tukey's test with $p < 0.05$. * $p < 0.05$ in comparison with C group. # $p < 0.05$ compared to the S group.

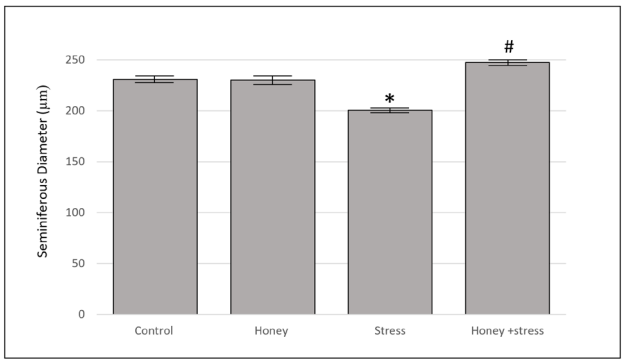


**Figure 7: Diameter (µm) of the rats' seminiferous tubules in control (C), honey supplemented control (H), stress (S) and honey supplemented stress (HS) groups.** Data are presented as means ± standard error of the means. Significant differences determined by one-way ANOVA followed by post-hoc Tukey's test with $p < 0.05$. * $p < 0.05$ in comparison with C group. # $p < 0.05$ compared to the S group.

## DISCUSSION

In stress, gonadotropin-releasing hormone (GnRH) in the HPG axis stimulates gonadotroph cells in the anterior pituitary to produce and release luteinizing hormone (LH) and follicular stimulating hormone (FSH). These hormones act on the testis to regulate and maintain testosterone production and spermatogenesis (20). LH stimulates the receptors of Leydig cells in the interstitial tissue for the synthesis and production of testosterone (21). In the present study, the serum testosterone concentrations in the stress group were significantly lower than in the control group, which might suggest that combined stress tests decreased the testosterone level in the rat models. This finding is consistent with previous studies done by Son et al. (2016) and Rai et al. (2004), showing that the serum testosterone levels decreased in the subjects imposed with chronic restraint stress than in the control rats (22, 23). Arun et al. (2016) and Al-Damegh (2014) also published that chronic restraint stress significantly reduced serum testosterone concentration in rats (6, 24).

On the other hand, the present study showed an increasing trend in the testosterone level following Tualang honey supplementation despite insignificant results. This result corresponds to a previous study which showed a similar pattern of changes in the testosterone level following chronic exposure to cigarette smoke (11). The insignificant effect of Tualang honey on the testosterone level in the present study might be due to the high level of stress inflicted on the rats, as the present study utilised two different types of stress, whereas other studies only typically imposed a single type of stress. A study reported that rats exposed to a chronic combinatory stress protocol (thirty minutes of RS and fifteen minutes of FST daily) showed elevation in corticosterone approximately 4.1 times higher than normal as early as the seventh day of the experiment and reached 6.8 folds on day twenty-eight, suggesting a constant rise in the hormone level (14). Comparably, a study done by imposing the rats with a single RS protocol (5 hours daily) reported 1.2 fold-rise in the corticosterone level on the fourteenth day of the experiment (23). This suggests that the chronic combinatory stress protocol used in the present study possibly had a high impact that inhibited the production of testosterone despite Tualang honey supplementation.

There is a reciprocal association between HPA and HPG whereby the activation of the stress axis causes suppression of testosterone production, while testosterone influences the response of the HPA axis (25). Chronic stress has a more pronounced effect on testosterone levels. The level of testosterone influences the higher part of the axis through its negative feedback mechanism on the hypothalamic neurons

and gonadotrophs in the anterior pituitary (3). Chronic stress can lead to dysregulation of the HPA axis, which increases the release of corticosterone. Dysregulation of the HPA axis can disrupt the normal production and regulation of testosterone, leading to a decrease in the testosterone level. Testosterone has been shown to have inhibitory effects on the HPA axis, reducing the release of corticosterone. In this study, the concentration of serum corticosterone was found to be higher in the stress group than in the control group. This might suggest that a combined stress test lowers testosterone and elevates the corticosterone level in rat models, demonstrating their reciprocity. Moreover, some studies also demonstrated that testosterone could reduce glucocorticoids and adrenocorticotropic hormones in response to chronic stress exposure (26, 27).

Studies showed that an increase in the corticosterone level and a decrease in the serum testosterone level caused impaired spermatogenesis (5-7). An increase in the level of corticosterone in reaction to stress contributes to the development of stress-related injury to the internal structure of the testis, which influences spermatogenesis, consequently affecting the spermatid count, Leydig cell count and epithelial height. These changes have been reported to be associated with abnormal reproductive function and infertility (7, 11, 28). Therefore, the number of spermatids is one of the indicators used to determine the effect of stress on the reproductive parameter in males. In the present study, it was found that the spermatid count in the testes of the rats exposed to the chronic combinatory stress protocol was significantly lower than those in the control group. This result showed that adequate stressor was given to the experimental rats. It is expected for the spermatid count to be reduced following exposure to chronic stress in keeping with some of the previous studies (28-30). Zou et al. (2019) suggested that two molecular factors, the arrestment of the cell cycle, particularly at the spermatogonia level and spermatids apoptosis, may contribute to spermatogenesis impairment in rats subjected to stress (30). It was stated that reduced differentiation of spermatogenic cells may lead to a decrease in the number of spermatids and mature sperms. In addition, the study reported that most of the stress-induced apoptotic cells were spermatids, which results in the reduction of spermatid count and sperm count in testicular seminiferous tubules. Hence, increased arrestment of spermatogonia and spermatid death may result in a reduction of spermatid counts in the seminiferous tubules, which then causes low sperm count. Another theory that might explain the reduction of spermatid count is the activity of the HPA axis.

A study suggested that glucocorticoid-mediated responses may cause negative effects on gonadal activity and fertility (27). Glucocorticoid receptors were detectable in a few testicular cell types, such as Leydig cells, Sertoli cells and the early spermatogenic cell populations (31). Therefore, it can be deduced that an increase in the glucocorticoids level, mainly the corticosterone, might lead to low testosterone production by Leydig cells, followed by inhibition of Sertoli cells activity in regulating spermatogenesis and eventually may result in inefficient spermatogenic cells differentiation to produce spermatids. Intriguingly, the present study found that the number of spermatids in the testes following supplementation of Tualang honey was significantly higher in the honey+stress group when compared to the stress group, suggesting that Tualang honey has a protective effect towards the stress imposed. This finding is in accordance with a previous study by Haron et al. (2015) in which the administration of Tualang honey to rats exposed to restraint stress had significantly improved the spermatid count, the sperm count and the testosterone level (12). In addition, Mohamed and colleagues (2010) suggested that the significance of the result was possibly due to the strong antioxidant properties of Tualang honey which acts locally at the testicular level (11). Thus, it can be postulated that Tualang honey is able to ameliorate the oxidative damage caused by chronic stress on the testicular tissue. More importantly, this condition may restore the viability and function of Leydig cells in producing testosterone.

Seminiferous epithelial height is referred to as the thickness of seminiferous epithelium from the basement membrane to the last epithelium visible near the lumen (19). The seminiferous thickness is one of the indicators to determine the effects of chronic stress on testicular germinal epithelial cells, which include the proliferating spermatogenic cell lineages and supporting non-dividing Sertoli cells. Mustafa et al. (2019) reported that the thickness of seminiferous epithelium is influenced by the changes in the total number of germinal epithelial cells of the seminiferous tubule (32). A decrease of either spermatogenic cells or supporting Sertoli cells may cause a reduction in epithelial height and vice versa. In the present study, the epithelial height and diameter of seminiferous tubules in the rats exposed to chronic stress were found to be significantly lower than those in the control group. Although no similar study has been conducted previously, a study by Mohamed et al. (2011) on cigarette smoke-induced stress demonstrated that stress exposure could cause a reduction in the epithelial height and diameter of seminiferous tubules, as well as the number of Leydig cells and testosterone concentration (11). The epithelial height and diameter are indirectly influenced by the testosterone concentration that is responsible for the regulation of spermatogenesis. Another study proposed that the epithelial height of seminiferous tubules is subjected to the change in the number of germ cells and degeneration of seminiferous epithelium, which were significantly decreased following restraint stress (7, 33, 34), suggesting impaired spermatogenesis. Hence, it can be concluded that the epithelial height and diameter of

seminiferous tubules are determined by the number of germ cells and the effectiveness of spermatogenesis.

In this study, supplementation of Tualang honey improved the epithelial thickness and diameter of the seminiferous tubule of the testes, which was significantly higher in the stress + honey group when compared to the stress-only group. Mohamed et al. (2011) showed a similar pattern of changes in epithelial height and diameter of seminiferous tubules following chronic exposure to cigarette smoke (11). Therefore, based on observations and analyses performed in the four study groups, it was shown that Tualang honey supplementation has a significant effect in improving the epithelial thickness, the diameter of seminiferous tubules and the spermatid count of the rats' testes that were imposed with chronic stress.

## CONCLUSION

In conclusion, our results showed that Tualang honey supplementation is able to improve the stress-induced atrophy of seminiferous tubules of the testis following exposure to chronic stress by improving the height and diameter of seminiferous tubules. In addition, Tualang honey can also improve the spermatid counts in the seminiferous tubules. To the best of our knowledge, this study is the first to show positive potential protective outcomes of Tualang honey in attenuating the effects of chronic combinatory stress on testosterone concentration, spermatid count, epithelial height and diameter of testicular seminiferous tubules in rat models. However, further study is needed to further delineate the result of testosterone.

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