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Research article

Effect of *Aquilaria malaccensis* leaves aqueous extract supplementation on testicular seminiferous tubules and testosterone level in adult male Sprague Dawley rats

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Abstract

Phytochemical screening was performed on *A. malaccensis* leaves to assess their impact on testicular seminiferous tubule diameter (STD) and seminiferous epithelial height (SEH), as well as testosterone level in adult male Sprague Dawley rats. The study involved 24 male rats, divided into four groups: Control (C: 0 g *A. malaccensis*/kg body weight, n=6), Treatment 1 (T1: 1 g *A. malaccensis*/kg body weight, n=6), Treatment 2 (T2: 2 g *A. malaccensis*/kg body weight, n=6), and Treatment 3 (T3: 3 g *A. malaccensis*/kg body weight, n=6), respectively. The extract of *A. malaccensis* leaves was administered orally once daily for 28 days and the rats were euthanized on Day 29 to assess their reproductive function. Phytochemical screening revealed the presence of various compounds in *A. malaccensis* leaves, including tannins, saponins, glycosides, flavonoids, terpenoids, steroids, and alkaloids. No significant differences were found in STD and SEH between the Control and Treated groups. A significant increase in testosterone level was observed in the T1 group (1.18 ng/mL) when compared to the C group. Incremental dosage of *A. malaccensis* appeared to significantly decrease testosterone level (0.92 ng/mL). No correlation was observed between the testicular seminiferous tubules and testosterone level. In conclusion, doses of 1, 2, and 3 g/kg of *A. malaccensis* did not appear to affect the seminiferous tubules of the testis. Specifically, 1 g/kg of *A. malaccensis* demonstrated potential benefits for male reproduction by elevating testosterone levels, whereas 2 and 3 g/kg exhibited potential harm by decreasing testosterone levels in male rats.

Keywords: *Aquilaria malaccensis*, Seminiferous tubules, Testosterone

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INTRODUCTION

In recent years, a global inclination has emerged towards the utilization of herbal plants as complementary medications, not only in developing countries but also in developed nations like Japan, Germany, and Australia (Dehelean et al., 2021). The widespread acceptance of herbal plants can be attributed to the common perception that all plant-based products are safe and harmless due to their natural origins (Wan et al., 2017; Pauzi et al., 2021). For instance, herbs like *Andrographis paniculata*, *Clinacanthus nutans*, *Ficus deltoidea*, and *Nigella sativa* are commonly used as dietary supplements to reduce fever, enhance blood circulation, and support diabetes management (Afzan et al., 2019; Uzbek and Shahidan, 2019). With the growing trend of using herbal remedies as supplements or treatments, which is also evident in Malaysia (Sharifuddin et al., 2018), it is important to acknowledge that men's dietary and nutritional practices can have an impact on their reproductive health. According to Durairajanayagam (2018), men's reproductive health can be significantly affected, either positively or negatively, by their dietary and nutritional habits.

Aquilaria malaccensis, locally known as karas or gaharu, has been used traditionally to treat a variety of ailments, including rheumatism, asthma, body pain, and smallpox (Li et al., 2021). People from different cultures have traditionally utilised *A. malaccensis* for multiple purposes, such as medicinal materials, perfumery, aromatic, and religious preparations (Kharnaior and Thomas, 2021). This plant has gained prominence due to its pharmacological characteristics, offering the potential to address a diverse range of medical conditions such as arthritis, gout, and diabetes (Indrisari et al., 2021). In addition, almost every part of *A. malaccensis*, such as the leaves, seeds, and roots, was considered beneficial in the pharmaceutical industry (Razak et al., 2019a). Studies have highlighted its significant antioxidant, pain-relieving, anti-fever, anti-inflammatory, anti-hyperglycemic, and antimicrobial properties (Hendra et al., 2016; Rashid et al., 2020). Various bioactive compounds, such as flavonoids, tannins, saponins, chromenes, lignans, and diterpenoids, have been identified within it (Mascarenhas et al., 2018; Musa et al., 2019a; Surjanto et al., 2019).

In recent years, there has been a growing interest in herbal remedies, particularly in addressing fertility issues in both men and women. Some examples of common herbal remedies used as supplements or treatments for reproductive health problems in Malaysia are *Eurycoma longifolia*, *Labisia pumila*, *Orthosiphon stamineus* and *Graptophyllum pictum* (Bokhari et al., 2018; Teh et al., 2021). To date, investigations into the impact of *A. malaccensis* on reproductive health remain limited, particularly in both males and females, with a specific focus on fertility, sexual function, and hormonal regulation. As male reproductive health and fertility are globally deteriorating, including within Malaysia (Jegasothy et al., 2020; Mann et al., 2020), it becomes imperative to enhance our comprehension of the influence of *A. malaccensis* on male reproductive health.

Recent studies have indicated that the consumption of *A. malaccensis* extracts at lower doses (≤ 1 g/kg) has shown improvement in sperm quality (Razak et al., 2019a; Zaidi et al., 2023) and enhanced libido (Musa et al., 2019) in male ICR mice and Sprague Dawley rats. However, no research has yet explored the effects on male reproduction when administering higher doses of *A. malaccensis* extracts (> 2 g/kg), especially on testis histology and reproductive hormones. Therefore, phytochemical screening was performed on *A. malaccensis* leaves to determine its effect on testicular seminiferous tubules and testosterone level in adult male Sprague Dawley rats. The findings of this study aim to offer scientific insights into the influence of *A. malaccensis* leaves on male reproduction and determine the optimal dosage for *A. malaccensis* leaves.

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MATERIALS AND METHODS

Plant material

A. malaccensis leaves were collected from Merchang Forest Reserve, Terengganu State Forestry Department, Terengganu, with the assistance of Assistant Field Officers for Aquilaria species identification. The collection of *A. malaccensis* leaves was done in the morning to prevent potential evaporation that could alter their composition. Leaves selection adhered to the guidelines provided by the Forestry Department Peninsular Malaysia (2015). Leaves of various ages, including both young and mature ones, were collected randomly without any specific order or pattern, and carefully packed into plastic bags. They were then transported to the Herbal Processing Laboratory at the Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (UniSZA). After removing 10 kg of fresh leaves from their stalks and washing them, the leaves were air-dried in an empty room (23-26°C) for three consecutive days. These dried leaves were subsequently ground into powder using a waring blender (Waring Commercial, USA) and stored in sealed plastic bags in a fridge at -80°C for future use.

Leaf extract preparation

The leaves extraction was carried out using distilled water as it was a common practice used by people of various cultures (Zulkifle et al., 2018; Anywar et al., 2020). The leaves powder (in grams) was soaked in distilled water (in millilitres) at a ratio of 1:50 for 24 hours (Nadilah et al., 2019). Subsequently, the mixture was subjected to 20 minutes of sonication to agitate the particles within the leaves sample. It was then filtered through Whatman Filter Paper No. 1 to remove the leaves powder. The resulting filtrate was centrifuged at 4000 rpm (25°C) for 20 minutes to collect the supernatant (Rashid et al., 2020). The supernatant was then collected, combined, and concentrated at 40°C by using rotary evaporator (Heidoph, Germany) to isolate the solvent from the crude extract (Samsulrizal et al., 2011). The resulting brown crude extract, with a total yield of 2 kilograms, was stored in universal bottles at 4°C until further use.

Phytochemical screening analysis

The extract of *A. malaccensis* leaves was screened for the presence of various secondary metabolites such as tannins, saponins, glycosides, flavonoids, terpenoids, steroids and alkaloids. The analysis was conducted as per standard methods described by Brain and Turner (1975) and Evans (1966).

Detection of tannins

Tannin was detected by using Braymer's test (Chooranam, 2017). A small quantity of *A. malaccensis* crude extract was dissolved with distilled water and filled into a test tube. Then, two drops of 5% of ferric chloride solution were added into the test tube. The formation of greenish-black precipitate indicated the presence of tannins.

Detection of saponins

Saponin was detected by using froth test (Singh and Kumar, 2017). Distilled water (20 mL) was added into 1 mL of *A. malaccensis* crude extract solution in a test tube. Then, the test tube was shaken vigorously for 15 minutes. The formation of one centimetre layer of foam indicated the presence of saponins.

Detection of glycosides

Glycoside was detected by using aqueous sodium hydroxide test (Jagessar, 2017). One mL of distilled water was added in 0.5 mL of *A. malaccensis* crude extract solution in a test tube. Then, a few drops of aqueous sodium hydroxide was

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added into the test tube. The formation of yellow colouration indicated the presence of glycosides.

Detection of flavonoids

Flavonoid was detected by using concentrated sulphuric acid test (Tyagi, 2017). A few drops of concentrated sulphuric acid were added into 0.5 mL of *A. malaccensis* crude extract solution in a test tube. A rapid development of red colour indicated the presence of flavonoids.

Detection of terpenoids

Terpenoid was detected by using Salkowski's test (Jani et al., 2020). In a test tube containing 2 mL of chloroform, 0.5 mL of *A. malaccensis* crude extract solution was added. Then, 3 mL of concentrated sulphuric acid was carefully added into the test tube to form a layer. An appearance of reddish-brown colour in the inner face indicated the presence of terpenoids (Santhi and Sengottuvel, 2016).

Detection of steroids

Steroid was detected by using steroid test (Shukla and Tyagi, 2017). Five mL of *A. malaccensis* crude extract solution was prepared and transferred to a test tube containing 2 mL of chloroform. Then, 2 mL of concentrated sulphuric acid was carefully added to the test tube. The formation of red ring at upper layer of the test tube and an appearance of yellow with green fluorescence at sulphuric acid layer indicated the presence of steroids.

Detection of alkaloids

Alkaloid was detected by using Dragendroff's test (De-Silva, 2017). Three mL of *A. malaccensis* crude extract solution was prepared and filled into a test tube. Then, a few drops of Dragendorff's reagent were added into the test tube. The formation of reddish-brown precipitate indicated the presence of alkaloids.

Animal preparation

The experiment involved selecting 24 adult male Sprague Dawley rats aged between 8 and 9 weeks, with body weights ranging from 250 to 300 grams. They were acclimatised for a week before starting the treatment. Animals were placed in standard-sized cages (19 x 13.5 x 8 inches) with six rats per cage. They were caged at 20-24°C and maintained under standard laboratory conditions with 50±10% humidity and a cycle of 12-h light and 12-h dark. Standard laboratory rat feed (Gold Coin Feed Mills Sdn Bhd, Malaysia) and water were given at *ad libitum*. The feed was specifically formulated to meet all their nutritional requirements. All ethical themes of animal study were conducted according to the Organisation for Economic Co-operation and Development (OECD, 2018) Test Guideline 407. Experimental procedures were approved by the Universiti Sultan Zainal Abidin Animal and Plant Research Ethics Committee under reference number UAPREC/04/044 (01/04/2019).

Feeding treatment

A total of 24 male rats were randomly divided into four groups, each containing six rats (n=6/group). The control group (C) received a supplementation of 1 mL of distilled water. Meanwhile, the treatment groups were provided with *A. malaccensis* extract at doses of 1 g/kg (T1), 2 g/kg (T2) and 3 g/kg (T3) based on the rats' body weight. The *A. malaccensis* extract was reconstituted in distilled water according to the dosage required for each treatment group. Individual body weights of rats in each group were measured before each feeding to determine the appropriate dose per kilogram of the rats' body weight. The formula used for dose calculation is as follows (Nair and Jacob, 2016):

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$$\text{Dose amount (mL)} = \frac{\text{dose desired } \left(\frac{\text{g}}{\text{kg}}\right) \times \text{animal weight (g)}}{\text{sample concentration } \left(\frac{\text{g}}{\text{mL}}\right)}$$

The *A. malaccensis* extract and distilled water were administered once daily in the morning for a continuous period of 28 days using force-feeding with an oral gavage (18 G, 2.25 mm). After 28 days of treatment, all rats were anaesthetized with 5% diethyl ether (BDH Laboratory supplies, England) and euthanized by cervical dislocation for the assessment on testicular seminiferous tubules and testosterone level (Shrestha et al., 2018).

Blood and serum collection

After the rats were euthanized, they were dissected using forceps and scissors, starting from the lower part of the body and gradually proceeding upwards. Blood samples were collected from each rat through inferior vena cava using a needle with a syringe (5 ml/cc). The drawn blood was placed into blood collection tubes containing clot activator (red caps). After collection, the blood samples were left for 30 minutes to allow complete clotting (Tuck et al., 2009; Rathkolb et al., 2013). Subsequently, they were centrifuged at 2500 rpm with 4°C for 15 minutes to separate the serum from the blood (Zakaria et al., 2023). The serum was then collected and stored at -20°C in a refrigerator until further use.

Histological assessment of seminiferous tubules

Testes from each rat were collected after dissection and immersed in Bouin's solution for 24 hours for preservation (Jenkins and Burg, 2003). Afterwards, the testis samples were cross-sectionally sliced in the middle with a sharp surgical blade and placed inside a histology cassette. The tissues were soaked in 65% ethanol for an hour to remove Bouin's fixative (Slaoui et al., 2017), followed by a series of ethanol concentration steps (80%, 95%, and 100%) for one hour each to dehydrate the tissues. Subsequently, the tissues were placed in xylene for two hours to remove ethanol (Akinloye and Morayo, 2010). Then, they were transferred to warm paraffin for two hours with two changes at one-hour intervals (Hamidian et al., 2018). Tissues were embedded in fresh molten paraffin wax using a paraffin dispenser (Shandon, model Citadel 1000, UK). The paraffin-embedded tissues were cut into 5 µm thick slices using a rotary microtome (Shandon, model AS325, UK) (Khayyat, 2011). These slices were expanded in a water bath at 50°C, mounted on glass slides, and any water droplets were removed by warming the slides at 60°C (Rolls, 2020; Siegfried and Steinfeld, 2021). The sections on the slides were stained using the standard haematoxylin and eosin (H&E) staining procedure (Loha et al., 2019), permanently mounted with Dibutylphthalate Polystyrene Xylene (DPX), and covered with coverslips for protection.

The measurement of STD and SEH was performed using the Las X image analyser (Leica Microsystems, Germany). At a magnification of 400x, the STD was measured at two different sites in 20 tubule cross-sections per slide, and the average diameter was subsequently calculated (McLachlan et al., 2007). The SEH was measured from the basement membrane to the surface of the epithelium at two different regions and expressed as mean of the two measurements (D'Souza, 2004). Histological section of the testis was shown in Figure 1.

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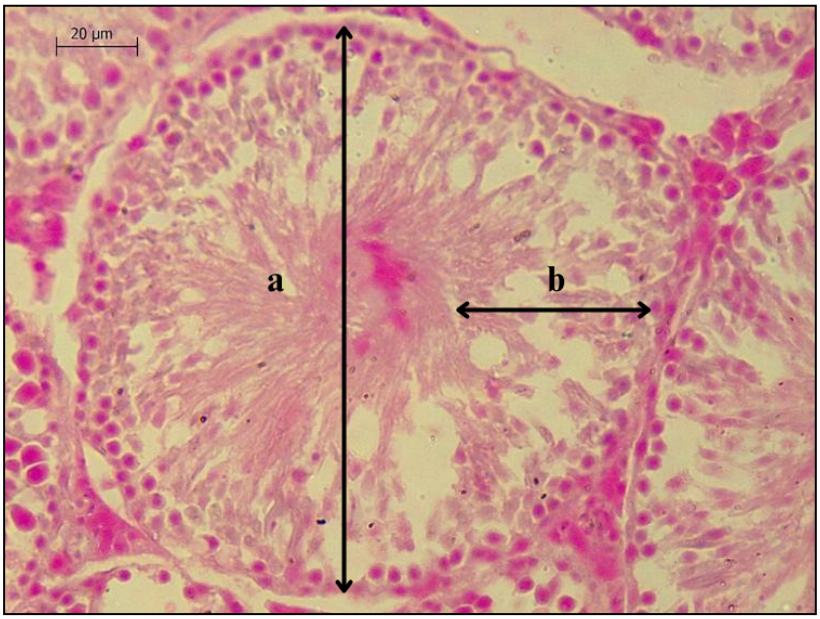

Figure 1 The example of rat testicular histomorphometry: (a) Seminiferous tubular diameter (STD) and (b) Seminiferous epithelial height (SEH), stained with H&E under a light microscope (400x magnification).

Testosterone level assessment

The concentration of testosterone in the serum was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, E-EL-R0155). The assay was conducted following the manufacturer's instructions provided by Elabscience Biotechnology Co. Ltd. (United States). In triplicate, 100 µL of each hormone standard (Testosterone: 0, 0.31, 0.62, 1.25, 2.5, 5, 10, and 20 ng/mL) and serum samples were pipetted into appropriate wells, followed by adding 50 µL of Biotinylated Detection Ab working solution. The plate was covered with a sealer provided in the kit and placed in an incubator for 45 minutes at 37°C. The contents of the wells were then rinsed with 350 µL of wash buffer per well in triplicate. After that, 100 µL of HRP-conjugated working solution was added to each well and incubated for 30 minutes at 37°C. The contents of the wells were then rinsed and drained, and this process was repeated five times. Then, 90 µL of substrate reagent was pipetted into each well of the plate and immediately covered with aluminium foil to protect from the light. The plate was then incubated for 15 minutes again at 37°C in the dark. The enzymatic reaction was stopped by the addition of 50 µL of stop solution to each well and the plate was gently tapped to ensure a thorough mixing. The plate was read at 450 nm using an Ultra Microplate Reader within 15-30 minutes after the addition of stop solution.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 21.0 software. Data distribution and variance were assessed through box-and-whisker plots and Levene's test, respectively. Testicular seminiferous tubules (STD and SEH) and testosterone level were subjected to one-way ANOVA parametric tests, followed by Tukey's post hoc test for pairwise comparisons. Results were presented as mean ± standard error of mean (SEM). The correlation between STD, SEH, and testosterone level was evaluated using Pearson's correlation analysis. A significance level of $p<0.05$ was used to determine statistical significance.

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RESULTS

Table 1 presents the results of phytochemical analysis of *A. malaccensis* leaves, revealing the presence of tannins, saponins, glycosides, flavonoids, terpenoids, steroids, and alkaloids in the *A. malaccensis* crude extract solution. Figure 2 displays the observations from the phytochemical screening conducted on *A. malaccensis* leaves.

Table 1 Results of phytochemical analysis of *Aquilaria malaccensis* leaves aqueous extract.

Test	*Aquilaria malaccensis* Leaves Aqueous Extracts
Tannins	+
Saponins	+
Glycosides	+
Flavonoids	+
Terpenoids	+
Steroids	+
Alkaloids	+

A positive sign (+) indicates the presence of the compound.

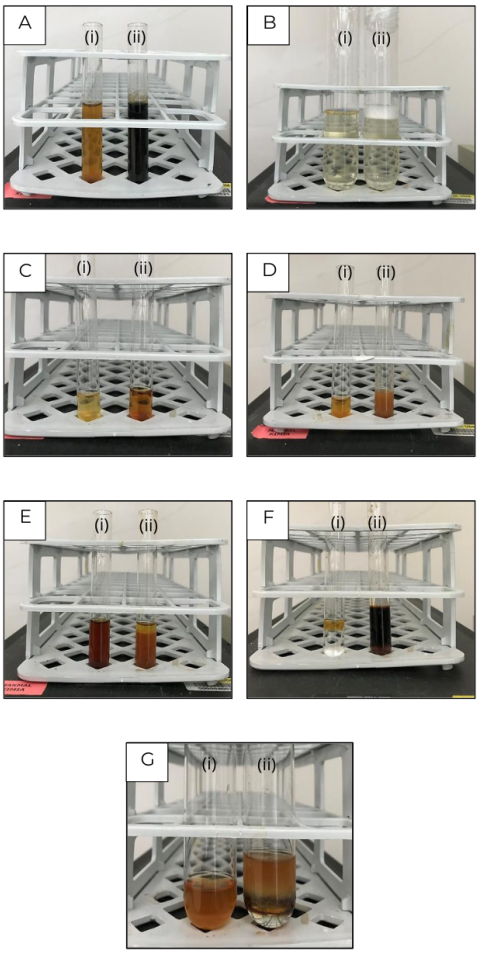

Figure 2 The observation of phytochemicals present in *Aquilaria malaccensis* leaves aqueous extract. A) Tannins, B) Saponins, C) Glycosides, D) Flavonoids, E) Terpenoids, F) Steroids and G) Alkaloids. i) Control, ii) Extract mixed with reagents.

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Figures 3 and 4 depict the mean values of STD and SEH in the Control and Treatment groups of male Sprague Dawley rats. No statistically significant differences (p>0.05) were observed among the experimental groups. Figure 5 illustrates the seminiferous tubule morphology in all groups. The T1 group exhibited normal and well-organized spacing between tubules, in contrast to the C, T2, and T3 groups. The T2 and T3 groups showed seminiferous tubules with less dense packing, featuring spermatogenic cells and wider emptied lumens. In contrast, the C and T1 groups exhibited densely packed tubules with small lumens densely filled with sperm tails. However, histomorphometric analysis of the STD and SEH did not reveal any significant abnormalities in the testicular tissue.

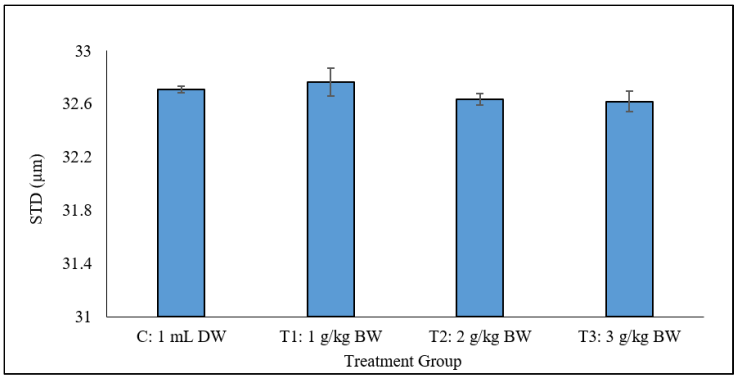

Figure 3 Seminiferous tubular diameter (STD) of testis in Control (C), Treatment 1 (T1), Treatment 2 (T2) and Treatment 3 (T3) groups. Data are presented as mean ± SEM (n = 6/group). No significant differences were found among the groups analysed by using one-way ANOVA test. The g/kg BW = gram of Aquilaria malaccensis extract per kilogram of rats' body weight.

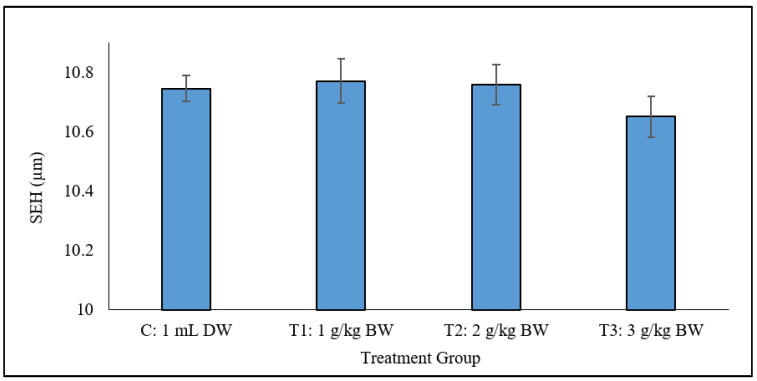

Figure 4 Seminiferous epithelial height (SEH) of testis in Control (C), Treatment 1 (T1), Treatment 2 (T2) and Treatment 3 (T3) groups. Data are presented as mean ± SEM (n = 6/group). No significant differences were found among the groups analysed by using one-way ANOVA test. The g/kg BW = gram of Aquilaria malaccensis extract per kilogram of rats' body weight.

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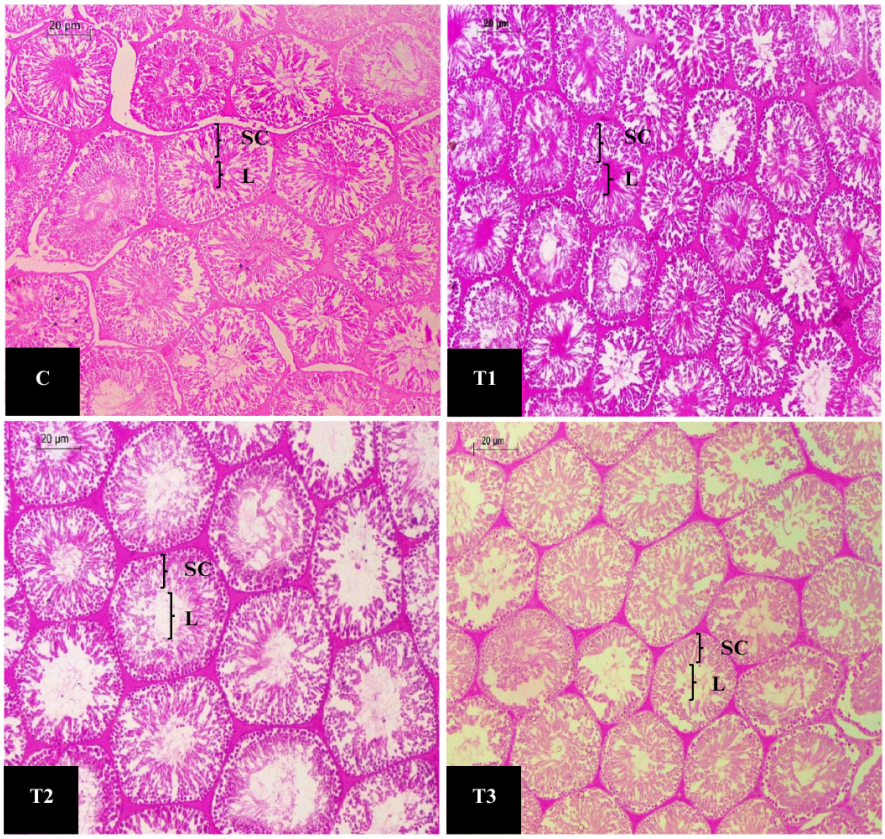

Figure 5 Light microscopic feature of seminiferous tubules in testis from Control (C: 1 mL distilled water), Treatment 1 (T1: 1 g/kg BW), Treatment 2 (T2: 2 g/kg BW) and Treatment 3 (T3: 3 g/kg BW) groups. SC=Spermatogenic cell; L=Lumen. [H&E staining, 400x magnification].

Figure 6 shows the mean values of testosterone level in the Control and Treatment groups of male Sprague Dawley rats. Significant differences ($p<0.05$) were observed between the C, T1, and T2 groups, with the T1 group (1.18 ng/mL) showing significantly higher level compared to the C (1.10 ng/mL) and T2 (0.92 ng/mL) groups.

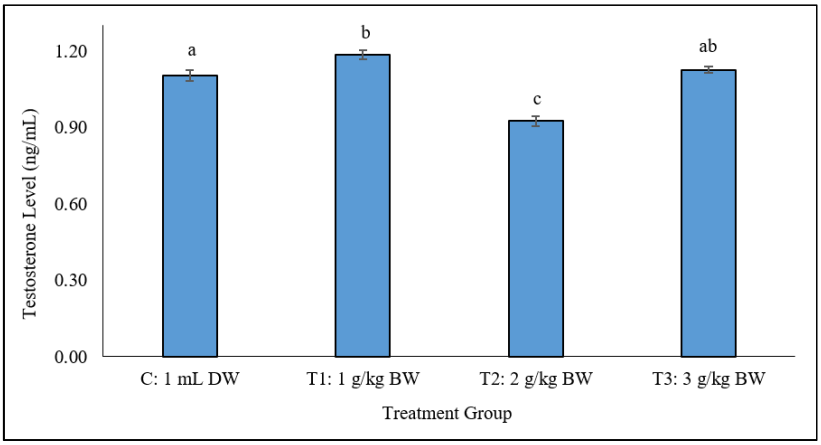

Figure 6 Testosterone level in Control (C), Treatment 1 (T1), Treatment 2 (T2) and Treatment 3 (T3) groups. Data are presented as mean ± SEM (n = 6/group). Different superscript (a, b, c) indicates a significant difference (p<0.05) as determined by one-way ANOVA followed by Tukey's post hoc test. The g/kg BW = gram of Aquilaria malaccensis extract per kilogram of rats' body weight.

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Table 2 presents the outcomes of the correlation analysis involving reproductive factors, encompassing STD, SEH, and testosterone level in male Sprague Dawley rats. The findings indicate that no significant correlations were observed among these results.

Table 2 Pearson's correlation findings for testicular seminiferous tubules and testosterone level in male Sprague Dawley rats.

Variable		STD	SEH	Testosterone
STD (µm)	Pearson's r *p*-value	-		
SEH (µm)	Pearson's r *p*-value	-0.146 0.650	-	
Testosterone (ng/mL)	Pearson's r *p*-value	0.265 0.404	-0.020 0.951	-

No significant correlations ($p>0.05$) were observed in the results.

DISCUSSION

As summarized in Table 1 and Figure 2, the results of phytochemical screening revealed the presence of various secondary metabolites in the aqueous extract of *A. malaccensis* leaves. These findings are consistent with the results of previous studies by Nik et al. (2014), Mascarenhas et al. (2018), Syamsul et al. (2020), and Batubara et al. (2021). Several phytochemical studies have also reported the presence of flavonoids, glycosides, terpenoids, and other chemical compounds, such as diterpenoids, triterpenoids, chromes, and lignans, in the leaves of *A. malaccensis* (Kristanti et al., 2018; Musa et al., 2019; Ramadan et al., 2022).

To date, no study has been found regarding the effects of *A. malaccensis* on seminiferous tubules of the testis. Although the STD (Figure 3) and SEH (Figure 4) in T1 group seemed to be higher compared to other groups, the differences were not statistically significant. Normal and well-organised spacing between tubules was also observed in T1 compared to C, T2, and T3 groups (Figure 5). As reported by Zhou et al. (2019), seminiferous tubules are highly related to the formation of sperm cells through a process called spermatogenesis. Since several previous studies have reported that no significant differences observed in the testis weight of male Sprague Dawley rats (Zulkifle et al., 2018; Razak et al., 2019a; Razak et al., 2019b; Zaidi et al., 2023), it might be because the seminiferous tubules in the testis are not being affected by the supplementation of *A. malaccensis*. It is important to note that the changes in the seminiferous tubules within the testis serve as essential indicators of physiological and pathological conditions in male reproduction.

As shown in Figure 6, the testosterone level in the T1 group was significantly higher (1.18 ng/mL) compared to the C and T2 groups. The high level of testosterone might be attributed to the presence of saponins (Shehzad et al., 2021; Abdelhameed et al., 2022), which are secondary metabolites found in *A. malaccensis* leaves. This was supported by Tauchen et al. (2021), who suggested that saponins may stimulate Leydig cells in the testis to enhance testosterone production. Studies by Leung and Wong (2013) and Fernandez-Lazaro et al. (2021) also revealed the beneficial effects of saponin compounds, namely ginsenosides, on male reproductive function. These components might be able to imitate the function of LH by stimulating the Leydig cells to produce testosterone. It is important to note that the high level of testosterone is closely related to the high concentration of LH, as it plays a crucial role in regulating testosterone production. However, we did not measure the levels of LH and FSH in this study. Furthermore, *A. malaccensis* leaves also contain phenolic compounds, such as flavonoids, which are the primary components responsible for potent antioxidants. These substances

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may also contribute to the synthesis of testosterone levels in male rats (Banihani, 2018; Zakaria et al., 2023).

To date, no study has been done to investigate the effects of *A. malaccensis* specifically on testosterone levels, except by Musa et al. (2019), which also reported a slight increment in the testosterone level (3.12 ng/mL) of male ICR mice after being treated with 200 mg/kg of *A. malaccensis* for 21 days. However, as shown in Figure 6, the testosterone level decreased as the *A. malaccensis* dosage was raised to 2 g/kg. STD and SEH in T2 and T3 groups (Figure 3 and 4) also exhibited a decreasing pattern with higher doses (2 and 3 g/kg), even though the differences were not statistically different. The decrease in testosterone level may be attributed to the excessive dosage of *A. malaccensis*, which the body may not tolerate due to potential toxicity or adverse reactions (Nasir et al., 2023). It is important to note that certain naturally occurring substances that are generally considered safe can have negative effects when exposed to specific doses or conditions (Bode and Dong, 2015). This was confirmed by Yulion et al. (2023), who reported that phytochemicals derived from plants, such as alkaloids and saponins, can damage cells and tissues of organs when improperly used. Additionally, prolonged consumption of high doses of alkaloids (1.5 g/kg) can also lead to a reduction in testicular testosterone production (Eyong and Braide, 2009). However, the reduction of testosterone hormone could still be affected by other chemical substances, which have not yet been discovered.

Emerging research suggests that certain chemical compounds found in plants, such as polyphenolic compounds, may promote testosterone production through different regulatory mechanisms. For instance, certain flavonoids have been found to influence testosterone synthesis, and such action may depend on specific structural features (Martin and Touaibia, 2020). Furthermore, the interaction between phytochemical compounds and the pituitary gland could potentially lead to a reduction in androgen production, consequently impacting testosterone levels (Zaidi et al., 2023). Therefore, further studies should be undertaken to investigate the specific mechanism for this reduction and explore the potential role of other chemical compounds in altering testosterone levels.

Although testosterone plays a crucial role in spermatogenesis that occurs within the testicular seminiferous tubules, the relationship between STD, SEH, and testosterone level (Table 2) did not reveal any significant differences. This implies that the variations in STD and SEH observed across different dosages of *A. malaccensis* were not statistically linked to the corresponding changes in testosterone levels. In other words, while the sizes and structural aspects of the seminiferous tubules varied with *A. malaccensis* dosage, these variations did not align with significant alterations in testosterone production. Therefore, further research is warranted to fully understand the relationship between *A. malaccensis* dosage, spermatogenesis, and testosterone production.

CONCLUSIONS

In summary, this study highlights the presence of various compounds, including tannins, saponins, glycosides, flavonoids, terpenoids, steroids, and alkaloids, within the aqueous extract of *A. malaccensis* leaves. Despite these constituents, the supplementation of *A. malaccensis* at dosages ranging from 1 to 3 g/kg in Sprague Dawley rats did not yield significant changes in seminiferous tubular diameter and epithelial height within the testis. However, the administration of *A. malaccensis* extract at 1 g/kg to adult Sprague Dawley rats showed potential enhancement in the performance of the male reproductive system by increasing testosterone levels. Nonetheless, caution is advised when considering higher concentrations of *A. malaccensis* (2 g/kg), as they may potentially lead to detrimental effects on male reproductive function, resulting in decreased testosterone levels.

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AUTHOR CONTRIBUTIONS

Norahidah Zaidi Performed the sampling and experiments, participated in data analysis and contributed to manuscript writing until the final version as well as all necessary requirements for journal's submission. **Asmad Kari** Contributed to endorse all experiment's material, critically reviewed the manuscript. Performed final revision, reviewed the manuscript and approved the final version. **Nurul Amalina Mohamad Nasir** Participated in data analysis. Reviewed the manuscript and approved the final version. **Mohd Nizam Haron** Devised the project and main conceptual ideas.

CONFLICT OF INTEREST

We have no conflict of interest.

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