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Research Article

Antifertility Effects of Curcumin on Sperm Quality, Morphology of Testicular, and Seminal Vesicle in Rats (*Rattus norvegicus*)

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Abstract | Several studies report that curcumin has antifertility effects and has the potential to regulate male fertility (contraception). However, determining the initial dose is preliminary studies based on spermatozoa quality parameters, morphology, and morphometry of testes and seminal vesicles that have never been reported. The main objective of this research is to examine the effects of curcumin on several aspects of reproduction and determine the optimal dose for using curcumin as an antifertility agent. The test animals in this study were Wistar rats (*Rattus norvegicus*) three-month-old males weighing 300 g. Rats were acclimatized for 7 days and given standard feed and water ad libitum. During the research, rats were given standard 552SP feed. A total of six rats were used in this study, then divided into two treatment groups, namely C1 (given standard feed and distilled water orally (without curcumin)); and C2 (given standard feed and oral curcumin 150 mg /kg body weight). The length of treatment followed the duration (cycle) of spermatogenesis in rats, namely 52 days. Data were analysed using the T-test independent. The results indicate a rise in variables related to spermatozoa abnormality and a reduction in spermatozoa concentration, showing no statistically significant differences (P>0.05). Similarly, there were no significant variations (P>0.05) observed in all variables related to testis and seminal vesicle morphology. The administration of curcumin did not yield a statistically significant effect (P>0.05) on decreasing spermatozoa quality concerning testicular morphology, morphometry, and seminal vesicle weight. However, a trend was observed indicating an increase in spermatozoa abnormalities and a decrease in spermatozoa concentration.

Keywords | Antifertility, Curcumin, Male contraception, Reproduction, Mouse, Turmeric

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INTRODUCTION

Indonesia and other Southeast Asian countries possess abundant natural resources with significant economic value on the global market, notably an extensive array of medicinal plants and spices. A substantial portion of the world's medicinal plants is situated in Indonesia (Jadid *et al*., 2020; Navia *et al*., 2022). Among these, turmeric, particularly its rhizome, stands out as a medicinal plant with a rich history of traditional use spanning generations.

Turmeric (*Curcuma longa*) is a spice or medicinal plant

originating from Asia, especially Southeast Asia (Ide, 2013). This plant is widely cultivated in South Asia, especially in India, Nepal, Pakistan, Bangladesh, Indonesia, Malaysia, Thailand, and the Philippines (Ahmad *et al*., 2020; Dosoky *et al*., 2019; Fithriyya *et al*., 2021). In Indonesia, turmeric grows easily in almost all regions, including Sumatra, Java, Bali, Kalimantan, Sulawesi, Maluku, and Papua (Rahmat *et al*., 2021).

Turmeric is renowned as a kitchen spice with high demand both domestically and internationally. Beyond its culinary use, turmeric holds significant potential in the field of medicine (Das, 2016; Tanvir *et al*., 2017). The active compound within turmeric, curcumin, exhibits diverse biological effects, including anti-carcinogenic, anti-inflammatory, potent antioxidant, anti-angiogenic, and antifertility properties (Maiti *et al*., 2021).

In the male reproductive system, certain reports assert that curcumin administration is protective, potentially enhancing spermatozoa quality, including total sperm count, spermatozoa concentration, and spermatozoa (Alizadeh *et al*., 2018; Aparnak and Saberivand, 2019; Riahi *et al*., 2021). Conversely, several studies suggest that curcumin may inhibit (reduce) spermatozoa quality, affecting motility, spermatozoa viability, capacitation, acrosome reaction, and increasing spermatozoa abnormalities (Ashok and Meenakshi, 2004; Joshi *et al*., 2011; Naz, 2011, 2014; Putra, 2012). In *in vitro* studies, human and mouse spermatozoa incubated with curcumin exhibited decreased motility, capacitation, and acrosome reaction (Naz, 2014). The active compound curcumin is believed to exert its influence either directly by affecting spermatogenesis in the testicles or indirectly by inhibiting the secretion of gonadotropin hormones (follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Şentürk and Sandıkçı, 2022).

Spermatogenesis is an essential process involving a series of high-level genetic and epigenetic events in germ cells that play an important role in converting spermatogonia into spermatozoa (Yuen *et al*., 2020). Antifertility effect of curcumin on spermatozoa caused a decrease in sperm count, sperm motility, sperm morphology and viability (Putra, 2012; Shah *et al*., 2008). This process is controlled by gonadotropin hormones, namely FSH and LH (Yuen *et al*., 2020). Inhibition of FSH secretion results in disruption of FSH and FSH-R binding which has an impact on the induction of a series of key regulatory molecules of spermatogenesis which will induce spermatogenesis disorders and a decrease in sperm quality which ultimately results in infertility. This phenomenon can be used as an opportunity to develop male contraceptive candidates (de la Iglesia *et al*., 2022; Schneider *et al*., 2020). However, in vivo in male Wistar rats (*Rattus norvegicus*) so far there is no information regarding its effects on reproductive function. This limited information also occurs in determining the correct dose, thus requiring a comprehensive study for the development of curcumin as an antifertility agent that can be used as a candidate for herbal contraception in men.

Based on existing studies, curcumin has the potential to regulate male fertility (herbal contraception) (Daniyal and Akram, 2015). According to Purwaningsih (2016) and (Nora *et al*., 2023), curcumin has the potential to control fertility, especially as an antifertility agent in men. Antifertility effect curcumin is reversible. In addition, the administration of curcumin to rats resulted in reversible inhibition of spermatogenic processes and fertility, thus suggesting the viability of this herb in male contraception (Mishra *et al*., 2018).

Based on these problems, it is necessary to study the antifertility effect of curcumin for determining the initial dose as a preliminary study based on spermatozoa quality parameters and testicular and morphometry of seminal vesicle. We hypothesized that increasing the existing dose would reduce the quality of spermatozoa, without disturbing the morphology of the testes and seminal vesicle.

MATERIALS AND METHODS

Animal samples

In this study, male white rats (*Rattus norvegicus*) served as the experimental animals. They underwent a 7-day acclimatization period and were provided with standard 552SP feed and ad libitum access to water. The male white rats weighed between 200-300 g, with an age range of 2-3 months. Selection of male white rats for treatment was done randomly based on the experimental design. Throughout the study, the male white rats received standard 552SP feed. Subsequently, they were divided into two treatment groups, each consisting of three experimental animals.

Treatment of curcumin

The curcumin utilized in this study is sourced from *Curcuma longa* (Turmeric) powder (Sigma-Aldrich®). The white rats were categorized into two treatment groups: C1, receiving standard feed and distilled water orally (without curcumin); and C2, receiving standard feed with oral curcumin at a dosage of 150 mg/kg body weight. The treatment duration aligns with the spermatogenesis cycle in rats, spanning 52 days (Opuwari and Monsees, 2020).

Body weight measurement

Brand digital analytical scale (taffware digipounds®) 12000 carried out every week during maintenance to determine the treatment dose.

Sampling and observation of rat testicular organs

Following the treatment period, euthanasia was conducted on male white rats using a cervical dislocation procedure. Subsequently, semen was collected from the epididymis, specifically from the cauda epididymis, to microscopically observe the condition of the spermatozoa post-treatment.

Macroscopic examination in anatomical pathology

Testicular length is assessed by aligning a tape with a measuring tool and calculating the length, while testicular diameter is measured using a caliper. Testicular volume is determined by placing the testicles in a measuring cup filled with water, and the volume is calculated based on the water displaced. Meanwhile, the weight of the seminal vesicle is measured using digital scales.

Evaluation of spermatozoa quality

Spermatozoa motility

Spermatozoa motility was assessed by placing the samples on a glass surface, followed by the addition of one drop of physiological NaCl. The observation was conducted through a microscope with a 40x magnification. The calculation of the number of motile sperm was based on their movement, categorized as fast progressive (A), slow progressive (B), circular (C), and vibratory (D) motions (Husnurrizal *et al*., 2023). The percentage was determined using the following formula:

$$\% \text{ Motility} = \frac{A}{A+B+C+D} \times 100\ \%$$

Spermatozoa viability

The examination of viability was performed by introducing one drop of spermatozoa on a glass slide, followed by the addition of one staining eosin-nigrosine drop. A smear preparation was made and fixed on a spirit lamp, then evaluated using a microscope of 40x magnification. The dead cells absorb a red pigmentation, while the live spermatozoa tend not to absorb anything colour, leading to a white appearance. The spermatozoa were then counted and divided by the total visible and presented as a percentage value (Hafizuddin *et al*., 2023).

$$\% \text{ Live} = \frac{\text{Total of live spermatozoa}}{\text{Total live and dead spermatozoa}} \times 100\%$$

Spermatozoa morphology

This observation was performed by dripping spermatozoa and eosin-nigrosine on the object glass, fixed on a spirit lamp, and observing in a microscope with 40x magnification. The morphological examination identified deformities that are categorized as primary (small/large head size, double head or double tail, and abnormal head shape) and secondary abnormalities (head rupture, tail breaking at the neck or middle, and folded tail) (Hafizuddin *et al*., 2021). The minimum spermatozoa observed were 200 cells, and the calculations were conducted using the following formula:

$$\% \text{ Normal sperm morphology} = \frac{\text{Normal sperm morphology}}{\text{Normal sperm morphology} + \text{Abnormality}} \times 100\%$$

Spermatozoa concentration

The concentration of spermatozoa is determined by sucking the spermatozoa to a scale of 0.5 and adding 3% NaCl solution to the 101 marks. The mixture is homogenized for 2-3 minutes and a few drops of the semen solution are discarded. Counting is prepared by preparing the Neubauer counting chamber and covering it with a cover glass. On the inside of Neubauer's counting chamber, semen solution was dripped, and then observed under a microscope (Olympus CX21). Sperm counts were carried out in five large boxes, with a magnification of 40x. The spermatozoa concentration was calculated using the formula N x 5 x 200 x 10,000 (N= number of spermatozoa in 5 boxes) (Syafruddin *et al*., 2020).

Data analysis

All data were presented as mean ± standard deviation. The comparison between treatment groups (C1 vs C2) was analysed using an independent-sample T test, p-value ≤ 0.05 considered significant (Ramadan *et al*., 2023).

RESULTS AND DISCUSSIONS

Evaluation of spermatozoa quality

The results of evaluating the quality of spermatozoa consist of Spermatozoa motility (%), spermatozoa viability (%), spermatozoa abnormalities (%), and spermatozoa concentration (x 10^6 cells / mL) are presented in Table 1.

Table 1: Characteristics of rat spermatozoa.

Parameter	Curcumin Dosage	
	C1 (without curcumin)	C2 (150 mg / kg BW)
Spermatozoa motility (%)	66.92 [a] ± 9.79	69.50 [a] ±10.17
Spermatozoa viability (%)	65.50 [a] ±13.43	86.67 [a] ±4.50
Spermatozoa abnormalities (%)	4.25 [a] ± 3.18	8.50 [a] ± 2.64
Spermatozoa concentration (x 10^6 cells / mL)	760 [a] ± 537.40	607 [a] ±240.06

[a]the same superscript indicates non-significant differences

Based on the T-test with a 95% confidence level, it shows that there is no significant difference (P>0.05). However, the variables abnormality of spermatozoa (%) and concentration of spermatozoa (x 10^6 cells / mL) showed that the decrease in the quality of spermatozoa was not significant.

Table 2: Morphometry of testes and seminal vesicle of rat.

Parameter	Curcumin dosage	
	C1 (without curcumin)	C2 (150 mg/kg BW)
Testicle length (cm)	2.00[a]±0.00	1.93 [a] ±0.05
Testicle width (cm)	0.90[a]±0.00	0.87 [a] ±0.05
Testicular diameter (cm)	3.75[a]±0.35	4.00 [a] ± 0.00
Testicular volume (mL)	3.00[a]± 0.00	2.67 [a] ± 0.57
Seminal vesicle weights (g)	1.10[a] ±0.14	1.00 [a] ± 0.35

[a] the same superscript indicates non-significant differences.

This study found that the quality of spermatozoa was not significantly different (P>0.05). This probably happened because the dose given was still the minimum dose (Belhan *et al*., 2020). Results of research Santonastaso *et al*. (2021) with a dose of 20 µM curcumin in freezing spermatozoa medium caused a progressive increase in motility, a significant decrease in intracellular ROS, and a decrease in DNA fragmentation of frozen sperm cells. Other research conducted by Roshankhah *et al*. (2017) also found this. The results of the study showed that curcumin at minimum doses (10, 30, and 60 mg/kg), significantly increased the average percentage of sperm motility, number, weight of testicles, and serum testosterone levels compared to the control group. However, research results in mice with high doses (600 mg/kg) can significantly reduce the quality of spermatozoa (Mishra and Singh, 2009). Other studies also show the same thing, that high doses of curcumin can reduce the quality of spermatozoa and reduce fertility (Hembrom *et al*., 2015; Naz, 2011). Based on this research, in future research, it is necessary to try giving high doses of curcumin, but not up to a lethal dose of 50, by looking for references again.

In this study, although sperm quality was not significantly different, there was a tendency for spermatozoa quality to decrease in variables of spermatozoa abnormalities, and spermatozoa concentration. This can be an indicator that there is an impact antifertility of curcumin by suppressing spermatogenesis. The suppressive effect of curcumin on the spermatogenesis stage will result in more sperm abnormalities. Likewise, it will cause the concentration of spermatozoa to decrease in number (Karakus *et al*., 2021; Rithaporn *et al*., 2003). Previous research has also reported that the effect of curcumin can improve spermatogenesis defects in dexamethasone-treated rats (Khorsandi *et al*., 2013).

Research by Naz (2011) found that curcumin inhibits sperm function, fertilisation and fertility. Curcumin blocks conception, reduces sperm motility, sperm capacitation and acrosome reaction. At high concentrations, its ability to block sperm motility and function lasts for 5-15 minutes. Curcumin can be developed as an ideal contraceptive in the future. The antifertility effect of curcumin is reversible, and at the time it was the first study to report the inhibitory effect of curcumin on sperm function, fertilisation and fertility.

In another study, it was reported that the administration of low concentrations of curcumin (30g/mL) decreased motility without decreasing viability and the highest concentration of 300g/mL decreased total sperm motility after 60 minutes. Curcumin inhibits human sperm protein kinase C which plays a role in sperm flagellum movement (Shah *et al*., 2008).

Although curcumin has the potential to reduce spermatozoa quality, more research is still needed to determine the optimal dose and duration of use in the development of male contraception.

Morphology of testes and seminal vesicle

Average morphometric measurements of testes and seminal vesicle white rat strain Wistar in both treatment groups are shown in Table 2.

All morphometric parameters measured (testicular length, testicular width, testicular diameter, testicular volume) and weight of seminal vesicle showed no significant difference (P>0.05). This shows that the curcumin given did not harm the morphology of the testes and seminal vesicle.

This study is in line with research by Roshankhah *et al*. (2017), who stated that curcumin had a positive effect on testicular morphometry. Curcumin has been shown to also have a positive effect on testicular morphology and morphometry. This is based on research by Taba *et al*. (2019), supplementation with curcumin can improve testicular structure and weight. This mechanism occurs with the effect of curcumin which can protect the testicles from oxidative stress damage.

The cause of seminal vesicle did not experience significant changes (P>0.05) because in certain doses curcumin is protective. This is in accordance with the statement Li *et al*. (2019), that curcumin treatment can reduce oxidative stress in seminal vesicles by reducing the expression of NOX1, NOX2 and NOX4, thus improving apoptosis and atrophy of seminal vesicles.

The results of other studies indicate that Curcumin has a strong protective effect against testicular toxicity and may be clinically useful. This mechanism occurs through its ability to increase the expression of BCL-2 protein, an important anti (Khorsandi *et al*., 2013). Recent research also reported that curcumin not only demonstrated superior protective effects as a protective measure but also opened new avenues to tie the benefits of curcumin in the

face of reproductive toxicity and related health challenges (Mohamed *et al*., 2023).

According to Mohebbati *et al*. (2017) turmeric and curcumin have protective effects on reproductive organ activities such as, anti-inflammatory, anti-apoptotic, and antioxidant effects in normal cells but show pro-apoptotic effects in malignant cells. Therefore, the different effects of turmeric and curcumin depend on the dose and type of cells used in the various models studied.

CONCLUSIONS AND RECOMMENDATIONS

The administration of curcumin at a dose of 150 mg/kg BW did not have a significant effect (P>0.05) on several aspects of male rat reproduction. Therefore, it is necessary to treat with various higher doses for further research development as an antifertility agent.

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NOVELTY STATEMENT

This study can provide new information about the antifertility effects of curcumin on spermatozoa quality, morphology, and morphometry of testes and seminal vesicles of male rats.

AUTHOR'S CONTRIBUTION

HN and MF: Participated in performing, selecting samples, sample collection, and writing the initial manuscript. HH: Performed manuscript revision and data analysis. RS: Conducted the research and performed practical experiments. RR: developed the original idea and protocol and revised the final manuscript.

Animals ethics

This study was approved by the Veterinary Ethics Committee, Faculty of Veterinary Medicine Universitas Syiah Kuala Ref: 217/KEPH/V/2023.

Conflict of interests

The authors have declared no conflict of interest.

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