ORIGINAL ARTICLE

Effect of 96% Ethanol Turmeric Extract as an Antifertility Agent on the Testicular Weight of Sprague Dawley Rats

Rizka Angrainy,[1] Asita Elengoe,[2] Rathimalar Ayakannu,[2] Berliana Irianti,[1] Manisha[1] and Aida Fitria[1]

[1]*School of Nursing and Applied Science, Lincoln University College, Malaysia*
[2]*Department of Biotechnology, Faculty of Applied Science, Lincoln University College, Malaysia*

ABSTRACT

Background. Turmeric (Curcuma longa Linn, synonym Curcuma domestica Val.) is a plant from Southeast Asia which has been consumed as a complement to cooking spices, herbal medicine, or as a medicine to maintain health and beauty such as skin and facial care.

Objective. The study aimed to determine the effect of turmeric extract on the testicular weight of rats.

Methods. This is an experimental research. After going through an adaptation period of two weeks, the animals were given turmeric extract orally for 30 days. The testicles were weighed, and sperm was taken from the vas deferens of male rats.

Results. Normality test (Kolmogorov-Sminorv) and homogeneity test (Homogeneity of Variances) were done. A significance value of P>0.05 was obtained, indicating that the data on the proportion of testicular weights at all test doses compared to the control were not statistically significant.

Conclusion. The administration of 96% ethanol turmeric extract at a dose of 25 mg/KgBW, 50 mg/KgBW, and 100 mg/KgBW for 30 days did not affect the weight of rat testicles. It is necessary to isolate compounds in the 96% ethanol extract of turmeric to determine its antifertility effect.

Keywords: ethanol 96%, turmeric, antifertility, testicular weight, rats

eISSN 2094-9278 (Online)
Published: December 12, 2025
https://doi.org/10.47895/amp.vi0.10969

Corresponding author: Rizka Angrainy
Lincoln University College
47301 Petaling Jaya, Malaysia
Email: rizkaangrainy@gmail.com
ORCiD: https://orcid.org/0000-0003-2403-1648

INTRODUCTION

Turmeric (Curcuma longa Linn, synonym Curcuma domestica Val) is a spice and medicinal plant from Southeast Asia that has spread to Malaysia, Indonesia, Australia, and even Africa. Almost every Indonesian and Indian person and Asian society, in general, has consumed this spice plant, either as a complement to cooking spices or herbal medicine or as a medicine to maintain health and beauty, such as use in skin and facial care.[1]

Some of turmeric's biological activities include anti-inflammatory, antioxidant, anticancer, antimutagenic, antifungal, antibacterial, antiparasitic, antiviral/anti-HIV, anticoagulant, antidiabetic, anticholesterol, anti-infective, antiproliferative, and antifertility. In addition, turmeric can protect the liver, is antifibrinolytic, and can protect against myocardial infarction.[2]

There are two ways antifertility compounds work: by providing a cytotoxic or cytostatic effect and by providing a hormonal effect. If the compound has a toxic effect, then the

cells exposed to it can die, especially the medium cells experiencing development. If the compound has a hormonal effect, then the compound will bind to the receptors found in the reproductive organs, which should be occupied by hormones. This is because these compounds have the same group as hormones that bind to receptors in the reproductive organs.[3,4]

Administration of turmeric extract in the form of alcohol and water to albino mice showed a decrease in epididymal weight, testicular weight, seminal vesicle weight, and prostate gland weight. In addition, turmeric also caused a decrease in sperm count and sperm motility, sperm morphology, and viability. It also caused a decrease in the diameter of the seminiferous tubules and Leidig cell nuclei.[5]

In the female reproductive system, turmeric extract is reported to be abortive and has the potential to be a contraceptive agent, thus functioning as a fertility regulator.[6]

Testicular weight is the best assessment of spermatogenesis. The testes are genital organs that can produce spermatozoa and sex hormones. Inside the testes are seminiferous tubules, connective tissue, and blood vessels. The seminiferous tubules are the largest components of the testes. If there is damage or atrophy of the cells that make up the seminiferous tubules, it will cause a decrease in testicular weight; changes in the spermatogenesis microscopically can be seen from changes in testicular weight.[7]

OBJECTIVE

The general objective of this study is to determine the effect of turmeric extract on the testicular weight of rats.

METHODS

Materials

Plant Determination

Plant determination was carried out by "Natural Materials" of the Riau College of Pharmacy. Results showed that the test sample plant was indeed a turmeric plant (Curcuma longa Linn. syn. Curcuma domestica Val.).

Extraction

A total of 250 grams of turmeric powder (Curcuma longa Linn. syn. Curcuma domestica Val.) was macerated with 90% ethanol to produce a dark brownish macerate. The extract obtained was concentrated using a vacuum rotary evaporator. Since a thick extract was not obtained, the extract was thickened with a freeze dryer to produce 62.9666 grams of thick extract.

Phytochemical Screening of the Extract

Based on the results of phytochemical screening carried out on the turmeric extract (Curcuma longa Linn. syn. Curcuma domestica Val.) contains alkaloids, saponins, terpenoids, steroids, flavonoids and tannins.

This research was conducted at the Pharmacy Laboratory of the University of West Sumatra from December 2023 to February 2024. Twenty-four male rats aged 8-12 weeks were used. These rats were obtained from the Animal House of the Faculty of Pharmacy, Andalas University, West Sumatra, Indonesia.

After going through an adaptation period of two weeks, the experimental animals were given turmeric extract orally for 30 days. The testicles were weighed, and sperm was taken from the vas deferens of male rats.

Measurement of testicular weight was carried out by weighing the testes using analytical scales. Then the results of the testicular weights of treated rats were compared with the weights of the control rats. The testicular weight was compared with the rat's body weight after treatment to obtain the proportion of testicular weight to the rat's body weight.[8,9]

Data Analysis

Statistical data analysis was carried out to anticipate differences in results between treatments in each experimental group. The results of the experiment were analyzed to see if there was a significant difference in the weight of the testes of each group of rats that were treated. Analysis of the data obtained was processed using the SPSS statistical data processing program version 26 including normality tests, homogeneity tests, and parametric tests (One-way ANOVA, Paired Sample T-Test).

Ethical Considerations

This research complied with the declaration of Pharmaceutical Laboratory ethical principles for medical research involving animals subjects. The study was approved by the Ethics Review Board of the University of Andalas, Indonesia (No.22/UN.16.2/KEP-FK/2024).

RESULTS

Based on the phytochemical screenings done to 96% ethanol turmeric extract (Curcuma longa Linn, syn. Curcuma domestica Val.), the group compounds found can be seen in Table 1.

Generally, compounds that can potentially act as antifertility agents include steroids, alkaloids, flavonoids, and triterpenoids. After conducting phytochemical screening tests, it was found that turmeric contains alkaloids, flavanoids, tannins, saponins, and triterpenoids so there was a possibility that turmeric has an antifertility activity.

Results of testicular weight measurements in control rats at a low dose of 25 mg/KgBW, a medium dose of 50mg/KgBW, and a high dose of 100 mg/KgBW after administering 96% ethanol turmeric extract for 30 days were shown in Table 2. There was an increase and decrease in testicular weight in the test group compared to the control group.

The average weight of the testes at a dose of 25 mg/KgBB was 1.31 grams with a standard deviation of 0.04 grams. At

a dose of 50 mg/KgBB the average weight of the testes was 1.24 grams, a standard deviation of 0.10 grams, while at a dose of 100 mg/KgBB it was 1.24 grams with a standard deviation of 0.07 grams (Table 2).

Table 3 shows that were no significant differences in the weight of the right testicle (p = 0.081) and left testicle (p = 0.089) of the rat.

The results of the Bonferroni multiple comparison test show that the weight of the right and left testicles at 25 mg/KgBB, 50 mg/KgBB, and 100 mg/KgBB doses were not significantly different (Table 4).

The results of the ANOVA P value show that that there was no difference in the average weight of the rat testicles (p = 0.079) (Table 5).

The results of the Bonferroni multiple comparison test show that the average weight of the rat testicles at 25 mg/KgBB, 50 mg/KgBB, and 100 mg/KgBB doses were not significantly different (Table 6).

The testicular weight were compared with the body weight of the test animals on the 31st day to obtain the proportion of testicular weight to body weight. The tests performed were the normality test (Kolmogorov-Sminorv) and the homogeneity test (Homogeneity of Variance). A significance value of P>0.05 was obtained, which indicated that the data on the proportion of testicular weight at all test doses compared to the control were not statistically significant.

Table 1. Screening Results Phytochemicals

No.	Identification Group Compound	Criteria	Test Results	Information
1	Alkaloid	Formed sediment white	There was sediment white formed when Meyer's reagent was added	Positive
2	Flavonoid	Color orange, red young, red	Orange color became red	Positive
3	Tannin	Color blackish green / blue	Orange color became green black	Positive
4	Saponins	Formed 5 minutes foam	There was foam that was durable and stable	Positive
5	Triterpenoid	Formed brownish or violet ring on the border solution	Brownish ring formed on the border solution	Positive
6	Steroid	Formed blue greenish ring	No blue greenish ring formed	Negative

Table 2. Mean Proportion of Testicular Weight in Control and Treatment Rats

Group	Testicular Weight		
	Correct Mean±SD	Left Mean±SD	Average testicular weight Mean±SD
Control	1.12 ± 0.17	1.11 ± 0.19	1.11 ± 0.18
Dosage 25 mg/KgBB	1.30 ± 0.03	1.31 ± 0.05	1.31 ± 0.04
Dosage 50 mg/KgBB	1.24 ± 0.93	1.24 ± 0.10	1.24 ± 0.10
Dosage 100 mg/KgBB	1.25 ± 0.09	1.22 ± 0.07	1.24 ± 0.07

Table 3. Results of One-way ANOVA Test on Rats' Right and Left Testicular Weights

Group	N	Right	*P* Value	Left	*P* Value
Control	5	1.12 ± 0.17	0.081	1.11 ± 0.19	0.089
Dosage 25 mg/KgBB	5	1.30 ± 0.03		1.31 ± 0.05	
Dosage 50 mg/KgBB	5	1.24 ± 0.93		1.24 ± 0.10	
Dosage 100 mg/KgBB	5	1.25 ± 0.09		1.22 ± 0.07	

Table 4. Post Hoc Multiple Comparison Test (Bonferroni) on the Rats' Right and Left Testicular Weight

Group	Right				Left			
	Control	Dosage 25 mg/KgBB	Dosage 50 mg/KgBB	Dosage 100 mg/KgBB	Control	Dosage 25 mg/KgBB	Dosage 50 mg/KgBB	Dosage 100 mg/KgBB
Control	-	0.089	0.52	0.38	-	0.08	0.53	0.86
Dosage 25 mg/KgBB	0.89	-	1.00	1.00	0.087	-	1.00	1.00
Dosage 50 mg/KgBB	0.52	1.000	-	1.00	0.53	1.00	-	1.00
Dosage 100 mg/KgBB	0.38	1.000	1.00	-	0.86	1.00	1.00	-

Table 5. Results of One-way ANOVA Test on Average Weight of Rat Testicles

Group	N	Average Testicular Weight	*P* value
Control	5	1.11 ± 0.18	0.079
Dosage 25 mg/KgBB	5	1.31 ± 0.04	
Dosage 50 mg/KgBB	5	1.24 ± 0.10	
Dosage 100 mg/KgBB	5	1.24 ± 0.07	

Table 6. Post Hoc Multiple Comparison Test (Bonferroni) on Average Weight of Rat Testicles

Group	Control	Dosage 25 mg/KgBB	Dosage 50 mg/KgBB	Dosage 100 mg/KgBB
Control	-	0.07	0.49	0.55
Dosage 25 mg/KgBB	0.07	-	1.00	1.00
Dosage 50 mg/KgBB	0.49	1.00	-	1.00
Dosage 100 mg/KgBB	0.55	1.00	1.00	-

DISCUSSION

The results of the testicular weight measurement showed a decrease but were not significantly different P>0.05. The decrease in testicular weight occurs due to the loss of epithelial germ cells that cannot regenerate due to age factors. This is in accordance with the results of additional observations, namely the calculation of the number of pachytene cells which are germ cells in the seminiferous tubules. From the data, it can be seen that there was a decrease in the number of germ cells along with the increasing dose given, but the decrease was not significantly different (p>0.05). This was also thought to be due to the fact that the optimal dose of 96% ethanol extract from turmeric has not been achieved for it to reduce testicular weight.[10]

The disruption of the spermatogenesis process and the decrease in the diameter of the seminiferous tubules, spermatozoa concentration and testicular weight from the observations above are closely related to the activity of the compounds contained in the 96% ethanol extract of turmeric itself. The results of phytochemical screening showed that there were alkaloids, saponins and steroids in 96% ethanol extract of turmeric. Although further research is needed on the types of alkaloids and saponins contained in turmeric, these compounds have been reported in several studies to interfere with the spermatogenesis process with different mechanisms. Alkaloids in the extract of oyong seeds, bitter melon fruit extract and beluntas leaf extract were reported to be able to suppress the secretion of reproductive hormones, namely testosterone, so that the spermatogenesis process is disrupted.[11-13] In addition, reported that alkaloids are also used as short-term contraceptive agents.[14]

Saponins founds in starfruit extract, bitter melon fruit extract, and pudding leaf extract were reported to be able to increase testosterone levels in the blood. Increased testosterone levels cause a feedback mechanism to the hypothalamus and pituitary. Testosterone will inhibit the hypothalamus from producing GnRH and inhibit the anterior pituitary from producing LH, decreased LH causes decreased testosterone levels and decreased testosterone can cause epididymal atrophy.[15]

One type of steroid compound found in pomegranate seeds is estrone. Estrone (E1 or also known as oestrone) is an estrogenic hormone which is one of several natural estrogens, which also includes estriol and estradiol. Estrogen in the body comes from testosterone produced by Sertoli cells when stimulated FSH, which plays a role in spermiogenesis, namely the process of forming spermatid cells into spermatozoa. Increased amounts of estrogen can result in feedback reactions. Estrogen reduces FSH secretion in certain circumstances that will inhibit LH, thus affecting the spermatogenesis process.[16]

From the explanation above, it can be seen that the compounds contained in 96% ethanol extract of turmeric, namely alkaloids, saponins, and steroids, have a direct and indirect effect on the body's hormonal balance, especially those responsible for stimulating the spermatogenesis process, namely testosterone, luteinizing hormone, follicle stimulating hormone, estrogen and growth hormone.[17]

Testosterone, secreted by Leydig cells located between the seminiferous tubules in the testes, is important for the growth and division of germ cells in the testes, which is the first stage in sperm formation. Luteinizing Hormone (LH), secreted by the anterior pituitary gland, stimulates Leydig cells to secrete testosterone. Follicle Stimulating Hormone (FSH), is also secreted by the anterior pituitary gland, stimulating Sertoli cells; without this stimulation, the change of spermatids into sperm (spermiogenesis process) will not occur. Estrogen, formed from testosterone by Sertoli cells when stimulated by FSH hormone, is also important for spermiogenesis. Growth Hormone (GH) is needed to initiate the initial division of spermatogonia.[18]

Testicular weight and size are the best initial assessments for spermatogenesis. The testes are genital organs that can produce spermatozoa and sex hormones. Inside the testes are seminiferous tubules, connective tissue, and blood vessels. The seminiferous tubules are the largest components of the testes. a condition when the testes shrink in size and function, which causes damage or atrophy to the cells that make up the seminiferous tubules, resulting in a decrease in testicular weight. A decrease in testicular weight reflects microscopic changes in spermatogenesis (sperm production) and atrophy of the cells that make up the seminiferous tubules that play a role in sperm formation.[19]

The average results of the proportion of testicular weight in the medium dose group (50 mg/KgBW) experienced an insignificant decrease compared to the control group. The insignificant decrease in testicular weight is likely due to the loss of spermatogenic elements in the testes. Changes in testicular weight can reflect changes in the seminiferous

tubules and interstitial edema, so it is suspected that the administration of the extract affects the spermatogenesis process in mice. A correlation was seen between testicular villi, germ cells, and tubule diameter. Condorelli et al. stated that normal spermatogenesis is only possible in normal-sized testes. A progressive decrease in sperm count and total sperm count has been reported in infertile men with a decrease in total testicular volume. A significant correlation has been found between testicular volume and spermatogenesis which is used as a consideration as an indicator of testicular function.[20]

The imbalance of these hormones can reduce or even prevent spermatogenesis from occurring, thus causing infertility. Disruption of the spermatogenesis process will then affect the decrease in the diameter of the seminiferous tubules so that the number of mature spermatozoa in the epididymis decreases and results in a decrease in testicular weight.[21]

CONCLUSION AND RECOMMENDATIONS

The decrease and increase in testicular weight were insignificant compared to the control. It can be concluded that administration of 96% ethanol turmeric extract at doses of 25 mg/KgBW, 50 mg/KgBW, and 100 mg/KgBW for 30 days did not affect the weight of rat testes. Further research is needed on the potential of 96% ethanol turmeric extract as an antifertility agent, including the optimal dose of use, the presence or absence of a recovery process after stopping the extract, the side effects, and the content of active compounds responsible for the antifertility effect and its mechanism.

Isolation of compounds in 96% ethanol turmeric extract needs to be done to determine its antifertility effect as well as the microscopic examination of the testes to confirm spermatogenesis or pathological changes in the testes as an effect of the extract.

Acknowledgments

The authors would like to thank their entire team and supervisors who have helped them in completing this manuscript.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

All authors declared no conflicts of interest.

Funding Source

None.

REFERENCES

1. Ekere SO, Ihedioha JI, Obidike IR, Njoku AL, Anya KO. Effects of oral administration of graded doses of Curcuma longa rhizome (turmeric) powder on the testis, epididymis and blood levels of selected reproduction related biochemical parameters of rabbit bucks. Journal Of Veterinary And Applied Sciences [Internet]. 2024;14(2):651–67. Available from: www.jvasonline.com
2. Agrawal S, Goel RK. Curcumin and its protective and therapeutic uses. 2016.
3. Maiti R, Roy U, Subhayan D, AK D. Antifertility Effects of Curcumin, an Indigenous Medicine in Rats. Int J Basic Clin Pharmacol. 2021;10(2):167–71.
4. Noorafshan A, Karbalay-Doust S, Valizadeh A, Aliabadi E, Mirkhani H. Ameliorative Effects of Curcumin on the Seminiferous Epithelium in Metronidazole-Treated Mice: A Stereological Study. Toxicol Pathol. 2010;38(3):366–71.
5. Ashok P, Manaeskhi B. Contraceptive Effect of Curcuma Longa in Male Albino Rat. Asian J Androl. 2013;71–4.
6. Shah GM, Khan Ma, Ahmad M, Zafar M, Khan AA. Observation on Antifertility and abortifacient herbal drugs. Afr J Biotechnol. 2015;159–64.
7. Zhou R, Wu J, Liu B, Jiang Y, Chen W, Li J, et al. The roles and mechanisms of Leydig cells and myoid cells in regulating spermatogenesis. Vol. 76, Cellular and Molecular Life Sciences. Birkhauser Verlag AG; 2019. p. 2681–95.
8. Aktas C, Kanter M, Erboga M, Ozturk S. Anti-Apoptotic Effect of Curcumin on Cadmium Induced Apoptosis in Rat Testes. Toxicol Ind Health. 2012;28(2):122–30.
9. Ganiger S, Mallesshappa HMN, Krisnappa H, Rajashekhar G, Rao VR, Sullivan F. A Two Generation Reproductive Toxcity Study with Curcumin. Turmeric Yellow in Wistar Rat. Food Chem Toxicol. 2015;64–9.
10. Gupta H, Maheshwari KK, Kumar N. Reversible germ cell toxicity of sulphasalazine and ampicillin combination in male rats. J Reprod Infertil. 2013;14(3):126–32.
11. Faisal S, Badshah SL, Kubra B, Emwas AH, Jaremko M. Alkaloids as potential antivirals. A comprehensive review. Nat. Prod. Bioprospect. 13, 4 (2023). doi: 10.1007/s13659-022-00366-9
12. Garg S, Talwar GP, Upadhyay SN. Comparison of extraction procedures on the immunocontraceptive activity of neem seed extracts. J Ethnopharmacol. 2016;44(2):87–92.
13. Siddiqui SA, Ali Redha A, Salauddin M, Harahap IA, Rupasinghe HPV. Factors Affecting the Extraction of (Poly)Phenols from Natural Resources Using Deep Eutectic Solvents Combined with Ultrasound-Assisted Extraction. Critical Reviews in Analytical Chemistry, 55(1), 139–160. doi: 10.1080/10408347.2023.2266846
14. Ozkavukcu S, Ibis E, Kizil S, Isbacar S, Aydos K. A laboratory modification to testicular sperm preparation technique improves spermatogenic cell yield. Asian J Androl. 2014 Nov 1;16(6):852–7.
15. Lee KS, Kim HP, Park HJ, Yoon YG. Improvement of testosterone deficiency by fermented Momordica charantia extracts in aging male rats. Food Sci Biotechnol. 2021 Feb 6;30(3):443-454. doi: 10.1007/s10068-020-00872-x. PMID: 33868755. PMCID: PMC8017075.
16. Sreekumar S, Sithul H, Muraleedharan P, Azeez JM, Sreeharshan S. Pomegranate fruit as a rich source of biologically active compounds. Vol. 2014, BioMed Research International. Hindawi Publishing Corporation; 2014.
17. Momin MAM, Bellah SF, Rahman SMR, Rahman AA, Murshid GMM, Emran T Bin. Phytopharmacological evaluation of ethanol extract of Sida cordifolia L. roots. Asian Pac J Trop Biomed. 2014 Jan;4(1):18–24.
18. Oduwole OO, Huhtaniemi IT, Misrahi M. The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. Vol. 22, International Journal of Molecular Sciences. MDPI; 2021.
19. Smith LB, Walker WH. The regulation of spermatogenesis by androgens. Vol. 30, Seminars in Cell and Developmental Biology. Elsevier Ltd; 2014. p. 2–13.
20. Condorelli R, Calogero AE, La Vignera S. Relationship between testicular volume and conventional or nonconventional sperm parameters. Int J Endocrinol. 2013;2013.
21. Li L, Lin W, Wang Z, Huang R, Xia H, Li Z, et al. Hormone Regulation in Testicular Development and Function. Vol. 25, International Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute (MDPI); 2024.