Research Article

Antifertility Effects of Ethanol-Extracted Turmeric on Male Rats: ELISA-Based Evaluation

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Abstract

Objective: To determine the effect of administering 96% ethanol extract of turmeric (Curcuma longa Linn, syn. Curcuma domestica Val.) on the antifertility effect on male rats.

Methods: 18 Male and 18 Female Sprague-Dawley Rats were grouped into 6 Groups, namely the Control Group, Positive Control Group (Birth Control Pills), Treatment Group 1 with a dose of 25 mg/KgBW, Treatment Group 2 with a dose of 50 mg/KgBW, Treatment Group 3 with a dose of 25 mg/KgBW, Treatment Group Mating of Male and Female Rats given turmeric with a dose of 50 mg/KgBW, Treatment Group Mating of Male and Female Rats given turmeric is Male with a dose of 50 mg/KgBW. Turmeric extract was given orally every day for 30 days.

Results: The data obtained were statistically analyzed using a one-way ANOVA test. Testosterone levels increased compared to the control group, indicating no significant difference, with a P value of 0.317 ($p>0.05$), and in vivo male sperm motility at doses of 25 mg/kgBW, 50 mg/kgBW, and 100 mg/kgBW was significantly different from the control group ($p\ 0.00 < 0.05$).

Conclusion: Giving 96% turmeric ethanol extract (Curcuma longa Linn, syn. Curcuma domestica Val.) did not affect testosterone levels in male rats in vivo at doses of 25 mg/kgBW, 50 mg/kgBW, and 100 mg/kgBW, with a significance value of $p\ 0.317 > 0.05$, indicating no significant difference. Meanwhile, sperm motility testing showed a significant difference with a result of $p = 0.00 < 0.05$. For further research, it is hoped that further observations will be made regarding the potential of turmeric ethanol extract by conducting histological tests, and a mating test will be carried out as evidence of whether or not the treated rats are fertile.

Keywords: antifertility, concentration testosterone, effectiveness, Sprague-Dawley Rats, turmeric extract.

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INTRODUCTION

Couples of reproductive age are husband and wife, whose wife is 15-49 years old and is still menstruating, or married couples, whose wife is less than 15 years old and has menstruated, or the wife is 50 years old but still menstruating[1]. Under normal conditions, it is easy for couples of reproductive age to get offspring, so they need or require fertility regulation, pregnancy care, and knowledge of safe childbirth. Couples of reproductive age can reduce birth rates by participating in family planning programs using effective methods, one of which is using hormonal contraception, so that the number of intervals in limiting childbirths can be calculated to improve the quality of reproduction.[2]

According to data from the World Population Prospects, the Total Fertility Rate (TFR) in Indonesia has declined in the last three decades. In 1990, Indonesia's TFR was still at the level of 3.10. That is, every woman, on average, gives birth to three children during her childbearing age. Then in subsequent years, the TFR will continue to decrease until it reaches 2.15 in 2022. Cumulatively, Indonesia's birth rate has fallen by 30.64% during the 1990-2022 period. Even though there is a decrease in the birth rate, the National Population and Family Planning Agency considers that Indonesia is not experiencing a sex

recession.[3]

The birth rate in Indonesia is high enough that the government created a family planning program to limit birth rates. Health profile data in 2018, the number of births in Indonesia reached 4,840,411 people, of which 2,423,786 were male and 2,322,652 were female. According to the National Population and Family Planning Agency, the family planning program plans to increase welfare through birth control three children per family, and in its development term, 2 children per family.[4]

Family planning aims to regulate the number and spacing of children through contraceptive methods. Both partners have the right to access safe, effective, and affordable options. Contraceptive choice is influenced by factors such as spousal support, health conditions, and guidance from medical staff.[5]

METHODS

Tools and materials

Preparation of Test Extracts

The simple drug is extracted by maceration with 96% ethanol.

Phytochemical Screening

Phytochemical screening is carried out to determine the presence of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, triterpenoids, and steroids.

ELISA examination

The tools used in this study were the Bio-Rad xMark Microplate Absorbance Reader, equipped with Microplate Manager software, 27.3^0 C Incubator, Absorbent Paper, Precision Pipettes, and disposable pipette tips. The materials used in this study were the ELISA Kit Bioassay Technology Laboratory, which consisted of Standard estrogen solution, ELISA plate coated with antibodies, Standard diluent solution, Streptavidin-HRP, Stop Solution, Substrate A solution, Substrate B solution, Wash buffer solution, Plate sealer, and Blood serum taken from Female Rats, 96% Ethanol, Turmeric Extract

Preparation

All reagents in the ELISA Kit are placed at room temperature, and then the standard solution is prepared. The working standard solution to be used is 6 different concentrations. The Testosterone Standard is diluted and made into several concentrations, namely 0, 1.5, 3, 6, 12, 24, 48 ng/mL. In addition, a washing solution is also prepared because this solution is available in concentrated form and must be diluted. Each plate requires 5 washes with a washing solution of 0.35 mL.

RESULTS

Extraction

As much as 1 Kg of powder turmeric (Curcuma longa Linn, syn. Curcuma domestica Val.) macerated in ethanol 96% produces colored macerated Chocolate. The extract obtained was more concentrated with a vacuum rotary evaporator to obtain a thick extract, which produced 62 grams of extract.

Filtering Phytochemicals Extract

Based on the results of screening phytochemicals that have been done to extract ethanol 96% turmeric (Curcuma longa Linn, syn. Curcuma domestica Val.), there are some group compounds, including alkaloids, flavonoids, tannins, saponins, triterpenoids, and steroids. The results can be seen in Table 1.

Table 1. Phytochemicals Extract

Identification Group Compound	Information
Alkaloid	Positive
Flavonoid	Positive
Tannin	Positive
Saponins	Positive
Triterpenoid	Positive
Steroid	Negative

Based on test results performed on screening phytochemicals shown that extract ethanol turmeric (Curcuma longa Linn, syn. Curcuma domestica Val.) contains alkaloids, saponins, terpenoids, steroids, flavonoids, and tannins.

Concentration Testosterone

Calculation of serum testosterone concentration on days 0 and 31 is done using a testosterone ELISA kit. Calculation data concentration testosterone on the day from 0 to 31 can be seen table below. Calculation results in

testosterone concentration on days 0 and 31 in each test group showed decreased and increased testosterone concentration

Table 2. Results of Level vs Optical Density

Standard Level Testosteron (ng/mL)	Optical Density (OD)	Mean
0	1.688	1.688
1.5	1.246	1.246
3	1.255	1.255
6	0.922	0.922
12	0.539	0.539
24	0.178	0.178
48	0.062	0.062

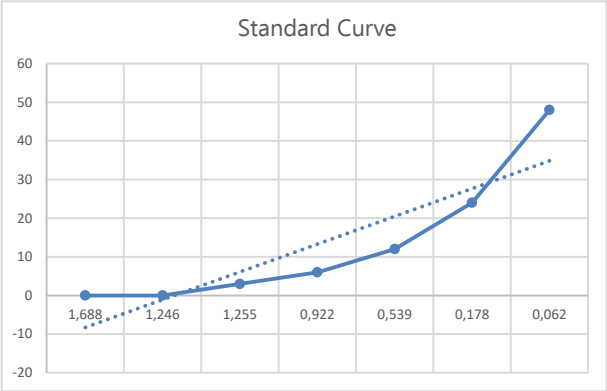

Figure 1. Standard Curve

In Table 2, the standard curve was made with 7 graded concentrations, namely 0 ng/mL, 1.5 ng/ mL, 3 ng/mL, 6 ng/mL, 12 ng/mL, 24 ng/mL, and 48 ng/ mL. used to obtain a linear standard curve with r = 0.841 Figure 1), and used to measure testosterone hormone levels.

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.445[a.]	.198	.037	598.33499

a. Predictors: (Constant), Standard Testosterone Level (ng/mL)

ANOVA[a]

Model		Sum of Squares	Df	Mean Square	F	Sig
1	Regression	441526.334	1	441526.334	1.233	.317[b]
	Residual	1790023.796	5	358004.759		
	Total	2231550.131	6			

a. Dependent Variable: OD (Optical Density)
b. Predictors: (Constant), Standard Testosterone Level (ng/mL)

Coefficients[a]

Model		Unstandardized B	Coefficients Std. Error	Standardized Coefficients Beta	T	Sig.
1	(Constant)	569.469	295.774		1.925	.112
	Kadar Standar Testosteron (ng/mL)	-15.681	14.121	-.445	-1.111	.317

a. Dependent Variable: OD (Optical Density)

Figure 2. Model Summary, Anova, Coefficients

The results in Table 1 were then analyzed using SPSS for linear regression testing. The results of the linear regression are shown in Figure 2. The results of the linear regression were then evaluated based on the significance value in the coefficient table in Figure 2, obtaining a significance value of 0.317 > 0.05, which means there is no significant difference.

Measurement Sperm Motility

Measurement results in rat spermatozoa motility control, dose low (25 mg kgBW), dose medium (50 mg/ kgBW), and dose high (100 mg kgBW) after giving extract ethanol 96% turmeric for 30 days. It looks like a decline in spermatozoa motility in the test group against control group.

The average result of spermatozoa motility can be seen in the Table.

Table 4. Average Sperm Motility of each Group

Group	Motility (%) ± SD
Control	87.7 ± 3.01
25 mg/KgBW	70.9 ± 5.99
50 mg/KgBW	41 ± 3.29
100 mg/KgBW	19.1 ± 5.42

The average spermatozoa motility data were obtained by counting 100 spermatozoa across six field views from left to right. Once the data were collected, normality testing (Shapiro-Wilk) and homogeneity of variance testing were conducted, yielding a significance value of $p > 0.5$, indicating that the data were normally distributed and homogeneous. Spermatozoa motility decreased progressively from the control group (73.33%) to the low-dose group (70.9%), followed by a sharp decline in the medium-dose group (41%) and the high-dose group (19.1%). This reduction was statistically significant based on the results of a one-way ANOVA test, which showed a significance value of 0.000 ($p < 0.5$). Further analysis using the LSD/BNT test revealed significant differences ($p < 0.5$) between the control group and both the medium-dose (50 mg/kgBW) and high-dose (100 mg/kgBW) groups, as well as between the low-dose group (25 mg/kgBW) and the medium- and high-dose groups. However, no significant difference ($p > 0.5$) was found between the medium-dose and high-dose groups.

DISCUSSION

This study was conducted to evaluate the administration of 96% turmeric ethanol extract on testosterone hormone levels and sperm motility. In male rats, the antifertility components considered are whether they can inhibit spermatogenesis, inhibit testosterone from affecting gonadotropin organs, or cause sperm to be unsafe.

Turmeric extract (96% ethanol) was obtained via maceration at room temperature in a dark bottle, stopped when the filtrate became pale. Maceration was chosen for its simplicity and non-heating process, making it suitable for thermolabile compounds. Ethanol was used for its broad solubility and ability to inhibit enzymes and prevent hydrolysis and oxidation.[7]The use of 90% ethanol solvent in this study is related to the compounds contained in it, where it is known that there are groups of compounds that can be used as antifertility agents, namely steroids, alkaloids, flavonoids, and tannins.[8] Phytochemical screening of the tested extract against the tannin, saponin, steroid, alkaloid, flavonoid, and terpenoid groups. The purpose of this study was to test the presence of certain groups of compounds that are suspected to be the compounds responsible for the antifertility activity of the 96% ethanol extract of turmeric.[9]

The results obtained showed that there were tannins, saponins, steroids, alkaloids, flavonoids, and terpenoids. It was found that the positive test extract contained tannins, saponins, steroids, alkaloids, and flavonoids.[10] The group of compounds that can be used as antifertility agents includes steroids, alkaloids, flavonoids, and tannins, all of which are found in the 96% ethanol extract of turmeric. This strengthens the assumption that this extract has antifertility activity.[11]

The extract was orally administered using a feeding tube, with volume adjusted daily based on body weight. It was suspended in 0.5% NaCMC, a recommended dispersing agent. Mice were weighed daily to determine dosage. Body weight fluctuated in both control and test groups during the 30-day administration of 96% ethanol turmeric extract.

However, there was an increase in the initial weight of the mice to the final weight of the mice after 30 days of being given the extract. This shows that the dose used did not cause any harmful effects and did not affect the metabolic process.[12] The study also showed that there was no significant change in the weight of the mice after being given 96% ethanol extract of Turmeric for 30 days.[13] The mice were dissected 24 hours after the final dose was given, namely on the 31st day. [13]

The mice were anesthetized using ether, and then their blood was taken through the orbital sinus of the eye, which is the source of blood collection with the largest amount. The mice were terminated, and their testes and epididymis were taken for observation of the following parameters:

Phytochemical Test

Based on the phytochemical tests that have been carried out on simplified turmeric (*Curcuma longa Linn. syn. Curcuma domestica Val.)* show

results are positive for alkaloids, flavonoids, tannins, saponins, and triterpenoids. In the extract, ethanol rhizome turmeric shows positive results in alkaloids, flavonoids, phenols, terpenoids, and tannins. Compounds phenols and tannins, which are not detected in simple drugs, can be caused by differences in polarity from the solvent used with the compound. [14] The Solvent can extract compounds that have the same polarity or are similar to the polarity solvent used. [15]

The choice of solvent and extraction method influences the results of phytochemical screening. The principle of "like dissolves like" explains that nonpolar compounds dissolve in nonpolar solvents, while polar compounds dissolve in polar solvents. Ethanol, as a polar solvent with a low boiling point (79°C), is commonly used to extract polar components from natural materials and is known as a universal solvent.[16]

Polar compounds from natural materials can be extracted using ethanol through a separation process. Ethanol is more effective than other organic solvents and requires minimal heat for concentration due to its low boiling point (79°C). Alkaloid testing was performed using Mayer and Dragendorff reagents, based on a precipitation reaction caused by ligand replacement.[17]

Positive results for alkaloids with Mayer's reagent are marked by the formation of white sediment, formed as a potassium-alkaloid complex. Alkaloids contain nitrogen atoms that have a partner electron free so that they can be used to form a coordinate covalent bond with metal ions. In the alkaloid test with Mayer's reagent, it is estimated that nitrogen in alkaloids will react with the metal ion K+ from potassium tetraiodomercurate (II) to form a potassium-alkaloid complex that precipitates. The results of the alkaloid test with the use of reagent Dragendorff is the formation of sediment or turbidity colored orange. [18]

Reagent Dragendorff contains bismuth nitrate and mercury chloride in a sour nitrate watery. In the experiment, sediment or turbidity was colored orange with the reagent Dragendorff. In the alkaloid test with the reagent Dragendorff, nitrogen is used to form a covalent coordinate bond with K+, which is a metal ion [19].

In the Flavonoid test, it produced a positive reaction that formed the color red. Reaction suspicion of Flavonoid compounds with Mg and HCl. Flavonoids are in group of compounds of phenol which have lots of OH groups with different high electronegativity, so that polar nature. Group compound. This is easy to extract in solvent ethanol, which has a polar nature because existence of the group hydroxyl, which can form hydrogen bonds.[20]

The saponin test was identified with reaction foam, where the results of the reaction to the extract showed a positive reaction. Saponins are generally present in the form of glycosides, so they tend to be polar. The foam produced in the saponin test is caused by the existence of glycosides that can form foam in water and are hydrolyzed to become glucose and compounds that form foam.[21]

The foam produced by saponin is not affected by acid, so after adding 1N HCl remains stable and foam No is lost. The terpenoid and steroid tests were carried out with the use of Liebermann-Buchard reagent, which will later show positive results obtained in the form of ring brownish or violet ring at the border of two solvents, so extracts that show the presence of triterpenoids. If the results obtained form a color green bluish, then the extract shows the presence of steroids.[22]

In the research, this is obtained positive results are obtained with a formation ring. This is based on the ability of triterpenoid and steroid compounds to form a color with H_2SO_4 in a solvent, such as acetate anhydride. Differences in the color produced by triterpenoids and steroids are due to the different groups on the C-4 atom.[23]

Tannin, a phenolic compound, is soluble in water and polar solvents. Its presence is tested using $FeCl_3$, which reacts with hydroxyl groups in tannins to form a green complex via coordinate covalent bonding between iron ions and nonmetal atos.[24]

Concentration Testosterone

Testosterone regulates spermatogenesis, sperm maturation, and male libido. In this study, serum testosterone was measured via ELISA on days 0 and 30. Results showed no significant difference ($p > 0.05$) between groups, with concentrations remaining within the normal range (0.66–5.4 ng/mL). Thus, 96% ethanol turmeric extract had no effect on testosterone levels or libido. 25 Testosterone levels in men vary widely due to secretion timing, diurnal cycles, glucose intake, and seasonal changes. Levels peak in the morning and decline by afternoon, with drops up to 15%, or over 50% in younger men though this is not consistently supported by studies. Genetic

factors and Sex Hormone Binding Globulin (SHBG) also influence testosterone fluctuations, including changes observed between day 0 and day 30. [26]

Antifertility compounds from plants act via cytotoxic and hormonal pathways. In this study, serum testosterone levels remained within the normal range, indicating that 96% ethanol turmeric extract likely works through cytotoxic effects, not hormonal disruption. This is supported by evidence of cell death via apoptosis. [27] The antifertility mechanism primarily involves apoptosis a programmed, controlled process that does not cause permanent effects or damage to normal cell function. [28]

Sperm Motility

Sperm are taken from the epididymis part cauda, where in the part This is place spermatozoa maturation before Ready ejaculated. It is estimated that the spermatozoa that are most ripe in the horse epididymis. [29]

An important part of spermatozoa movement is the neck. In the part neck, there are mitochondria, which are a source of energy for sperm that are ATP producers. Sperm movement involves molecular dimensions, namely protein macromolecules found in the parts of the axoneme tail of sperm. Molecule in the morning has its own ATPase activity. By ATPase, ATP is hydrolyzed into ADP and phosphate. The energy produced from this ATP hydrolysis is what will then be used for spermatozoa movement. [30]

Infertility in men can happen if the spermatozoa are not motile. Sperm motility was introduced as a factor important in successful fertilization naturally and experimentally. In fertile individuals. Spermatozoa motility is related directly to the ability to fertilize. [31]

Research result; This shows that giving extract can cause disturbance in spermatozoa motility, visible from the percentage motile spermatozoa motility test animal group dose medium (50mg/ kgBW) and high (100mg/ kgBW) loss of 60%. Motility is said to be normal when 50% of the spermatozoa are moving. progressive. This is supported by the statement of the Harvard MIT Division of Health Science and Technology, that spermatozoa must have adequate motility (> 60 %) to pass the female genital tract, and its ugliness. Sperm motility (<60%) is one of the reasons from the amount causes of infertility in men. The most effective dose in giving a decline in percent motility lowest in research. This is at a dose of 50mg/ kgBW. It is seen a decline in motility of 35.10% is seen % which is not significantly different significant with a decline in motility at a dose of 100 mg/ kgBW, which is by 22.90%. [32]

Mechanism; The extract of ethanol 96% turmeric to decline spermatozoa motility has not been known, but a number of researchers report that immobility of spermatozoa caused by the compounds in plants can result in the form of death, damage to cell membranes, a decrease in ATP, and damage to chromatin. Decreased spermatozoa motility is suspected due to the existence group of compounds in the soil inside the extract. [33]

It is known that soil has activity, among others can coagulate protein. Use of compound: The active ingredients in bitter melon leaves can be used as an antifertility in animals. Tannin apparently hinders protein synthesis. Presumably, dynein protein experiences damage due to the presence of compound tannin, so the mechanism of energy liberation for impaired sperm motility. This protein is important because it has ATPase activity that functions to maintain internal homeostasis for Na-K ions. [34]

Tannins also have properties toxic to spermatozoa. Tannins are astringents that cause the occurrence of cell shrinkage, so they can be influential to the permeability of the sperm cell sperm. The disruption permeability membrane also affects the viability of sperm, because the need for nutrition is disturbed, which results in the turn off of sperm. It can be concluded that giving extract ethanol 96% turmeric with doses 50mg/ kgBW and 100 mg/ kgBW can lower spermatozoa motility significantly compared to the control, but there is no difference between the second dose, so the best dose for lower spermatozoa motility in this research namely 50 mg/ kgBW.

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CONCLUSION

Based on the results of research that has been done, we can draw the following conclusion as follows: Screening Phytochemicals, The chemical compounds in the 96% ethanol extract of Turmeric (Curcuma longa Linn, syn. Curcuma domestica Val.) are compounds of the alkaloid, triterpenoid, saponin, tannin, and flavonoid groups.Giving of 96% Turmeric Ethanol Extract (Curcuma longa Linn, syn. Curcuma domestica Val.) did not affect testosterone levels in male rats in vivo at doses of 25 mg/KgBW, 50 mg/KgBW, 100 mg/KgBW, with a significance value of p 0.317 > 0.05, which means there is no significant difference.Giving extract ethanol 96% of Turmeric (Curcuma longa Linn, syn. Curcuma domestica Val.) can lower in vivo motility of male spermatozoa with a dose of 25 mg/ KgBB, 50 mg/ KgBB, 100 mg/ KgBB, with a real difference if compared to with control group with significant difference (p 0.00 < 0.05)

PRACTICAL IMPLICATIONS

For further research, it is hoped that further observations will be made regarding the potential of turmeric ethanol extract by conducting histological tests, and a mating test will be carried out as evidence of whether or not the treated rats are fertile.

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