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by Salni Salni

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The Efficacy of Temu Putih Fraction (*Curcuma Zedoaria* (Berg) Roscoe) Related Quality and Quantity of Spermatozoa in Male Wistar Rats

Tiara Fatrin[1#], Salni[2], Sri Nita[3], Triwani[3], Joko Marwoto[3], Ziske Maritska[3], Rachmat Hidayat[4], Mgs Irsan Saleh[5], Nita Parisa[5], Nia Savitri[5], Evi Lusiana[5], Debby Handayati Harahap[5], Ayeshah A Rosdah[5]

[1] Biomedical Science Student, Faculty of Medicine, Universitas Sriwijaya
[2] Department of Biology, Faculty of Science, Universitas Sriwijaya
[3] Department of Biology, Faculty of Medicine, Universitas Sriwijaya
[4] Biomolecular Laboratory, Faculty of Medicine, Universitas Sriwijaya
[5] Department of Pharmacology, Faculty of Medicine, Universitas Sriwijaya

 # Correspondence : tiara_fatrin88@yahoo.com

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Abstract

Background
Male participation in KB is still relatively low wh compared to the participationof women. Researchers have to do research to find the contraception drug. Temu putih *(Curcuma Zedoaria* (Berg) Roscoe*)* is one of traditional herb that used as antifertility.

Aim of Study
Aim of this study to examine change in the amount, motility, morphology, and viability spermatozoa male rats (*rattus norvegicus)* due to temu putih fraction suplementation.

Methods
This study was an experimental study using a completely randomized design (CRD), post test with control group design. The sample in this study was 30 male rats, 10 weeks old, weight 150-200 gram. Rats were given temu putih fraction (n hexan, etylacetate and methanol-water) at dose 300 mg/kgBB/day for 48 days. Temu putih was extracted by ethanol and did fractionation by liquid-liquid methods. The results of this study were assayed by SPSS 18.

Results
The amount motility, morphology and viability of spermatozoa in the group of metanol fraction of water decreased compared with the control group (p= 0,000), motilitas of spermatozoa in the group of metanol water fraction decreased compared with the control group.

Conclusion
Temu putih fraction can reduce the amount, motility, morphology, and viability of spermatozoa in male rats.

Keywords: *Fraction, Temu Putih, Amount of spermatozoa, Motility of spermatozoa, Viability Of Spermatozoa*

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Background

Population problems remain an important issue, as it is closely related to the aspects of population quantity control, population quality improvement and population mobility direction, and it has potention to become uncontrol population growth. Keluarga Berencana (KB) is a programme from Indonesia government to control the growth of populations. KB has a goal to increase the quality of life in family by forming qualified small family. Survei Demografi Kesehatan Indonesia (SDKI) 2012 showed the amount of KB participant was 75.025 and in 2013, the amount of participant was 63.945.[1]

Female contraceptive methods is much greater than male contraceptive methods. The female method is 93.66%, while the male method is only 6.34%. The participation of men in using contraceptives is still very small. The use of contraceptives is still dominantly done by women.[1] Limitations of contraceptive methods is one of the main reasons for lowering participation of men in family planning. The ideal contraceptive device for men should be able to prevent fertilization, safe, fast performance, no side effects, and does not affect the potential for sex or libido. Researchers continue to develope in order to find the ideal method of contraception. One of the things that can be developed today is the use of Indonesian natural medicinal plants as an alternative to male antifertility.[2,3]

Various methods are being developed to reduce male fertility by the use of antifertility compounds, both of which can decrease the number of spermatozoa as well as those associated with hormone regulation. One of the most widely grown herbs in Indonesia and used for traditional medicine is temu putih [Curcuma zedoaria (Berg) Roscoe]. Temu putih (Curcuma zedoaria (Berg.) Roscoe) is included in the Zingiberaceae family, which has a chemical content of 1-1.5% essential oils, curcumin, gum, resin, starch, and tannins. Temu putih rhizomes contain saponins, flavonoids, and polyphenols. Other compounds are also found in temu putih rhizomes such as: tannins, glycosides, triterpenoids and alkaloids.[4-6]

Alkaloids can affect the weight of the testes, the secretion of the reproductive hormones that necessary for the spermatogenesis process, the essential oil does not work in the spermatogenesis process but in sperm transport, the flavonoids can agglomerate the sperm thereby decreasing the motility and sperm life. Temu putih extract (C. Zedoaria) can affect spermatogenesis of mice by decreasing spermatogonia, spermatocyte, spermatid, and spermatogenic cells, and decreasing the quality of mouse spermatozoa by decreasing motion speed, motility and viability. Temu putih extract doses intake of 300 mg / kg BW / day significantly affected spermatogenesis and quality of spermatozoa.[7-9]

Methods

The research design was experimental study, post test with control group design. The study had been approved by bioethic humaniora Faculty of Medicine Sriwijaya University .

Preparation Extract and Fraction of Temu Putih

Temu putih was provided by Indonesia Traditional Herbal Research Center, Tawangmangu, Central Java, Indonesia. Temu putih was washed, dried and drilled. After that Temu putih was extracted by maceration methode used methanol and it would get temu putih extract. After that extract was added aquadest (7:3). Fractination was done by liquid-liquid fraction method. N-hexan was added to extract methanol-aquadest (1:1) in separator tube and became two layer, the lower layer was collected as n-hexan fraction. After that, ethylacetate was added to extract methanol-aquadest (1:1) in separator tube and became two layer, the lower layer was collected as ethylacetate fraction and the upper layer was collected as methanol-water fraction.

Procedure of Experimental

Thirty rats were used in this study. Inclusion criteria were male Sprague Dawley Rats , eight weeks old, weight 150-200 gram and health. Rats were devided into 5 group, every group 6 rats, group 1 : control group, group 2 : rats were given temu putih extract 300 mg/kgBW for 48 days, group 3 : rats were given temu putih n-hexan fraction 300 mg/kgBW for 48 days, group 4 : rats were given temu putih etylacetate fraction 300 mg/kgBW for 48 days, group 5 : rats were given temu putih metanol-water fraction 300 mg/kgBW for 48 days.

Quantity and Quality of Spermatozoa Assay

Spermatozoa was taken in the cauda section of the epididymis on the right side, then cauda epididymis was placed in a petri dish containing 0.9% 1 ml NaCl and cut into small pieces and then allowed 1-2 minutes to allow the spermatozoa to escape from the epididymis.

The amount of spermatozoa was assayed by Improved Neubaeur (hemocytometer). Around 10 µL sperm suspension was taken with a pipette, then placed inside the hemocytometer and then covered with a cover glass after which it was allowed 10-15 minutes for the sperm to be absorbed and settled in the calculation plane. The calculation of sperm numbers is done by 400x magnification using a light microscope. At the center of the hemositometer counting space there are 25 large plots. Once obtained the number of spermatozoa then multiplied by 1 million. The commonly used for the total number of spermatozoa is million / ml.[11]

The sperm motility is measured by looking at the sperm velocity in the count chamber neubaure. One drop of sperm suspension in a 0.9% NaCl solution dripped on the count chamber was then observed under a 400 times magnification microscope. The number of motile sperm was quickly calculated based on the WHO criteria, namely; Progressive motility (PR): the spermatozoa move actively, either linearly or in a large circle, regardless of speed. Non-progressive motility (NP): all other patterns of motility in the absence of progress, such as swimming in small circles, flagellar strength barely displacing the head, or when only flagellar beats can be observed. Immotility (IM): no movement. Observations made on 200 sperms, then repeated as much as 3 times for one rat and the result is averaged. The sperm motility is expressed in percent units. The percentage of motile sperm count was determined by summing the PR + NP category, divided by the number of categories PR + NP + IM then multiplied by 100%.[11]

Sperm morphology was observed from the smear preparations made on a clear glass object by dripping one drop of sperm suspension. After drying the preparation, it was fixed with 40% methanol for 5 min. Then it was rinsed with aquades and dried. Then the object glass wass dropped with 3% giemsa dye and left for 30

minutes, then rinsed again with tap water and dried at room temperature. The observations were performed with a 400 times magnification microscope of 200 sperm per treatment group, the results expressed in percent.[11]

The viability of sperm was observed using Eosin Y dye. It was dripped on the tip of the object glass and then added 1 drops of semen of rat (10µl), homogenized and made smear preparations. Observation of spermatozoa viability was performed on 200 spermatozoa cells under a light microscope with 400x enlargement, observed that live spermatozoa will not be colored by Eosin Y but dead spermatozoa will be reddish due to damage to plasma membrane of spermatozoa cells. Determination of spermatozoa viability is expressed in percent 100%.[11]

Phytochemical Analysis

The sample solution was bottled using capillary tube on Silica GF silent phase 254 which wass activated by heating at 105ºC - 110º C for 1 hour then eluted with methanol: chloroform phase (1:39) v/v. Chromatogram results were observed in UV254 nm. Spotting is detected by H_2SO_4 spray.

Analysis of Data

The results of this study were assayed by SPSS 18. Data was assayed for bivariate and multivariate analysis. Bivariate analysis was used T test and multivariate test was used pos hoc test.

Results

The Efficacy of Temu Putih in Quantity and Quality of Spermatozoa

There was a decreasing in the amount of spermatozoa in the group 2 (extract), group 3 (n-hexane fraction), group 4 (ethyl acetate fraction) and group 5 (methanol water fraction), but the most significant decreasing spermatozoa in group 5. Methanol water fraction decreased the amount of male rat spermatozoa, and in the fraction of n-hexane, spermatozoa more than the other treatment groups but still lower the number of spermatozoa significantly from the control group.

Table 1. The Efficacy of Temu Putih in Amount of Spermatozoa

Group	Treatment	Mean of Spermatozoa Amount (million/mL) ± SD	p Value for ANOVA test
1	Contol	80,77 ± 1,761	0,001
2	Temu putih extract 300 mg/kgBW	39,72 ± 1,230**	
3	Temu putih n hexan fraction 300 mg/kgBW	64,12 ± 1,816 **	
4	Temu putih etylacetate fraction 300 mg/kgBW	49,57 ± 3,424**	
5	Temu putih methanol-water fraction 300 mg/kgBW	30,02 ± 3,100 **	

**p<0,05 pos hoc bonferroni test, vs Group 1

There was a decreasing in the motility of spermatozoa in the group 2 (extract), group 3 (n-hexane fraction), group 4 (ethyl acetate fraction) and group 5 (methanol water fraction), but the most significant decreasing of motility spermatozoa in group 5. Methanol water fraction decreased the motility of male rat spermatozoa.

Table 2. The Efficacy of Temu Putih in Motility of Spermatozoa

Group	Treatment	Mean of Motility of Spermatozoa (%) ± SD	p Value for ANOVA test
1	Contol	80,88 ± 1,994	0,001
2	Temu putih extract 300 mg/kgBW	39,80 ± 1,289 **	
3	Temu putih n hexan fraction 300 mg/kgBW	64,04 ± 1,724 **	
4	Temu putih etylacetate fraction 300 mg/kgBW	49,35 ± 3,502 **	
5	Temu putih methanol-water fraction 300 mg/kgBW	30,00 ± 3,095**	

**p<0,05 pos hoc bonferroni test, vs Group 1

There was a decreasing in the morphology of spermatozoa in the group 2 (extract), group 3 (n-hexane fraction), group 4 (ethyl acetate fraction) and group 5 (methanol water fraction), but the most significant decreasing of morphology spermatozoa in group 5. Methanol water fraction decreased the morphology of male rat spermatozoa.

Table 3. The Efficacy of Temu Putih in Normal Morphology of Spermatozoa

Group	Treatment	Mean of Spermatozoa Normal Morphology (%) ± SD	p Value for ANOVA test
1	Contol	71,55 ± 1,651	0,001
2	Temu putih extract 300 mg/kgBW	32,60 ± 1,051**	
3	Temu putih n hexan fraction 300 mg/kgBW	38,79 ± 0,939**	
4	Temu putih etylacetate fraction 300 mg/kgBW	17,47 ± 1,687**	
5	Temu putih methanol-water fraction 300 mg/kgBW	16,50 ± 0,976**	

**p<0,05 pos hoc bonferroni test, vs Group 1

There was a decreasing in the viability of spermatozoa in the group 2 (extract), group 3 (n-hexane fraction), group 4 (ethyl acetate fraction) and group 5 (methanol water fraction), but the most significant

decreasing of viability of spermatozoa in group 5. Methanol water fraction decreased the viability of male rat spermatozoa.

Table 4. The Efficacy of Temu Putih in Viability of Spermatozoa

Group	Treatment	Mean of Spermatozoa Viability (%) ± SD	p Value for ANOVA test
1	Contol	69,43 ± 4,940	0,001
2	Temu putih extract 300 mg/kgBW	41,87 ± 13,672**	
3	Temu putih n hexan fraction 300 mg/kgBW	40,02 ± 4,038**	
4	Temu putih etylacetate fraction 300 mg/kgBW	37,71 ± 4,095**	
5	Temu putih methanol-water fraction 300 mg/kgBW	30,15 ± 2,530**	

**p<0,05 pos hoc bonferroni test, vs Group 1

Phytochemical Analysis

Based on qualitative test of phytochemical component showed on extract and fractional fraction there were alkoloid component, steroid / ternoid (essential oil), and flavonoid.

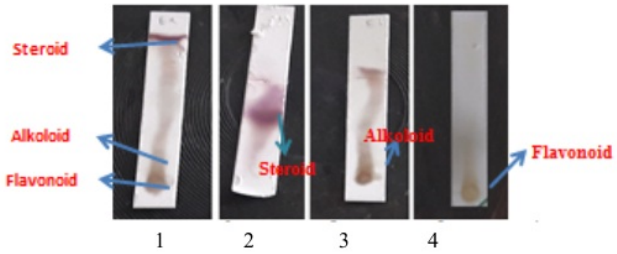

Figure 1. TLC Analysis of Temu Putih (1) extrcat (2) n hexan fraction (3) etylacetate fraction (4) methanol-water fraction

Discussion

Methanol-water fraction contained flavonoid compounds that can stimulate estrogen formation in mammals and its structure estrogenic compounds will provide negative feedback to hypothalamus-hipophysis-testis so that will decrease the level of secretion of LH and FSH hence spermatogenesis process disruption result of spermatozoa formation will be hampered. The disruption of spermatogenesis is probably due to the presence of a competitive white compound substance in the FSH (Follicle Stimulating Hormone) receptors, thus disrupting the FSH balance in the hypothalamic-pituitary axis and further inhibiting spermatogenesis. Spermatogenic decreasing can also be caused by cytotoxic substances in temu putih, thus disrupting the growth

and development of cell tissue. As a result of disruption of growth and development of this network, the number of spermatogenic cells decreased because spermatogenic cells are actively dividing cells.[12-15]

In ethyl acetate fraction, the amount of live spermatozoa decreased by 49.6 million / ml compared with the control of the possibility because ethyl acetate fraction there was alkoloid compounds that can suppress the secretion of reproductive hormone testosterone so that the testosterone levels in the blood become low , the decreasing testosterone levels can result in changes in the composition of epididymal fluid, cause decreasing the quality of spermatozoa. N-Hexan Faction experienced decreasing in the amount of live spermatozoa from the control group of 64.1 million / ml probably because the steroid compound in n-hexane fraction decreased the number of live spermatozoa caused by disruption of testosterone secretion by leydig hormone cells testosterone plays a role in maintaining the survival of spermatozoa in the epididymis. The process of spermatogenesis occurs in the testis seminiferous tubule. This process is influenced by the FSH hormone that triggers the ongoing process of spermatogenesis and testosterone play a role in activating genes in sertoli cells that trigger spermatogonia differentiation to initiate spermatogenesis process. Spermiogenesis is the process of spermatozoa formation of spermatids influenced by FSH and testosterone, FSH effect on sertoli cell proliferation that produce ABP to transport testosterone hormone which will stimulate spermatogonia to initiate spermatogenesis.[16-18]

Conclusion

Temu putih fraction can reduce the amount, motility, morphology, and viability of spermatozoa in male rats.

Acknowledgments

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