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POTENCY OF TURMERIC RHIZOME DECOCTION ON SPERM INFERTILITY OF MALE MICE TO SUCCEED FAMILY PLANNING PROGRAM IN WEST JAVA SOCIETY

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ABSTRACT

Objective: The study aimed to evaluate the effect of turmeric rhizome decoction on sperm fertility of male mice.

Methods: Ten male mice were divided randomly into two groups, control, and tested groups. The control and the tested groups were given water as vehicle and turmeric rhizome decoction at the dose 517.4 mg/kg body weight, respectively for 30 d. The mice were put under anesthesia by urethane, and the percentage of sperm motility, morphology, and sperm concentration were calculated, respectively. The results were compared with seminiferous tubule cell images.

Results: The rapid progressive sperm motility of the control and the tested groups were 53±6.4 % and 13±3.6 %, respectively; the difference was significant (P<0.001). The normal sperm morphology in both the control and the tested groups were 57.8±5.76 % and 40.8±2.72 %, respectively; the difference was significant (P<0.01). However, the differences of sperm concentration of both groups were not considered significant. Seminiferous tubule cell images of the tested group showed disruption of sertoli cells along with the present of cellular vacuolization and pyknotic nuclei.

Conclusion: Intake of the turmeric rhizome decoction at the dose of 517.4 mg/kg-bw in mice has a significant effect in decreasing the sperm motility and morphology. The decoction caused sperm abnormality or asthenoteratozoospermia. The habit of consuming turmeric decoction can succeed family planning program.

Keywords: Asthenoteratozoospermia, Sperm infertility, *Curcuma longa* L., Turmeric, Decoction of turmeric rhizome

INTRODUCTION

Indonesia is one of the five countries with the largest population in the world according to the World population data sheet of 2013. This becomes a burden for the government to support the people's prosperities. The government should make a strategic policy to solve the overpopulation problems, including family planning program. Family planning program is one of the key elements due to reducing the population growth. Population Data and Family Planning reported that the proportion of contraceptive method is 93.66 % for women and 6.34 % for men. According to gender, the contraceptive method used by women is much larger than men. Male participation in the use of contraception is still very small that the use of contraceptives is predominantly done by women. The type contraceptives in male contraceptives are vasectomy and condom [1].

Consuming traditional medicine becomes a tradition in Indonesia. Turmeric is one of the most popular ingredients and is widely used as a spice and coloring agent of many foods such as chicken curry and others. Turmeric can be processed traditionally into the form of beverage, namely jamu. Jamu is consumed to cure amenorrhea, colds, diarrhea, dyspepsia, fever, hyperlipidemia, jaundice, rheumatic, and others [2].

Several studies stated that turmeric has positive effects to humans' health. Therefore, many turmeric products are widely sold and registered as traditional medicine at the National Agency for Food & Drug Control. There are several turmeric effects, which were published e. g. the decoction of *Curcuma longa* L. rhizome at 145 mg/kg-bw is effective in reducing the blood-glucose level in rats; it had a significant effect (P<0.02) in decreasing the glucose absorption level in the intestine [3]. At the dose of 6 g had a significant effect in increasing postprandial serum insulin levels, it was suggested that it affected insulin secretion, but had not been the significant effect on the glycemic index or the response of plasma glucose [4].

Curcumin was suggested to have a palliative effect on diabetes mellitus. Curcumin at the doses of 50 and 300 mg/kg had the effect to restore glycemic indices in diabetic rats, which were induced with streptozotocin, and it was found that it increased the activity of pancreatic G6PDH and level of GSH [5]. Curcumin at a dose of 1 g/kg body weight resulted decreased intestinal motility of albino rats [6], and decreased the gastric emptying time [7]. Curcumin at the dose of 2 g/kg-bw once a week of a-two-week treatment significantly reduces atrophy of soleus muscle in rats immobilized [8]. The decoction of 3.98 g of the dried rhizome of *Curcuma longa* had antioxidant activity; EC_{50} was 18.4 µg/ml with 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [9]. Modern *in vitro* studies showed that turmeric is a potent antioxidant and other activities. Turmeric extracts remove free radicals, inhibit lipid peroxidation, and increase antioxidant enzymes. It is an anti-cancer agent, anti-inflammatory, anti-microbial, anti-mutagenic and others. Based on *in vivo* studies, both preventive and therapeutic effects found in turmeric reported that the yellow spice showed anti-cancer, hypoglycemic, hepatoprotective, anti-arthritic, and cardioprotective properties" [10].

The derivatives of curcumin which was produced by docking and modeling had activity against the infection of influenza (H1N1) virus [11]. The study reported that curcumin at the dose of 5 mg/kg body weight has a protective effect on the harmful effects of ultraviolet C radiation [12]. Another study reported that addition of curcumin at 2.5 mM during freezing resulted in positive effects on sperm parameters (DNA integrity, sperm motility, viability, and TAC/sperm total antioxidant capacity level) after thawing [13]. *In vitro* study, the addition of curcumin in different concentrations (10-40 µg/ml) to sperm suspension along with diethanolamine caused a significant increase in sperm motility and viability. The results indicate that curcumin ameliorates spermato-toxic effect on human spermatozoa from diethanolamine [14].

Turmeric contains more than 100 phytoconstituents. A volatile oil containing turmerone, which is the main component from the turmeric

root. The root also contains coloring agents namely curcuminoids which consists of curcumin demethoxycurcumin, 5'-methoxycurcumin, and dihydro-curcumin as a natural antioxidant [10, 15].

Indonesian people have a tradition of drinking jamu for a treatment of diseases or to improve their health. The treatment or health care should be maintained as a routine activity. In addition, turmeric is widely used for various purposes, especially in jamu formula. Turmeric decoction is an herbal medicine which be easily made. Registered turmeric products in the form of traditional medicine are widely sold in the market. This leads to the condition that turmeric consumptions cannot be controlled in the society. Unexpectedly, long-term turmeric consumption has not been studied. Therefore, the study of the turmeric decoction effects in the traditional medicine on sperm health in male mice is important to determine both positive and negative effects.

MATERIALS AND METHODS

Sample

Curcuma longa L. (turmeric) rhizome was identified at the School of Life Sciences and Technology, Institut Teknologi Bandung, cultivated in Wonogiri (Central Java), and harvested in October, 2012.

It has several distinctive characteristics, such as 7.5–9.0 cm length of its rhizome, 3.5–4.5 cm diameter, and 1,460.6 g weight in fresh (wet) condition or 279.27 g weight in dried condition.

Animals

All mice were acclimatized to laboratory conditions for 7 d prior to the experiments. The mice were housed under standard environmental conditions, including the room temperature, with a-12 h dark-light cycle, and allowed free access for eating standard pellet diet and drinking water.

All experimental protocols were approved by the Committee of Animal Ethics, Faculty of Medicine, Maranatha Christian University-Immanuel Hospital No. 168/KEP/V/2014 on May 22, 2014.

Ten male mice DDY strain divided into two groups, five mice (n=5) of each and 29–31 g weight.

Chemicals

E. Merck: potassium chloride, potassium dihydrogen phosphate, sodium chloride, disodium hydrogen phosphate, sodium hydroxide, methanol, acetic acid. Acetic buffer pH 2.9; phosphate buffer saline (PBS) pH 6.8, reagents for haematoxylin-eosin staining.

Equipment

Microscope (Olympus, CX21LEDFS1), Haematocytometer Neubauer, balance (Sartorius 2442), micropipette (Ecopipette by CAPP), surgery equipment, Decoction equipment. Petri disk. Microtome (Reichert-Jung 820 II).

Preparation of turmeric rhizome decoction (as tested solution)

Traditional human dose: 3.98 g dried turmeric [2].

Dose for mice = 3.98 g × 0.0026 = 10.348 mg/mice or 517.4 mg/kg body weight of mice. The decoction of the dried turmeric *rhizome* with the dose of 517.4 mg/kg in 25 ml water was made. The decoction is prepared by boiling at 90 °C for 30 min, and water is added to the decoction until the volume is 25 ml.

Urethane solution for a mouse (20 g)

Anesthetic solution is made by dissolving 0.039 g of urethane in 0.5 ml water for injection [15].

Procedure

Sperm analysis

The assessment of sperm motility, morphology and concentration were done according to the WHO laboratory manual protocol for the examination of human semen and sperm-cervical mucus interaction [16].

Semen collection

Mouse sperms were collected by slicing the epididymis in 10 ml phosphate buffer saline (PBS) pH 6.8.

Sperm motility analysis

10 µl of the sperm suspension was placed on the semen analysis chamber (Haematocytometer Neubauer). A maximum of six microscopic fields was assessed to evaluate sperm motility on at least 200 sperms for each animal.

Sperm morphology analysis

For the analysis of morphological abnormalities, sperm specimen was smeared on clean and grease-free slides and allowed to air-dry. The slides were stained with 1% safranin in water and 0.25% violet crystal in acetic buffer pH 2.9, and then examined at 400 × for morphological abnormalities in each sample.

Sperm concentration analysis

10 µl of the sperm suspension was placed on the semen analysis chamber (Haematocytometer Neubauer). A maximum of sixteen microscopic fields was assessed to evaluate sperm concentration.

The equation: 0,100 mm^3 × 10.000 mm^3× total sperm account in 16 boxes × ml of dilution

Statistical analysis

The result of each parameter from control and tested groups were statistically analyzed with Student T test.

Histology of mice testis analysis

The mice testis of tested and control groups were fixed in formalin. Then histology slides were made on histology protocol and stained with haematoxylin and eosin [17].

The images of seminiferous tubules from both groups were observed to find the changes in the histoarchitecture.

RESULTS

Due to examine the effect of turmeric rhizome decoction on sperm fertility, a distinctive test was performed. The examination used three parameters, i.e. sperm motility, morphology, and concentration.

Table 1: Sperm motility, morphology, and concentration comparison between control group and turmeric rhizome decoction (517.4 mg/kg-bw) group

Parameters	Control	Turmeric rhizome decoction
Sperm motility (%)	53±6.4	13±3.6*
Sperm morphology (%)	57.8±5.76	40.8±2.72**
Sperm concentration (10^6/ml)	27.06±1.71	26.26±2.45

Data are presented as mean±standard error mean (n=5). Asterisk (*) indicated p<0.001 and (**) indicated p<0.01 compared to control.

The first parameter is sperm motility. The rapid progressive percentage of the control group was higher than the tested group with the result was 53±6.4 % and 13±3.6 %, respectively. The differences of both groups' sperm motility were considered statistically significant (P<0.001).

The second parameter is sperm morphology that the group control percentage of sperm morphology was higher than the tested group with the result was 57.8±5.76 % and 40.8±2.72 %, respectively. The differences of both groups' sperm morphology were considered statistically significant (P<0.01).

The third parameter is sperm concentration. The sperm concentration of the control group was higher than the tested group; these were 27.06±1.71 and 26.26±2.45 million, respectively. However, the differences of both groups' sperm concentration were not considered statistically significant).

All parameters of sperm fertility of both groups showed that control group was higher than the tested group (table 1). This result showed that turmeric rhizome decoction at the dose of 517.4 mg/kg-bw has the effect on sperm fertility.

To support this result, the histology analysis was done in mice seminiferous tubules. The histology images of mice testis seminiferous tubules of control and tested groups were shown in fig. 1 and fig. 2, respectively.

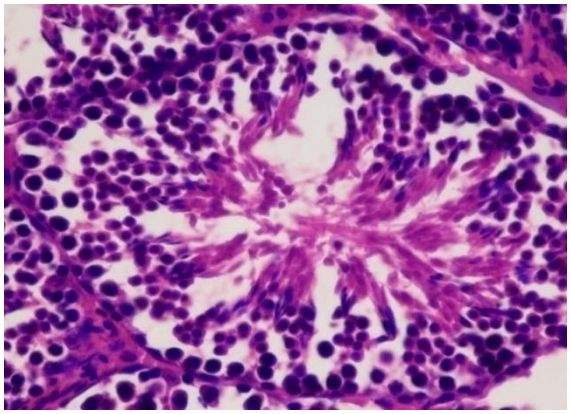

Fig. 1: Seminiferous tubule of the normal control mice group showed the normal spermatogenesis (from spermatogonia at the basal lamina of the epithelium until spermatozoa in the lumen) (H & E × 400) (n=5)

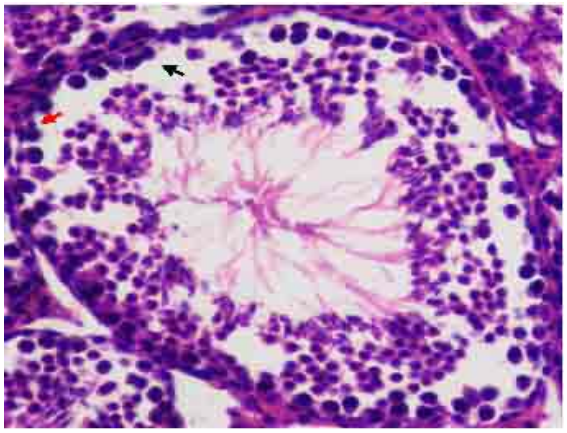

Fig. 2: Seminiferous tubule of the tested mice group showed the disruption occurs in seminiferous tubules and spermatogenesis that are presented by cellular vacuolization (black arrow) and pyknotic nuclei (red arrow) (H & E × 400) (n=5)

The disruption occurs in seminiferous tubules and spermatogenesis that are presented by cellular vacuolization (black arrow) and pyknotic nuclei (red arrow) (fig. 2). This condition causes the disruption of sperm parameters, which is shown by decreases the percentage normal sperm morphology and sperm concentration.

DISCUSSION

In testis, the seminiferous tubules are lined by germinal or seminiferous epithelium. The seminiferous epithelium contains two cells, germ cell for spermatogenesis and sertoli as supporting cells. Sertoli cells have important roles in sperm development and maturation of complete processes of spermatogenesis (fig. 1) [17-19].

The fertility parameters in this study are sperm morphology, concentration, and motility, which were influenced by the process in testis. All parameters of the control group were higher than the tested group (table 1) and histology analysis (fig. 1 and 2) showed disruption in the seminiferous epithelium. Both results showed that turmeric rhizome decoction at the dose of 517.4 mg/kg-bw cause effects on the process in testis or spermatogenesis.

Effect of turmeric rhizome decoction at the dose of 517.4 mg/kg-bw on spermatogenesis was caused by phytoconstituents in decoction, which penetrated into seminiferous tubules or sertoli cells. Later phytoconstituents caused disruption of sertoli cells secretion and constituent from the lumen of seminiferous tubules. Sertoli cells secretion and constituent from the lumen of seminiferous tubules are important to support spermatogonia, spermatocyte, spermatid, and spermatozoa to live and grow in spermatogenesis. Disruption in their lives and growths led to higher spermatozoa quantity with abnormal morphology and low sperm concentration. Disruption in seminiferous tubules could be detected by cellular vacuolization (fig. 2) and pyknotic nuclei (fig. 3), and the decreasing of normal sperm morphology and concentration in tested groups is depicted in table 1.

Spermatozoa from seminiferous tubules enter the epididymis; the processes in epididymis cells have important roles in spermatozoa maturation. The sperm was removed from the seminiferous tubules and enter to the epididymis. The sperm lacks the ability to swim forward (motility) and fertilize an ovum. In the epididymis, the sperm develops the capability of motility. The epithelial cell secretions and constituents of the luminal fluids of epididymis cell increase the potential for sperm motility and fertility [19].

The percentage reduction of sperm motility of the tested group was not only caused by the process in seminiferous tubules but also was caused by the process in the epididymis which the sperm cannot develop the capability of motility or the maturity (table 1). The decreased ability of sperm motility in the tested group when compared to the control group may occur due to disruption of epididymis cells (table 1). The disruption was caused by the effects of turmeric phytochemical compounds that penetrated in epididymis cells, which led to disrupt the luminal fluid secretions and constituents of the epididymis cells.

In this study, the sperm motility, sperm morphology, and concentration sperm decreases were described. These conditions were caused by the phytoconstituents of turmeric rhizome decoction that penetrated into sertoli cells and epididymis cells and disrupt both cell's functions.

The results revealed that the turmeric rhizome decoction at the dose of 517.4 mg/kg-bw in mice has the significant effect in decreasing the sperm motility percentages or asthenozoospermia ($P<0.001$), and sperm morphology or teratozoospermia ($P<0.01$). However, on the sperm concentration was not considered significant.

The results showed that the turmeric rhizome decoction at the dose of 517.4 mg/kg-bw which was given to mice during a-30 d treatment reduces the sperm fertility.

CONCLUSION

The histology images show the disruptions of spermatogenesis. These images support the results of this study that identified the lower level of sperm motility, normal sperm morphology, and sperm concentration.

Turmeric rhizome decoction at the dose of 517.4 mg/kg-bw that given to mice orally during a-30 d treatment showed the significant effect to decrease sperm motility and morphology. The turmeric rhizome decoction causes mice sperm abnormality or asthenoteratozoospermia condition.

The turmeric rhizome decoction becomes a strategic prospect to develop a male oral contraception. It comes from the habit of drinking turmeric rhizome decoction that can succeed family planning program.

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CONFLICT OF INTERESTS

Declared none

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