Combination Formulated Herbals (*Pimpinella alpina*, *Eurycoma longifolia*, *and Curcuma xanthorrhiza*) Increases Number and Improves the Quality of Sperm in Male Rat

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***Abstract*—Number fertility cases increased significantly within the decade all over the world including Indonesia, therefore finding a new agent which can stimulate or improve fertility condition is very important. In the present study, we explore effect of combination formulated herbals (*Pimpinella Alpina, Eurycoma longifolia, Curcuma xanthorrhoea) which is used traditionally in Indonesia as anti infertility agent.* Twelve male wistar rats were divided into 2 groups control and treatment for 2 weeks. Rat was treated with formulated herbal with dose concentration is 0.009 g/rats via gavaging, then rats were sacrificed under ether anesthesia. Epididymis was excised and sperm was collected, stained using 2% for exploring changes in motility and viability of sperm under a light microscope. There was significant increment of motility by 13% and number of sperm by 40% compared to control, however there is no change in viability in both groups. Simultaneously, formulated herbals increased the number and motility (quality) of the sperm and potentially in solving infertility problem.**

***Index Terms*—formulated herbals, mobility, viability of sperm**

I. Introduction

Sperm abnormality causes and leads a serious physic and mental health problem among young as well as old men. In the world infertility for aged persons also involved other factors such as; degenerative disease, worse injury, and stress [1]. In Indonesia, Infertility cases caused by sperm abnormality are increasing within the decade, therefore finding a new agent which can stimulate or improve sperm quality and quantity is very important.

Sperm quality was determined by its capability to move along the female genital tract and its motility has a positive correlation with ROS control balance. Zandieh *et al*. 2017 had reported that there was an enhancement between the level of H_2O_2 and sperm DNA fragmentation in the unexplained infertile group [1]. Besides, reduced sperm motility in the unexplained infertility group was significantly correlated with elevated levels of ROS. An T *et al.* 2017 had reported that sperm infertility also can be caused by gene mutation. According to gene ontology enrichment and pathway analyses, many of these different expressed proteins were associated with sperm function, including sperm binding to the zona pellucida and proteasome function; interestingly, half of these proteins were related to mitochondrial metabolism [2]. Protein-interaction networks revealed that a decrease in Cystatin C and Dipeptidyl peptidase 4 in the mitochondria may be sources of the decrease sperm motility from diabetic patients. By maintaining the ROS balance, it may contribute to the improvement of infertility cases.

Nature provides many plants which has antioxidant effects to solve the infertility problem. As tropical country, Indonesia has various plant diversity and become the mega-center of herbal medicines in the world. Indonesian since centuries ago, has been using traditional herbal medicines called “jamu” for treatment many health problems. However, the effectiveness, formulation and safety of many herbal medicine has not yet been supported by comprehensive research.

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Pimpinella alpina, Eurycoma longifolia, Curcuma xanthorrhiza have been reported traditionally used for enhancing sexual function in man. Unfortunately, the current information regarding the consumption safety of the formula made from the three herbal are still limited. In the present study, we examined a formulation which was consisted of three different kind of herbals: *Pimpinella alpina, Eurycoma longifolia, Curcuma xanthorrhiza*. In West Java,the three herbals have been reported possess an active agent and some eldest modalities for enhancing sexual function. Optimising formulation using these three herbals is believed can stimulate the sperm quality in fertility case.

Overall, understanding and analyzing the effect of the herbal formulation on number, mobility and viability of the sperms in male rats will be a new alternative treatment for infertility problem.

II. Method

A. Animal

The animal experiment protocol in the present study was approved by the Ethic Research Committee, Faculty of Medicine, Universitas Padjadjaran. Fifteen male Wistar rats at the age of 14 weeks (body weight 260 ± 10 g, n=105) used in the present study were purchased from Biofarma (Bandung, Indonesia). Rats were divided into 2 groups which were control and 2 weeks. They were kept in standard individual cages and provided with food and water *ad libitum*. The room temperature was kept at 24°C under a 12h light - 12h dark cycle (light on : 06:00-18:00). Environment was maintained stable throughout the project.

B. Drug Dosage Preparation

The drug tablet contained herbal formula (40% *Pimpinella alpina, 40% Eurycoma longifolia, and 10% Curcuma zanthorrhiza).* The drug tablets were grounded into fine particle using mortar and pestle and then, it was diluted with solvent/mili-Q. After that, they were put into microtubes and stored at room temperature for further used. The amount of drug dosages were calculated based on rats individual body weight. Based on the calculation, every rats will receive around 0.009 g of formulated herbal per day.

C. Oral Feeding

Formulated herbals were orally fed right into rats esophagus using soft gavage (plastic made) every day for 2 weeks treatment. The amount of diluted formulated herbals given was approximately 2 ml. Sodium Chloride (NaCl) 0.9% was used in this experiment.

D. Sperm Motility and Viability Analysis

As the treatment reached 2 weeks, the animals were sacrificed under Ether anaesthesia and their testicles and epididymis were excised and collected to examine the sperm mobility and viability. The organ sample then cut into pieces and put into petri dish contained 1 mL NaCl in order to obtain some caudal epididymal sperm solution. After that, around 200 µl of caudal epididymal sperm solution was taken and put into object glass then covered with cover glass for analyzing the sperm motility. The sperm were analyzed under microscope with 200x magnification. Meanwhile, another 10 µl was dropped onto an object glass and stained with Eosin spread by using the glass edge. Then, the respective sample were rinsed using Methanol and air-dried at room temperature until it was ready for sperm viability observation. After that, all the object glasses of the samples were kept for further used (3).

E. Statictics

All data were presented as mean ± standard error. A two-tailed student's t-test was used for two-group comparison involving a single continuous variable and a one-way repeated measurement analysis of variance (ANOVA) test was used for intra-subjects multiple comparison. Differences between the experimental groups were considered statistically significant at $p < 0.05$. All analyzes used SPSS 19 for Windows.

III. Result

A. Body Weight

The average of body weight on control sample was 319.28±32 g and meanwhile, for the treatment sample was 282.28±79 g (Fig. 1). Based on the result, there is no significant change of body weight between control sample and treatment sample for 2 weeks.

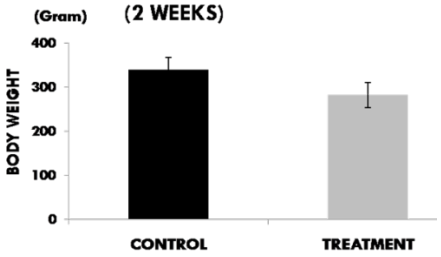

Figure 1. Formulated herbal combination doesnot change body weight after 2 weeks. Data was presented as Average ± SD with $p < 0.05$.

B. Formulated Herbal Increased Number of Rat's Sperm

There was significant increment number of sperms after 2 weeks treatment of formulated herbals experiment. Number of sperm was increased by 40% higher compared to control group. In viability, there is no significant change of control and treated group.

C. Formulated Herbal Increased Motility of Rat's Sperm

Based on the result in Fig. 3, there was significant increment in sperm motility after 2 weeks treatment. The percentage of control sample was only 6% and the percentage of treated sample was 13.33% which was higher than the control one. There is a significant increment almost 2 folds compared to control group after treatment.

Based on the result in Fig. 4, there was no significant changes in viability between control and treatment.

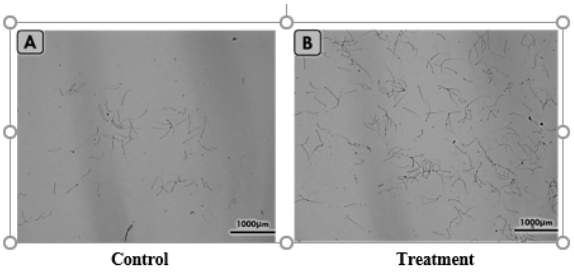

Figure 2. Two-weeks administration of herbal formula has increased number and density of rat's sperm. The images of sperm from control group (left) and treated group (right) under Axiocam microscope; magnified by 10x.

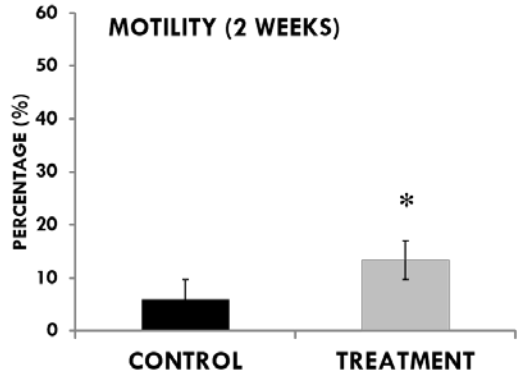

Figure 3. Formulated herbals increased the motility of rat's sperm after 2 weeks treatment. The data was presented as an Average ± Standard Error Minimum (SEM) with P<0.05.

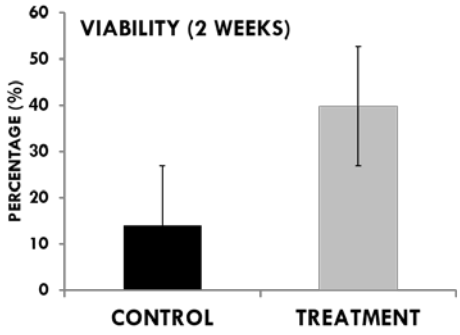

Figure 4. Formulated herbals increased viability of rat's sperm after 2 weeks treatment. The data was presented as Mean Average ± Standard Error Minimum (SEM) with $p<0.05$.

IV. Discussion

Male infertility, erectile and reproductive systems dysfunction are common health disorders during the process of male aging aside a stress that have been proven potentially affect the sexual dysfunction. A better understanding about sperm physiology and factors that affect its maturity on molecular levels will be very useful in improving male reproductive system.

A. The Effect of Formulated Herbals on Body Weight

Three type of herbals consist with *Curcuma xanthorrhiza (Java Turmeric), Eurycoma longifolia and Pimpinella alpina* may alters the body weight. One of the active compounds called Xanthorrizol is mainly exist in *Curcuma xanthorrhiza*. This component has been reported possesses a variety of biological activities such as antioxidant, antimicrobial, antihyperglycemic, anticancer, and so on. Mi-Bo *et al.* (2014) had reported whereby Xanthorrizol have protective effects against the development of obesity-induced insulin resistance and high blood glucose levels and could provide a suitable therapeutic approach to the treatment of type 2 diabetes mellitus [3]. Based on the result (Fig. 1), there is no significant body weight alteration after 2 weeks treatment of the formula. In the matter of fact, the rats did not suffers appetite alteration or high stress during treatment.

B. The Effect of Formulated Herbals on the Increment of Sperm Motility and Viability

In the present study, we used the formulated herbals consist of three different herbals (*Pimpinella alpina, Eurycoma longifolia, and Curcuma xanthorrhiza)* for stimulating and increasing male fertility. All these three herbals have similar active agent in enhancing the libido/sexual behavior : *Eurycoma longifolia.* It acts as a potential agent for reversing the effects of estrogen by increasing spermatogenesis and sperm counts in male rats [3]; *Pimpinella alpina,* a herb medicinal roots were reported as an aphrodisiac that able in increasing sexual desire and causes an erection on the respective group [4]. Lastly, *Curcuma xanthorrhiza,* it has an active compound called curcumin and it can increase sexual libidity [5].

Male fertility can be defined by sperm capability to move and fertilize the ovum in the female genitalia tract. The formulated herbal has significantly increased motility of the sperm (Fig. 3) that is an important parameter in investigating mens' infertility or its potential environmental exposures. Exposure to environmental and occupational toxicants may adversely affect sperm motility and motion characteristics, and thus affected by environmental factors during sperm development or epididymal storage. Sperm motility was resulted by depends largely on mitochondrial within the sperm mid area [6], [7]. It had been reported that several important factors that influenced sperms' flagellum movements such as : changes in ultrastructural features had reduced sperm motility [8]; a mtDNA deletion in patients with asthenozoospermia [9]; mtDNAencoded complexes (e.g., complexes I and IV) and nuclear DNA-encoded complexes (e.g., complex II) protein in the inner membrane which is affecting mitochondrial energy production partly control motility impairment [10]; or the well-known sensitivity of sperm to reactive oxygen species, xenobiotics toxicity [11]. These reports provide strong evidence that biomarkers, molecular protein and ultrastructures has correlation and play important roles to determine sperm motility. In addition, even though formulated herbal has antioxidant properties which may increase the sperm viability, we did not observe any significant change after 2 weeks treatment in our study (Fig. 4). Agarwal A et al (2008; 2014) had reported that semen parameters is associated with reactive oxygen species in men infertility case [12], [13]. Reactive Oxygen Species causes loss of sperm viability via axoneme damage which partly cause sperm

immobilization; enzymatic activities inhibition (cAMP Dependent Protein Kinase) (Lindeman, 1978); Protein methylase (Gagnon et al, 1986), Axonemal protease and Axonemal transglutaminase [14].

Moreover, this indicates that treatment for the treated groups were highly effective for increase the sexual libidity. It is more likely that the extract of the three kinds herbals helps in improving and increasing the testosterone availability in gonads/epididymis. Increase in testosterone level has been related with a significance moderate enhancement in sexual desire as well. Sperm maturation in epididymis will increase the capability of spermatozoa such as within capacity of conducting glycolysis whereby it will cause the improvement of spermatozoa motility and viability.

V. Conclusion

As conclusion, formulated herbals consisted of *Pimpinella alpina*, *Eurycoma longifolia*, *Curcuma xanthorrhiza* increased the amount, motility and viability of male rats sperm.

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