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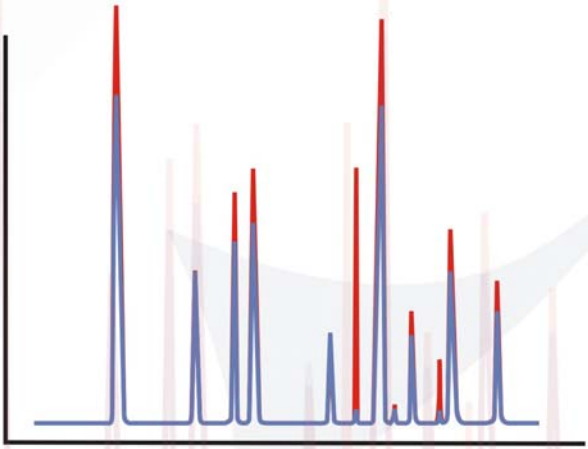

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FOREWORD

The International Conferences on Natural and Material Sciences 2009 (NAMES09) was conducted on the 3rd and 4th of July 2009 in Banjarmasin, Indonesia. The aim of the conference was to initiate international network of scientific collaboration in research and education of natural and material sciences. The conference was attended by 68 active participants that discussing diverse issues of natural and material sciences including Physics, Material and Chemistry, Applied Mathematics, Biology, Pharmacy, and Natural Products. In addition, 16 posters of research results on the topics were exhibited.

Editor

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BIOLOGY

THE EFFECTS OF EXTRACT *Piper retrofractum Vahl* EXPOSURE TO THE QUALITY OF SPERMATOGENESIS MICE (Mus musculus L) SWISS WEBSTER

***Yuanita Windusari*[(1)] *and Arum Setiawan*[(2)]**

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The research aims is studying the effects of extract Piper retrofractum Vahl exposure to the quality of spermatogenesis mice (Mus musculus L) Swiss Webster. This research has been done on June until December 2006 at Physiology laboratory, Department of Biology, Sriwijaya University. It was design using the Complete Randomized test at 5% rate of precision, then continue with Duncan's Multiple Range Test. There are four groups of mice with different treatment and each treatment was replicated 6 times. They were control by giving aquadest, treatment by giving a dosage of extract 0,25 mg/g bodyweight (bw), 0,33 mg/g bw, dan 0,50 mg/g bw. Extract was given at a volume 0,1 ml/10g bw and administrated by oral during 34 days. The result showed that extract of P retrofractum Vahl caused increase of spermatogonia average, spermatogenesis and increase quality of spermatozoon morfology , progressive motility speed, viability and progressive motility of spermatozoon.

Keywords : *Piper retrofractum Vahl, spermatogenesis*

THE EFFECTS EXPOSURE OF EXTRACT Piper retrofractum Vahl TO THE QUALITY OF SPERMATOGENESIS MICE (Mus musculus L) SWISS WEBSTER

by
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Keyword : Piper retrofractum Vahl, spermatogenesis

INTRODUCTION

Traditional medicine has been applied by public to fulfill requirement of health. Exploiting of traditional medicine in general more majored as step of preventive, though there is also effort as therapy a disease. The exclamation returns to nature (back to nature) has increased traditional medicine popularity. Usage of finite traditional medicine is based on experience result done traditionally and has not been based on circumspect research result. This thing claims doing of effort that usage of traditional medicine does not generate negative effect and can increase public health level. Scientific evidence about special quality, security and safety, and quality of tradisional medicine must be affirmed for the shake of well guaranteed of benefit clinic expected.

One of traditional medicine circulating in public is special stamina adder drug for man. This medicine contains material known as aphrodisiac, is working in hormonal and also nonhormonal. Aphrodisiac can be interpreted as drug or matter which can stimulate and increases ability of sexuality [(1)]. One of traditional medicine having special quality as aphrodisiac is cabe Jawa or *Piper retrofractum* Vahl.

P.retrofractum Vahl is one of drug crop type which many applied by Indonesian people. *P.retrofractum* Vahl included is the big ten of simplistic absorbent vegetation by traditional medicine industry, and occupies sixth rank or 95% from total simplistic consumed by inscribed has traditional medicine industry in Indonesia.

The fruit and root of this plant containing piperine and compound having the character of androgenic. Existence of piperine in nature equal to 93% there is at crop *P.retrofractum* Vahl , so that *P.retrofractum* Vahl is source of a real potential traditional aphrodisiac drug raw material [(2)]. Effect androgenic and anabolic fruit of *P.retrofractum* Vahl like piperine and essential oils contained from family Piperaceae to require further research [(4)]. This research done to know extract influence *P.retrofractum* Vahl to male sexual organ (*Mus musculus* L).

METHODS

This research executed in June- December 2006 at Animal Physiology Laboratory, Department of Biology, Mathematics and Natural Sciences Faculty, University Sriwijaya. Material applied is 48 mice (*Mus musculus* L) Swiss Webster, have never marries, age 2 month, weighing 25 - 30 g, pellet Par G for feed of mice, fruit of *P.retrofractum* Vahl, chloroform, Bouin's solution, absolute alcohol, alcohol 96 %, aquadest, physiological salt, Mayer's albumin, Hematoxylin-Eosin, toluol, xylol, canada balsam, and colourant giemsa.

Equipment applied is keeping cage of treat animal, disposable syringe 1 ml, dissection equipment, and photomicrography, equipment of making of paraffin method, flacon bottle, staining jar, hot plate, hemocytometer Neuter, stop watch, hand counter, spotting pipette, and light microscope.

Steps of research :

1. Preparation of test animal

Before given by treat white mouse is acclamation during 1 week with given by pellet and water. One day before treatment, white mouse is fasted.

2. Extraction

P.retrofractum Vahl is dried with sunshine indirectly, in blender and sieved with sieve 15 - 20 mesh. Extraction 540 g powder with ethanol 96 % 1500 cc applies mixer during 30 minutes. Result of extraction is macerate by during 24 hours,

dissociated the residue and filtrate by using Buchner funnel. Filtrate obtained packed into evaporator 40-50°C. Extract yielded thinned by using aquadest at retail 50 % [4].

3. Treatment

White mouse is grouped according to various extract doses. Based on treatment various dose is divided to become 4 group of with 6 restating. Treatment time depth is 34 days. Treatment dose at each group, that is group I (control), group II (0,25 mg/g bb), group III (0,33 mg/g bb), group IV (0,50 mg/g bb). Extract is given every day 0, 1 ml using disposable syringe [5].

4. Making of preparation and observation

Mice tested, fainted by using chloroform and cut open to be taken the testis organ and epididymis.

a. Spermatogenesis

Testis made preparation of slice with paraffin method and painting by using Hematoxylin Eosin . From every testis made three sectors. Every sector is selected 10 best slices. Each preparation is selected by 10 structure of tubulus seminiferus at random. Counted the spermatogenic cells consisted spermatogonia, primary spermatocyte cell, secondary spermatocyte cell, and spermatid cell. Observation is done with lenticular magnification 450 times.

b. Quality of spermatozoa

Spermatozoa from caudal epididymis, made sperm suspension with 1 ml condensation with temperature physiological solution 37-40°C. Parameter observed covers normal spermatozoa morphology, speed of progressive motility of spermatozoa, spermatozoa viability, and progressive motility of spermatozoa.

Normal morphology: sperm suspension dripped at object glass the preparation with wiping methods and painted with Giemsa 3%. Out of 100 sperm tails, calculated sperm percentage having normal morphology using hand counter.

Speed of progressive motility : Sperm suspensions dripped at haemocytometer calculate room Neubeur. Speed moved sperm is measured with calculating how much time required getting through two sides haemocytometer square. Set of speed expressed in µm/second. Sperm calculated is having progressive motion.

Spermatozoa viability: sperm dripped at object glass and preparation with wiping methods and the coloration with Giemsa 3%. Out of 100 spermatozoa, calculated alive sperm percentage (noncolourize) and dead sperm (red color) [6].

Progressive motility : sperm suspension dripped at haemocytometer calculate Neubeur rooms. Out of 100 sperms, calculated percentage having good motility by using hand counter.

5. *Making of Photograph Preparation*

Documentation is done by using equipment of photomicrography with ventricular magnification 10 x 10 and 10 x 40. For photograph, selected preparation representative from every group.

6. *Data Analyses*

Data obtained in the form of qualitative data and quantitative. Quantitative data is calculated to applies test ANOVA and continued [by] continuation test in the form of test DMRT (Duncan's Multiple Range Test)

RESULT AND DISCUSSION

Observation to spermatogonia

Tables 1. Giving influence of extract *P. retrofractum* Vahl during 34 days by gavage for average of spermatogonia (*Mus musculus* L) Swiss Webster.

Treatment (mg/g bb)	Averange of spermatogonia
Control	60,33 ± 8,18 a
0,25	61,67 ± 3,25 a
0,33	67,83 ± 3,29 ab
0,50	86,33 ± 4,50 bc

Tables is showed that real difference between group of controls with group of dose 0,50 mg/g bb, and between group of doses 0,25 mg/g bb and 0,33 mg/kg bb with group given dose 0,50 mg/kg bb. Increasing of dose because increase number of average spermatogonia and has not seen the happening of degradation. So inferential that extract makes positive influence to improvement of average number spermatogonia. In parallel increases dose anticipated causes number of active matters

which implied in extract is coming into body more and more. Thereby available enough material which can be utilized as trigger and proliferation contributor of cell and growth and development of spermatogonia becomes more effective.

Observation to spermatocyte

Table 2. Giving influence of extract *P.retrofractum* Vahl during 34 days by gavage for average of spermatocyte (*Mus musculus* L) Swiss Webster

Treatment (mg/g bb)	Averange of spermatosit
Control	73,17 ± 7,60 a
0,25	75,33 ± 4,71 a
0,33	86,83 ± 3,62 b
0,50	106,33 ± 4,11 c

At visible tables that at control there are no real difference compared to group of dose 0,25 mg/g bb. While between groups of controls with group of dose 0,50 mg/g bb, there is reality difference. Improvement of number of spermatocytes in tubules somniferous because of active matter which implied in extract which is estimable can increase mechanism of hormonal through process hipotalamus-hipofisis-testis with gonadotropin product increase. Gonadotropin hormone that is FSH and LH has a real important role in development of cell spermatogenic[7].

Observation to spermatid

Table 3. Giving influence of piperine from extract *P.retrofractum* Vahl during 34 days by gavage for average of spermatic (*Mus musculus* L) Swiss Webster

Treatment (mg/g bb)	Averange of spermatid
Control	127,83 ± 5,75 a
0,25	128,67 ± 9,79 a
0,33	136,67 ± 4,27 a
0,50	157,83 ± 5,52 b

Giving of dose 0,50 mg/g bb increases number of average of spermatic compared to control dose, 0,25 mg/g bb and 0,33 mg/g bb. Improvement of number of spermatic

in tubules somniferous because of active matter which implied in extract which is estimable influences cell Sterol activity under influence FSH to produce androgen binding protein (ABP) functioning to testosterone, later on applied to increase growth and development, and looks after structure and function of basal cells in tubules somniferous [8].

Observation to cells spermatogenic

Table 4. Giving influence of extract *P.retrofractum* Vahl during 34 days by gavage for spermatogenic cells (*Mus musculus* L) Swiss Webster.

Treatment (mg/g bb)	Averange spermatogenic cells
Control	276,17 ± 39,73 a
0,25	284,33 ± 28,00 a
0,33	319,83 ± 20,39 a
0,50	393,17 ± 8,47 b

Table shows the difference between groups of controls with group of dose 0,50 mg/g bb. Result from observation which has been done proves that extract can increase number of cells associations spermatogenic progressively. This is anticipated influenced by active compound content at extract causing looks after of tubules somniferous, cell Sertoli, cell Leydig, and function of seed cells, causing can increase spermatogenesis effectiveness. The difference having a meaning between structures histology tubules somniferous, network interstitial and closeness of association of spermatogenic cells control with treatment. Seen that formation of cells spermatogenic is each other squeeze one another but not overlap, this mean of the cells connected by cytoplasm bridge. Cytoplasm bridge stands in transfer of information between cells in coordination system of spermatogenesis[9]. This is showed that normal spermatogenesis after giving of extract, causing can be says that extract influential reality to spermatogenesis. The increasing of association amounts of spermatogenic cells is caused to increases the association of cells spermatogenic where this closeness tends to increase along with increasing of dose.

Observation to morphology of sperm

Table 5. Giving influence of extract *P.retrofractum* Vahl during 34 days by gavage for percentage normal morphology of spermatozoa (*Mus musculus* L) Swiss Webster.

Treatment (mg/g bb)	Morfology of spermatozoa (%)
Control	77,17 ± 3,44 a
0,25	81,50 ± 2,06 b
0,33	83,17 ± 2,27 bc
0,5	86,33 ± 1,49 c

Dose 0,25 mg/g bb gives low influence in improvement of normal spermatozoa morphology percentage. Highest influence is showed by group of dose 0,50 mg/g bb. In increasing normal spermatozoa morphology at mice, matter which implied in extract indirectly by the way of increasing spermatogenesis affectivity. Anticipated effect androgenic and anabolic happened when consuming *P retrofractum* Vahl to cause improvement of spermatogenesis affectivity. With existence of improvement of spermatogenesis effectivity, hence quality of spermatozoa also will increase. The matter works by the way of influencing protein synthesis at seed cells for the agenda of metamorphosis to form part of normal spermatozoa body.

Observation to progressive motility of spermatozoa

Table 6. Giving influence of extract *P.retrofractum* Vahl during 34 days by gavage for progressive motility of spermatozoa (*Mus musculus* L) Swiss Webster.

Treatmeant (mg/g bb)	Rate of progressive motility of spermatozoa (%)
Control	110,50± 2,81 a
0,25	138,83 ± 4,41 b
0,33	162,83± 5,05 c
0,5	243,83± 7,36 d

Dose 0,25 mg/g bb gives low influence in improvement percentage of normal spermatozoa morphology. Dose 0,33 mg/g bb shows real influence compared to dose

0,25 mg/g bb. The same as to dose 0,50 mg/g bb shows real influence with dose 0,33 mg/g bb. Improvement of spermatozoa motility with progressive motion is anticipated because of chemistry matter which implied in extract assisting energy facility essential to speed of spermatozoa. Speed of spermatozoa motility depend on movement of tail, whereas movement of spermatozoa tail depend on availability of energy from ATP decomposition[10].

Observation to spermatozoa viability

Table 7. Giving influence of extract *P.retrofractum* Vahl during 34 days by gavage for percentage viability of spermatozoa (*Mus musculus*) Swiss Webster.

Treatment (mg/g bb)	Viability of spermatozoa (%)
Control	86,50 ± 2,06 a
0,25	89,67 ± 1,11 b
0,33	91,83 ± 1,57 b
0,5	94,67 ± 1,80 c

A table is indicated that significance of influence average of normal spermatozoa morphology percentage compared to group of control. Dose 0,25 mg/g bb gives low influence in improvement of normal spermatozoa morphology percentage. Improvement of dose 0,33 mg/g bb shows different influence not real compared to dose 0,25 mg/g bb and dose 0,50 mg/g bb shows real influence with dose 0,33 mg/g bb. Improvement of spermatozoa viability percentage because of content standing in arranging spermatozoa membrane permeability. Expressing of membrane permeability of spermatozoa closely related with spermatozoa viability influencing transportation of nutrition for the life endurance[11].-Besides also, usage of extract causes the happening of addition progesterone from outside body which will be synthesis so that is formed androgen. Increasing of androgen means increasing testosterone rate followed with dilution product increase of prostate. Dilution of these prostate functions to protect spermatozoa from area that is not profits causing can increase spermatozoa viability[12].

Observation to progressive motility of spermatozoa

Table 8. Giving influence of extract *P.retrofractum* Vahl during 34 days by gavage for progressive motility percentage of spermatogonia (*Mus musculus* L) Swiss Webster

Treatment (mg/g bb)	Progress motility of spermatozoa (%)
Control	78,67± 1,97 a
0,25	82,00± 1,29 b
0,33	83,83 ± 1,86 b
0,5	86,83± 1,86 c

Dose 0,25 mg/g bb a little influence to improvement of normal spermatozoa morphology percentage. Improvement of dose 0,33 mg/g bb shows different influence unsignificant to dose 0,25 mg/g bb, while dose 0,5 mg/g bb significant to dose 0,33 mg/g bb. Improvement of progressive motility percentage is anticipated because of solvent estimable content of activation of motion of spermatozoa by increasing of ATP-ase enzymatic activity. Centered of tide spermatozoa compiled by microtubules containing substance fiber[13,14]. This fiber substance compiled by dynein protein in centre (*middle piece*) of spermatozoa cell membrane what results the happening of improvement motility of spermatozoa by maintaining ion sodium and potassium homeostatic.

CONCLUSION

Based on result of research hence inferential that exposure of extract *Piper retrofractum* Vahl to male mice sexual organ (*Mus musculus* L) Swiss Webster causes :

- Improvement average numbers of spermatogonia, spermatocyte, spermatide, and spermatogenic cells, and increases closeness of association of cells spermatogenic.
- Improvement number of normal spermatozoa morphologies, rate of progressive motility of spermatozoa, and viability of spermatozoa.

- Increasing of dose extract cause increase of spermatogenesis effectiveness, quality of spermatozoa, especially dose 0,50 mg/g bb compared to control and the other of group.

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