Effect of Red Seaweed (*Eucheuma cottonii*) Powder Administration to The Quantity and Quality of Spermatozoa of *Allethrin*-Exposed House Mice (*Mus Musculus*)

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ABSTRACT

Allethrin is an active chemical agent used in several variants of mosquito repellant products. When inhaled, allethrin is suspected to disrupt the quality of spermatozoa characterized by the reduction in amount, motility, and viability as well as the increase in morphological anomalies. This research is aimed to determine the effect of Eucheuma cottonii powder towards the quality and quantity of spermatozoa of house mice (Mus musculus) exposed by allethrin from electric mosquito repellant device. This research was carried out using laboratory experimental method with completely randomized design. The observation of the quality and quantity of spermatozoa were conducted right after acclimatization process (T_0), at day 14 (T_1), day 28 (T_2), and day 42 (T_3) after acclimatization process. Parameters of spermatozoa observed were the quantity, motility, morphology, and viability. Data were tested by One-way ANOVA and, if proven influential, were followed up by Tukey test information. The results showed that Eucheuma cottonii's powder could only increase the quantity and motility spermatozoa of mice exposed to allethrin.

Keywords: Eucheuma cottonii, allethrin, quantity and quality of spermatozoa, mus musculus

INTRODUCTION

Eucheuma is one of the red seaweed (*Rhodophyceae*) species commonly harvested and traded as a main commodity in both national and international trade. *Eucheuma cottonii* possesses macro minerals such as nitrogen, oxygen, calcium, and micro minerals such as iron, selenium, zinc, magnesium, and sodium. Contents of amino acids, vitamins, and minerals found in seaweed have been known to be as much as 10 – 20 times compared to that of the land-based flora (DKP, 2008).

Micro minerals such as calcium, zinc, selenium, magnesium, and sodium found in *Eucheuma cottonii* can be very useful as chemical compound which enhances the quality and quantity of sperm as those minerals are vital in the process of spermatozoa generation during spermatogenesis phase.

Vitamin C and E found in *Eucheuma cottonii* are valuable antioxidants. In a by Hsu *et al* (1998), it is found that intake of Vitamin E or Vitamin C as antioxidants can inhibit the formation of free radicals which prevents spermatozoa cell damage and increases motility as well as penetration capability of spermatozoid cells on mice oocyte (Sudjarwo *et al.*, 2006).

Free radicals may come from the excess of body metabolism process (internal) and from outside the body (external).

One of the chief sources of external free radicals today is pesticides (insecticides) such as electric mosquito repellants which are widely used by many people today. Many of these electric mosquito repellants contains *allethrin* as their active chemical agent. Prolonged inhalation of *allethrin*, aside from the fact that it may cause respiratory problems, may also form secondary metabolite which can act as free radical. The free radicals may spread itself via circulatory system to many areas of the body including testicles.

Testicles are vital reproduction organs in which spermatozoa are generated. If damaged, the quality of the sperm generated will suffer as well or, in other words, a state of infertility may occur.

Research by Sakr and Azab (2001) shows that *allethrin* causes histological changes of testicles, decreasing the mass and the diameter of tubulus seminiferus. This fact signifies that when testicles are damaged, formation of spermatozoa is disrupted, giving detrimental effect to the amount and quality of spermatozoa generated.

Eucheuma cottonii has been consumed as a traditional alternative which is known for its medical potency.

Various contents of antioxidant agents in *Eucheuma cottonii* such as Vitamin C and E can reduce the negative impacts of free radicals. Moreover, due to its high content of micro minerals, rmance of hormonal systems, even believed to elenium, and zinc enables *Eucheuma cottonii* to

increase both the quality and quantity of sperm.

This research is aimed to assess the effect of per-oral administration of *Eucheuma cottonii* red seaweed powder towards the quantity and quality of spermatozoa in *allethrin*-exposed male house mice (*Mus musculus*).

The expected outcome of this research is to provide scientific information in the effort of infertility prevention in males using one of the natural marine resources which is *Eucheuma cottonii*.

MATERIALS AND METHODOLOGY

This research lasted from November 2009 to March 2010 at the laboratory of Institut Bahan Obat Alam[1] Universitas Diponegoro with materials of *Eucheuma cottonii* powder, house mice (*Mus musculus*) test subjects, B11 Probiotic Poultry Supplement, and electric mosquito repellant containing *allethrin*.

Equipment and Materials

Equipment used in this research were powder-making equipments such as blenders, house mice keeping and treatment equipments, and sampling equipments for treatments on each powder dose consisting of 3 repetitive administrations with 3 mice for each repetitive administration. Feeding was given twice a day for 42 consecutive days. For each feeding, 0.8 grams of *Eucheuma cottonii* powder was added to 3 grams of pellets with 3 drops of water, 1.6 grams of *Eucheuma cottonii* powder was added to 2 grams of pellets with 3 drops of water, and 2.4 grams of *Eucheuma cottonii* powder was added to 1 gram of pellets with 3 drops of water.

Sperm analysis was performed by using hemocytometer and microscope. Materials used in this research were materials for animals keeping and treatment such as feeding as well as materials used in mice spermatozoa analysis which were physiological saline, giemsa stain, 95% alcohol, chloroform, 96% ethanol, and distilled water.

Research Methodology and Design

Samples of *Eucheuma cottonii* was collected from Karimunjawa waters using purposive method. The methodology employed in this research is laboratory experiment with Completely Randomized Design (CRD) using 3 treatments and 2 controls with three repetitive administrations for each treatment.

Collection and Preparation of *Eucheuma cottonii* Samples

Eucheuma cottonii samples were collected directly from farming site at Karimunjawa waters after which they were transported to Institut Bahan Obat Alam Universitas Diponegoro for treatment. Samples were rinsed with fresh water to remove dirt, sand, and organisms. Samples were then cut into strands of $\pm$ 1 mm in size and then were dehydrated on drying shelves covered in black screens to avoid direct contact with sunlight. Dried samples were blended and sieved through to obtain *Eucheuma cottonii* powder.

Preparation of Test Feeding

Eucheuma cottonii powder was scaled to batches of 0.8 grams, 1.6 grams, dan 2.4 grams after which the batches were put into 504 containers.

Preparation of Treatment Groups and Test Subjects

15 HDPE Plastic mouse cages with a size of 40 x 32 x 13 cm were readied at which the top of the cages were covered by net wire. Inside each cage were 5 cm-thick chaff as bedding as well as food and water bowls for the mice.

60 male house mice (*Mus musculus*) were put into acclimatization process for 7 days. Test subjects were grouped according to design of the research of 5 groups where each group consisted of 3 treatment cages with 4 mice kept in each cage.

Execution of Research

60 mice were randomly put into 5 groups, with each group consisting of 12 mice, and were housed into 3 cages to distinguish repetitive administration. All mice were measured for initial weight. All treatment groups were exposed to *allethrin* with the exception of group A which acts a a no administration of *Eucheuma cottonii* powder

in the feeding). Electric mosquito repellant containing 40 mg/mat of *allethrin* was carried out was exposed to the subject for 42 consecutive days after acclimatization process. 7 days after acclimatization process, a mouse from each treatment group was dissected for T_0 observation data. At day 8, mouse cages were divided into 3 compartments and then *Eucheuma cottonii* was added to the prepared feeding according to their treatment groups. Feeding of foodstuff containing *Eucheuma cottonii* powder was given twice a day for 42 consecutive days.

Observations of quantity and quality of spermatozoa of mice were carried out after acclimatization process (T_0), at day 14 (T_1), at day 28 (T_2), and at day 42 (T_3) after acclimatization process. Dissection of mice from each treatment group cage was conducted during the course of observations. Observation parameters sought were the quantity of spermatozoa per milliliter, percentage of motility, percentage of morphology, and percentage of viability of sperm. Observations of spermatozoa were conducted by hemocytometer and microscope with 400x magnification scale.

Analysis of Data

To determine whether per-oral administration of *Eucheuma cottoniii* powder affects the quantity and quality of sperm of *allethrin*-exposed house mice, statistical analysis with one-way ANOVA and follow-up analysis with Tukey test were performed to compare one variable with another before and after treatments were given.

RESULTS AND DISCUSSIONS

Results

Results of the research on the effects of *Eucheuma cottonii* powder towards the quantity of spermatozoa of *allethrin*-exposed house mice (*Mus musculus*) showed the tendency of *Eucheuma cottonii* powder to increase the quantity of spermatozoa particularly on day 24 and day 42 of treatment. The results are described in Table 1 as follows:

Table 1. Observation Results of Spermatozoa Quantity of *Mus musculus*

Treatment	Observation Periods							
	T_0		T_1		T_2		T_3	
	Sperm Quantity (10^6/ml)	Gain (%)	Sperm Quantity (10^6/ml)	Gain (%)	Sperm Quantity (10^6/ml)	Gain (%)	Sperm Quantity (10^6/ml)	Gain (%)
A (Control (-))	21.037 ± 4.122^{b}		22.203 ± 3.832^{a}		21.517 ± 2.835^{a}		22.667 ± 3.710^{a}	
B (Control (+))	8.74 ± 2.545^{a}	-58.45	16.260 ± 3.212^{a}	-26.77	21.15 ± 1.761^{a}	-1.71	19.667 ± 9.262^{a}	-13.24
C(Powder 0.8g)	18.407 ± 2.280^{ab}	-12.50	26.890 ± 3.836^{a}	65.38	21.3 ± 9.344^{a}	0.71	22.297 ± 5.753^{a}	13.37
D (Powder 1.6g)	16.557 ± 2.172^{ab}	-21.29	17.597 ± 3.591^{a}	8.22	24.963 ± 7.224^{a}	18.03	24.617 ± 8.730^{a}	25.17
E (Powder 2.4g)	17.427 ± 6.602^{ab}	-17.16	24.967 ± 6.885^{a}	53.55	32.187 ± 5.05^{a}	52.18	43.447 ± 5.4^{b}	120.91

Information:
- Values are deviation standard averages ±
- Similar letters after values under the same column mark insignificant differences (p>0.005)
- Gain (%) = {(Treatment value – Control value)/Control value} x 100%

Results of the research on the effects of *Eucheuma cottonii* powder towards the quality of spermatozoa of *allethrin*-exposed house mice (*Mus musculus*) showed the capability of *Eucheuma cottonii* powder to increase the motility of spermatozoa. However, the administration of *Eucheuma cottonii* powder did not result in significant effect towards the increase in morphology and viability of spermatozoa. The results are shown in table 2, 3, 4 respectively.

Table 2. Observation Results of Spermatozoa Motility of *Mus musculus*

Treatment	Observation Periods							
	T_0		T_1		T_2		T_3	
	Motile Sperm (%)	Gain (%)	Motile Sperm (%)	Gain (%)	Motile Sperm (%)	Gain (%)	Motile Sperm (%)	Gain (%)
A (Control (-))	21.023 ± 3.732^{b}		18.67 ± 6.714^{a}		20.14 ± 5.376^{c}		18.033 ± 2.961^{b}	
B (Control (+))	11.633 ± 2.557^{a}	-44.65	9.917 ± 2.765^{a}	-46.88	5.27 ± 3.231^{a}	-89.71	6.503 ± 3.582^{a}	-63.94
C(Powder 0.8g)	14.12 ± 4.831^{ab}	-32.84	11.303 ± 4.721^{a}	13.98	16.297 ± 2.243^{bc}	209.21	9.403 ± 1.8^{a}	44.59
D (Powder 1.6g)	14.983 ± 2.131^{ab}	-28.73	10.1 ± 1.994^{a}	1.85	10.263 ± 1.611^{ab}	94.74	7.96 ± 2.315^{a}	22.41
E (Powder 2.4g)	12.03 ± 1.213^{a}	-42.78	7.68 ± 2.556^{a}	-22.56	6.88 ± 2.697^{a}	30.55	4 ± 0.634^{a}	-12.73

Information:
- Values are deviation standard averages ±
- Similar letters after values under the same column mark insignificant differences (p>0.005)
- Gain (%) = {(Treatment value – Control value)/Control value} x 100%

Table 3. Observation Results of Normal Spermatozoa Morphology of *Mus musculus*

Treatment	Observation Periods							
	T_0		T_1		T_2		T_3	
	Normal Sperm (%)	Gain (%)	Normal Sperm (%)	Gain (%)	Normal Sperm (%)	Gain (%)	Normal Sperm (%)	Gain (%)
A (Control (-))	94.637± 7.187^{a}		99.31 ± 1.195^{a}		99.057 ± 1.634^{a}		99.463 ± 0.93^{a}	
B (Control (+))	98.147± 3.21^{a}	3.709	100^{a}	0.695	100^{a}	0.943	99.033 ± 1.674^{a}	-0.432
C(Powder 0.8g)	95.873± 4.356^{a}	1.306	98.767± 2.136^{a}	-1.233	99.557 ± 0.768^{a}	-0.443	99.207 ± 1.374^{a}	0.176
D (Powder 1.6g)	97.13 ± 3.336^{a}	2.634	99.307± 1.201^{a}	-0.693	98.927 ± 1^{a}	-1.073	97.97 ± 2.232^{a}	-1.073
E (Powder 2.4g)	94.393± 4.881^{a}	-0.258	99.63 ± 0.641^{a}	-0.370	99.63 ± 0.641^{a}	-0.370	100^{a}	0.976

Information:
- Values are deviation standard averages ±
- Similar letters after values under the same column mark insignificant differences (p>0.005)
- Gain (%) = {(Treatment value – Control value)/Control value} x 100%

Table 4. Observation Results of Spermatozoa Viability of *Mus musculus*

Treatment	**Observation Periods**							
	T_0		**T_1**		**T_2**		**T_3**	
	Trans parent Sperm (%)	**Gain (%)**	**Trans parent Sperm (%)**	**Gain (%)**	**Trans parent Sperm (%)**	**Gain (%)**	**Trans parent Sperm (%)**	**Gain (%)**
A (Control (-))	100^{a}		100^{a}		100^{a}		100^{b}	
B (Control (+))	99.307± 1.201^{a}	-0.693	97.907 ± 1.994^{a}	-2.093	97.293 ± 2.6^{a}	-2.707	97.597± 1.076^{a}	-2.403
C(Powder 0.8g)	99.603± 0.687^{a}	-0.397	100^{a}	2.138	98.93 ± 0.931^{a}	1.683	99.277± 0.989^{ab}	1.721
D (Powder 1.6g)	99.543± 0.418^{a}	-0.457	100^{a}	2.138	99.673± 0.566^{a}	2.446	99.103± 0.858^{ab}	1.543
E (Powder 2.4g)	99.46 ± 0.935^{a}	-0.540	100^{a}	2.138	100^{a}	2.782	99.45 ± 0.54^{ab}	1.899

Information:
- Values are deviation standard averages ±
- Similar letters after values under the same column

ol value} x 100%

DISCUSSIONS

Observation results showed that treatment groups with administration of *Eucheuma cottonii* powder experienced increase in the quantity of spermatozoa compared to positive control group which did not benefit from *Eucheuma cottonii* powder intake. Similarly, the quality of spermatozoa (motility) in that treatment groups with administration of *Eucheuma cottonii* powder showed better results compared to that of the positive control group.

Eucheuma cottonii red seaweed contains Vitamin C and E which function as antioxidant agents. These antioxidant agents are known to prevent the formation of free radicals. Free radicals may come from external source such as *allethrin* which is the active chemical compound found in mosquito repellants. Vitamin C has been known to neutralize free radicals such as that contained in mosquito repellants. Hsu *et al* (1998) conducted a research which proved that intake of Vitamin E or Vitamin C as antioxidants can inhibit the formation of free radicals which prevents spermatozoa cell damage and increases motility as well as penetration capability of spermatozoid cells on mice oocyte (Sudjarwo *et al.*, 2006).

In addition to antioxidant properties which neutralizes the effect of free radicals such as *allethrin*, *Eucheuma cottonii* powder has the potency to increase the quantity of spermatozoa due to its nutritional contents of zinc and selenium as well as the presence of arginine amino acids which are vital for healthy sperm. Nutritional contents if *Eucheuma cottonii* will increase spermatogenic cells which will eventually become spermatozoa during spermatogenesis phase.

Zinc plays an important role in increasing the quality of sperm as a part of co-factor enzymes needed for spermatogenesis. Selenium is an important element in the formation of glutathione peroxide enzymes (GSH-Px), enzymes whose main function is to counter the damage caused by the existence of hydrogen peroxide. Arginine amino acids content of *Eucheuma cottonii* is one of the vital precursors needed in testosterone hormone production which is expected to maintain the quantity and quality of spermatozoa (Haryanto *et al.,* 2001).

CONCLUSIONS

Based on the result of this research, it can be concluded that per-oral administration of *Eucheuma cottonii* powder can increase the quantity of spermatozoa of *allethrin*-exposed house mice with 0.8 gram and 1.6 gram powder treatment at day 28 and day 42. However, the per-oral administration of *Eucheuma cottonii* powder did not show significant effect towards the gain in normal morphology and viability of spermatozoa of *allethrin*-exposed house mice during the course of 42 day observation.

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