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Potency of Sandfish (*Holothuria scabra*) Powder to Increase Sperm Quality and Sperm Quantity in Mice (*Musmusculus*)

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Abstract

Objectives: This research is aimed to examine the effect of sandfish powder on the spermatozoa quality and genital weights of male mice. **Methods/Analysis:** In this study, laboratory experimental method was applied. Male mice were treated by giving sandfish powder in three dosage rates of its steroid content (10, 30, and 50µg of steroid/100g body weight) in 12 days. Whereas for control treatments as well as for comparison, the author utilizes some mature male mice without given any hormone (negative control) and some mature male mice that given methyl testosterone. **Findings**: The authors examined the quality of spermatozoa (concentration, normality, and motility of spermatozoa) and genital weight (testis and seminal vesicle weight) of each male mouse. It was discovered that administration of sandfish powder did not significantly affect its genital weights as well as normality and motility of the spermatozoa. However, it could increase the concentration of spermatozoa in administration of 10µg of steroid/100g body weight. **Application/Improvements:** The potential of sandfish powder as drug to enhance the quality of the male reproduction.

Keywords: Drug on Pharmacy, Men, Quality and Quantity of Sperm, Sandfish Powder, Steroid Hormone

1. Introduction

Sea cucumber is one of the sea creatures that contains some active compounds and has been investigated to be utilized as a food product, health supplement, depress some disease or a material for pharmaceuticalindustry[1]. Some studies showed this potential utilization. Sea cucumber (*Cucumaria frondosa*) was reported had antibacterial activity[2], antifungal disulfated triterpene glycoside from

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the sea cucumber *Psolus patagonicus*[3], isolation of arginine kinase from *Stichopus japonicas*[4], activity of serum amyloid A on the sea cucumber *Holothuria glaberrina*[5], structure of the major triterpene glycoside from the sea cucumber *Stichopus malls*[6], and isolation of fucan sulfates from the body wall of sea cucumber *Stichopus japonicas* and their ability to inhibit osteoclastogenesis[7].

Moreover, there is an indigenous knowledge in local people that sandfish (a kind of sea cucumber) has a potential as an aphrodisiac[8], it was suggested by the steroid content in the sea cucumber. In [9] stated that, sandfish powder contains steroid hormone which can be utilized as aphrodisiac. In [10] showed that steroid hormone from sandfish could be utilized for giant prawn masculinization. In [11] showed that steroid hormone from sandfish could be utilized for improve reproduction quality of man. In [12] reported that the administration of sandfish powder on male could increase their sexual behavior (kissing vagina and mounting). However, no study has been conducted on the effect of sandfish powder on the spermatozoa quality and genital weights of male mice. The study, therefore aimed to investigate the effect on the concentration, normality and motility of spermatozoa (spermatozoa quality); testis and seminal vesicle weights (genital weights). The research is expected to obtain the idea of the potency of sandfish to increase the quality as well as quantity of sperm and also as a fertility enhancer drugs for men in the pharmaceutical world.

2. Materials and Methods

Materials. The materials used for this research are mature sandfish *(Holothuria scabra)* which were collected from Bengkulu Province, Indonesia, with the body weight of 200 to 500g.

Male mice. Test organisms used for research were male mice *(Mus musculus) bulb c* type which genitally matures (approximately 2 months old). The mice were obtained from Faculty of Animal Science, Bogor Agricultural University. Before the treatment, acclimatization was required for about 7 days.

Treatment. The treatment applied was dosage of administration, i.e. 10µg/100g, 30µg/100g, and 50µg/100g of body weight of each mouse. The control used was administration of methyl testosterone hormone 42 µg/100 g of body weight[13] and without any administration of product or hormone. The solvent used was con oil. The administration of the product was applied daily for 12 days using volumetric pipette orally. Each treatment was done for 5 times replication.

The assessment of spermatozoa quality. The observation of the quality of mice spermatozoa was done for some spermatozoa characteristics, such as concentration, normality and motility. Spermatozoa of the mice were collected from cauda epididimis, which is the place for maturing and storage of spermatozoa. The collection of spermatozoa was done by cutting the cauda epididymis and pressing it gently.

Concentration of spermatozoa. The concentration of spermatozoa was measured using *hematocytometer* at *Neubauer* area under the microscope at 400 fold. The spermatozoa sampling points were four points at the side and one point in the middle. Concentration of the spermatozoa shows the number of sperm in 1mL of semen.

Normality of spermatozoa. Normality of spermatozoa was determined by dropping 2-3 drops of eosin 2% on the object glass with one drops of semen, and then gently homogenized using sterile glass bar. In order to observe the spermatozoa in dead condition, another thin preparate was prepared on the other object glass, air dried and followed by fixation using Bunsen burner and subsequently observed under the microscope at 400 fold.

Motility of spermatozoa. One drop of semen was put on the object glass and then added with 2-3 drops of NaCl solution, gently mixed using cover glass. The semen was subsequently put on the new object glass and then observed under the microscope at 400 fold. Motility of spermatozoa was measured in percentage of spermatozoa which are actively moving.

Weight of genital organ. In order to observe weight of the genital organ, male mice were dissected for isolating a part of testes and seminal vesicle. Subsequently, each sample was weighed by analytical balance scale and expressed in gram.

3. Results and Discussion

3.1 Concentration of Spermatozoa

The average of the mice spermatozoa concentration after the administration of sandfish powder is presented in Figure 1. The results showed that the highest concentration of mice spermatozoa was obtained by male mice with the administration of sandfish powder dosage of 10µg/100g body weight, which was containing approximately 208million/mL of sperm. This result showed that the administration of sandfish powder at the steroid concentration of 10µg/100g of body weight was the most appropriate concentration which resulting the proper number of hormone and nutrition. It was consequently necessary in the spermatozoa production. Therefore, the number of spermatozoa was high. Yet, the administration of the sandfish powder at the high concentration of steroid had decreased the spermatozoa concentration. This is due to the relatively high concentration of the added steroid hormone will inhibit the production of spermatozoa[14].

Concentration of the spermatozoa related to the spermatogenesis or sperm production in the tubuli seminiferi in the testis. The production of spermatozoa was initiated by the presence of *Follicle Stimulating Hormone* (FSH)[15–17], while testosterone hormone which is produced by leydig cells has important role in the formation and the development of the spermatozoa. The study showed that the administration of sandfish powder does not interrupt the spermatogenesis process; however, the concentration of steroid 10µg/100g of body weight could increase the concentration of spermatozoa.

3.2 Normality of Spermatozoa

Normality of spermatozoa is the percentage of the number of normal and abnormal spermatozoa. The results which present the normality of the spermatozoa after the administration of the sandfish powder at the different concentrations are shown in Figure 2. The average nor-

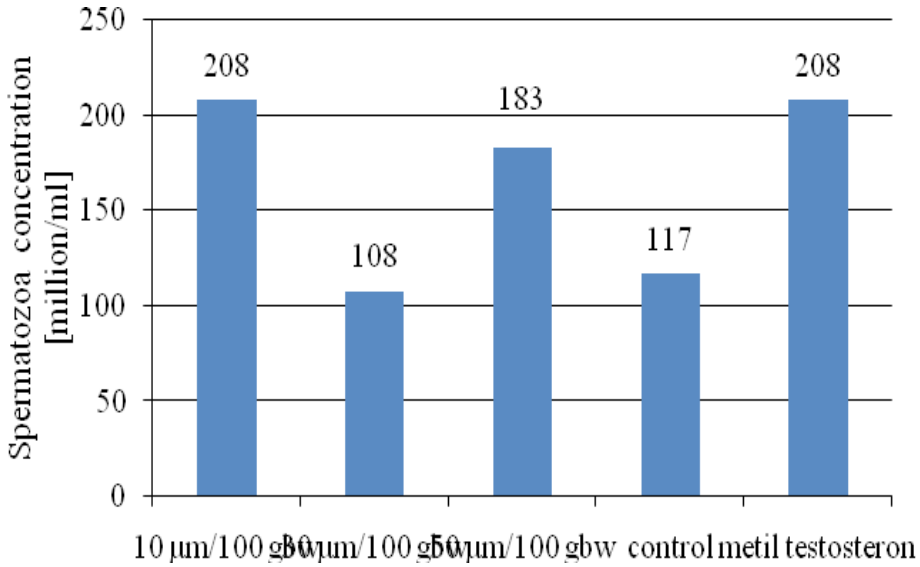

Figure 1. Concentration of the spermatozoa in male mice at the different dosages of the sea cucumber steroid and its comparison to control.

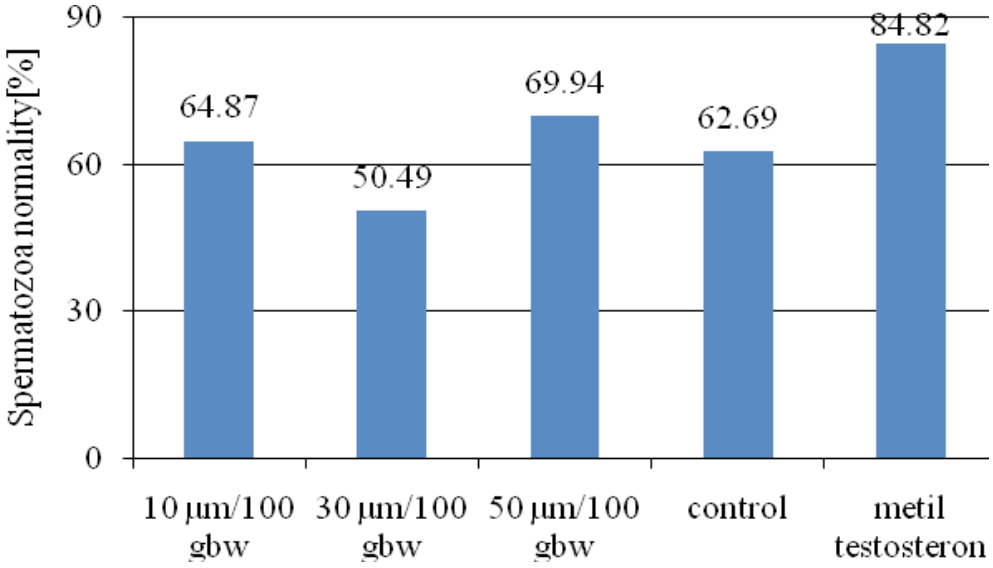

Figure 2. Normality of the spermatozoa in male mice at the different dosages of the sea cucumber steroid and its comparison to control.

mality of the spermatozoa ranged from 50.49 to 69.94%. Normality of the mice spermatozoa after the administration of sandfish powder was not significantly different with the average normality of the mice spermatozoa of the controls. This result showed that administration of sandfish powder with the steroid dosage of 10µg/100g of body weight to 50µg/100g of body weight had no effect on the normality of the spermatozoa.

Most of abnormality was cytoplasmic droplet, i.e. droplet or small bubble on the tail. The droplet was cytoplasm that will be disappeared from the spermatozoa tail during the development of spermatozoa in the epididimis to the transfer process of spermatozoa from epididimis into genital track[18]. In [19] reported that the droplet in the distal (tail of the spermatozoa) is not a serious problem, conversely if the droplet is located the proximal area (close to the head of the spermatozoa), it indicates the presence of abnormality during spermiogenesis. The occurrence of proximal droplet might indicate that the mice used in the study were still young, while in the old ones it indicates the degeneration process of epitel seminiferus. In addition, the occurrence of proximal or distal cytoplasm indicates that the semen of the mice is frequently used so that the development of the sperm was not mature enough. The study showed that abnormality mostly displayed by the droplet occurrence on the distal. This result might have been because the sperms had been frequently used, i.e. for five days' observation.

3.3 Motility of Spermatozoa

The average of motility of the mice spermatozoa after the administration of sandfish powder at different steroid concentrations are presented in Figure 3. The data showed that the average of motility of the spermatozoa was approximately 31.67% to 63.33%. This result indicated that the administration of sandfish powder at the steroid dosage of 10µg/100g of body weight to 50µg/100g of body weight had no effect on the motility of the spermatozoa.

Motility presents the activity of the spermatozoa; the higher number indicates the higher quality of the sperma-

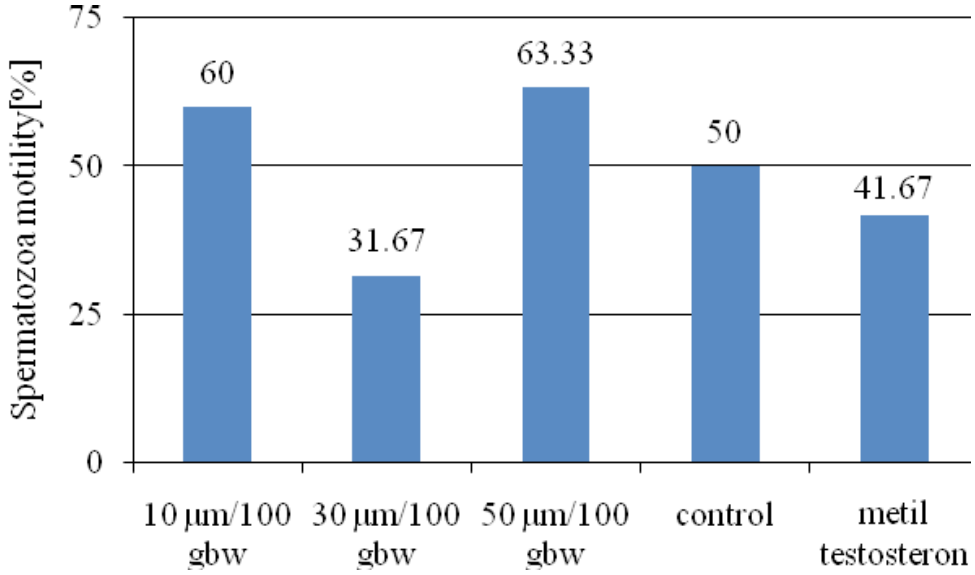

Figure 3. Motility of the spermatozoa in male mice after the administration of sandfish powder at different dosages of steroid and its comparison to control.

tozoa. Motility is the fundamental character to determine the quality and capability of the spermatozoa to fertilize the ovum[20]. Sperm which possess the high motility was required for fertilization process in the female genital track. Motility of the spermatozoa was determined by the tail of the sperm which is part of the production of energy and motility character of the sperm cells[21,22]. Tail of the sperm or flagella consists of fibrils which are contractile and generate the moving of the tail that create the motility of the sperm cells. This motility was generated by the longitudinal surf movement of the fibrils that form mitochondrial helix. Energy which was required to move was provided by the mitochondrion that was changed into kinetic energy, so that the tail cells of the sperm can move. The needed energy for the motility of the spermatozoa was from the conversion of Adenosine Tri-Phosphate (ATP) in the mitochondrial path through degradation reaction into Adenosine Di-Phosphate (ADP) and Adenosine Mono-Phosphate (AMP):

$$ATP \leftrightarrow ADP + HPO_3^{=} + Energy$$

$$ADP \leftrightarrow AMP + HPO_3^{=} + Energy$$

The alteration process reveals the energy that can be used as a kinetic energy. Organic compounds used by the spermatozoa as energy source for their motility and survival life are fructose, sorbitol, GPC (Glycerolphosphorylcholine) and plasmalogen[23]. Fructose, sorbitol and GPC are present in the semen, while plasmalogen present in the spermatozoa.

3.4 Testis Weight

The average of mice testis weight after sandfish powder administration with the different concentrations of steroid is shown in Figure 4. From the figure, it can be seen that testis weight ranged from 0.0855g to 0.1081g. The heaviest testis weight in this research was obtained by the mice after the sandfish administration with the steroid dosage of 10µg/100g of body weight, while the lowest one was obtained from the one which were given the steroid dosage of 30µg/100g of body weight. Analysis of variance

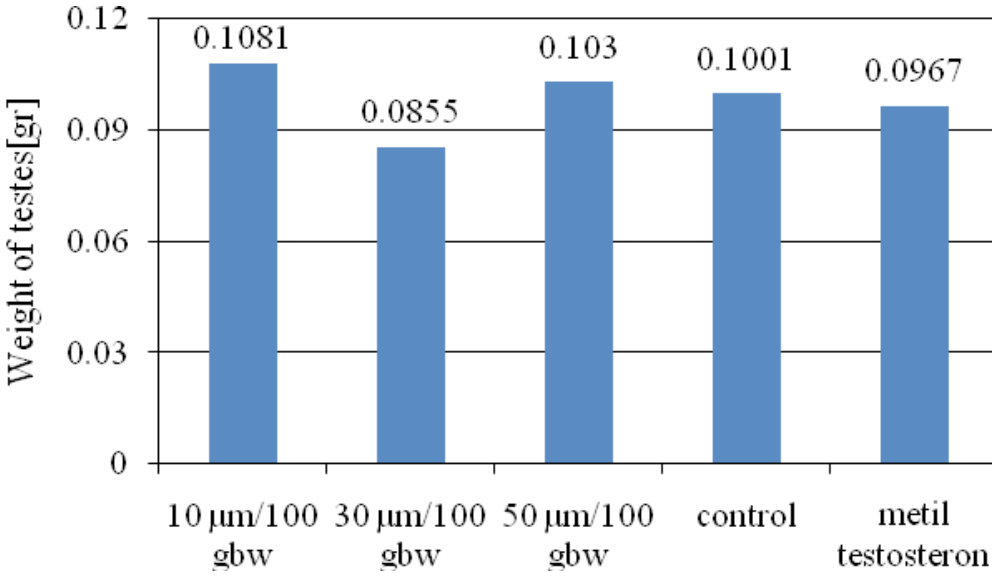

Figure 4. Weight of testes in male mice after the administration of the sandfish powder at different dosages of steroid and its comparison to control.

revealed that the administration of sandfish powder at different concentrations had no significant effect on testis weight of the mice ($p>0.05$). The same results showed by the controls and the mice after the administration of methyl testosterone.

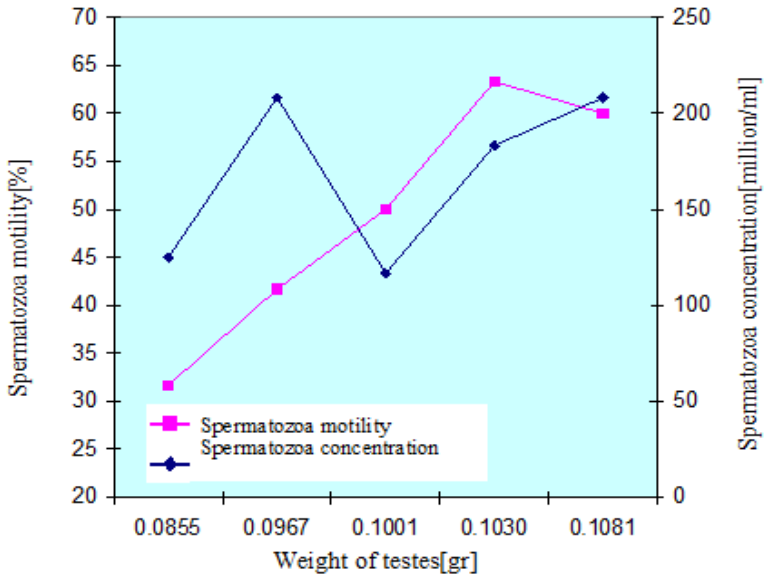

Figure 5. The correlation of testes weight and the mortality and concentration of the spermatozoa.

The data showed that the administration of sandfish powder had no effect on the testis weight. This result might be related to the maturity of the mice or the gonad. In this condition, the development of testis weight had achieved the maximum level; therefore, administration of the sandfish powder had no significant effect on the testis weight.

Testis is the primary sexual organ of male mice which possess multifunction, i.e. produce male sexual hormone or testosterone and produce spermatozoa[24]. Testosterone hormone is produced in the Leydig cells under the instruction of LH (*Luteinizing Hormone*) in the pituitary gland, while the production of spermatozoa in the seminiferitubuli was stimulated by FSH (*Follicle Stimulating Hormone*)[25].

Some literatures reported that testis weight correlates with the production of spermatozoa and the quality of semen of some animals, such as cow and sheep[26,27]. These results were due to the higher of testis weight, the more Leydig cells produced and the seminiferitubuli and subsequently affect the production of testosterone. The testosterone production level affects the quality and the number of spermatozoa produced. However, in some cases the testis weight possessed no effect on the number and quality of spermatozoa, such as in Saanen sheep[28]. Correlation test was performed to determine the correlation of testis weight and the quality of spermatozoa. The correlation of the testis weight and the concentration and the motility of spermatozoa are presented in Figure 5. The results showed that no positive correlation between the testis weight and the concentration and motility of spermatozoa was observed ($p>0.05$). It was suggested that the production of spermatozoa in the testis is affected by the genetic factor[29] and the number of sertoli cells during the development of the testis[30].

3.5 Seminal Vesicle Weight

The average of seminal vesicle weight of the mice after the administration of sandfish powder at the different concentrations of steroid compared to positive and negative controls are presented in Figure 6. The average of seminal vesicle weight ranged from 0.0564g to 0.1170g. The highest mean of the seminal vesicle weight (0.1170g) was obtained from the mice after the administration of

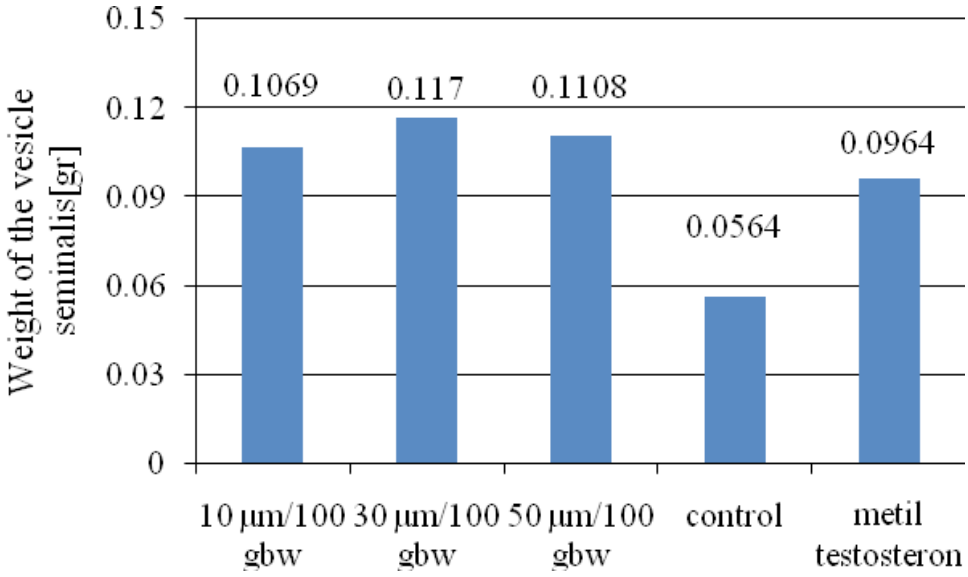

Figure 6. Weight of the seminal vesicle in male mice after the administration of the sandfish powder at different dosages of steroid and its comparison to control.

sandfish powder with the steroid dosage of 50µg/100g of body weight, while the lowest one was 0.0564g from the negative control. Analysis of variance revealed that the administration of sandfish powder had no effect on seminal vesicle weight of the mice (p>0.05).

The administration of sandfish powder displayed no significant effect on the seminal vesicle weight. It was proposed due to the mice used in this study were sexually mature, whereas the development of sexual organs had achieved the optimum level. Weight of seminal vesicle in the administration of sandfish powder at different concentrations of steroid was higher compared to the negative control. It indicated that the administration of sandfish powder did not inhibit the development of seminal vesicle in the male mice.

Seminal vesicle is the genital gland which produces opaque liquids containing protein, kalium, citric acid, fructose and enzymes in high concentration[21]; and the most concentrated are fructose and prostaglandin[31]. The liquid is part of the semen which is subsequently excreted at the same time with the spermatozoa from the testis. Metabolism activity and the motility of spermatozoa are then stimulated by this liquid[14].

4. Conclusion

In this paper the author has presented the study on the potency of sandfish (*Holothuria scabra*) powder to increase sperm quality and quantity for man. After discussing the administration of sandfish powder, the results show that sandfish powder does not significantly affect genital weights and quality of the spermatozoa of male mice. Yet, the author discovered that the administration of 10µg of steroid/100g as shown by the increasing of sperm concentration. Sandfish powder potential to be fertility enhancer drugs on pharmacy. Thus, it can be concluded that sandfish powder has the potency of increasing the quality as well as the quantity of sperm and could potentially be utilized in the pharmaceutical world as a fertility enhancer drugs for men. From the results discovered by the author, several things that need to be studied further, namely whether the sandfish powder really has impact on the quality of sperm; the administration of sandfish powder to immature male mice as well as its impact; conducting its sub-clinical trial and also its clinical trial.

5. Acknowledgement

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6. References

1. Dance SK, Lane I, Bell JD. Variation in short-term survival of cultured sandfish (Holothuriascabra) released in mangrove-seagrass and coral reef flat habitats in Solomon Islands.Aquaculture. 2003 Apr; 220:495–505. CrossRef.
2. Haug T, Kjuul AK, Styrvold OB, Sandsdalen E, Olsen OM, Stensvag K. Antibacterial Activity in Strongylocentrotus droebachiensis (Echinoidea), Cucumaria frondosa (Holothuroidea), and Asteriasrubens (Asteroidea). Journal of Invertebrate Pathology. 2002 Oct; 81:94–102. CrossRef.
3. Murray AP, Muniain C, Seldes AM, Maier M. Patagonicoside A: A novel antifungal disulfated triterpene glycoside from the Sandfish Psolus patagonicus. Tetrahedron. 2001 Oct; 57:9563–8. CrossRef.
4. Guo SY, Guo Z, Guo Q, Chen BY, Wang XC. Expression, purification and characterization of arginine kinase from the sandfish stichopus japonicus. Protein Expression and Purification. 2003 Jun; 29:230–4. CrossRef. https://doi.org/10.1016/S1046-5928(03)00013-5
5. Cardona PGS, Berrios CA, Ramirez F, Arrarás JEG. Lipopolysaccharides induce intestinal serum amyloid A expression in the Sandfish Holothuria glaberrima. Development and Comparative Immunology. 2003 Mar; 27:105–10. CrossRef.
6. Moraes G, Norchote PC, Kalinin VI, Avilov SA, Silchenko A, Dmitrenok PS, Stonik VA, Levin V. Structure of the major triterpene glycoside from the sandfish Stichopus malls and evidence to reclassify this species into the new genus

Australostichopus. Biochemical Systematic and Ecology. 2004 Jul; 32:637–50. CrossRef.

7. Kariya Y, Mulloy B, lmai K, Tominaga A, Kaneko T, Asari A, Suzuki K, Masuda H, Kyosashima M, lshii T. Isolation and partial characterization of fucan sulfates from the body wall of sandfish Stichopusjaponicus and their ability to inhibit osteoclastogenesis. Carbohydrate Research. 2004 May; 339:1339–46. CrossRef.
8. Riani E, Syamsu K, Kaseno. Use of steroid as natural aphrodisiac in human. Report of Graduate Research Grant. Bogor: Bogor Agricultural University; 2005. [In Indonesian]
9. Riani E, Gumbira-Said E, Syamsu K, Kustiariyah, Kaseno, Cordova MR. The potency of sandfish (Holothuria scraba) as a source of natural aphrodisiacs. World Academy of Science, Engineering and Technology (WASET). 2013 May; 77:1684–92.
10. Riani E, Sudrajat AO, Triajie H. Effectiveness of sandfish extract that has been formulated to prawn masculinize. Bionatura. 2010 Nov; 12(3):145–54 (in Indonesia).
11. Riani E, Nurjanah S, Gumbira-Said E, Syamsu K, Kaseno, Cordova MR. 2014. Potency of sandfish (Holothuriascraba) flesh powder to improve reproduction quality of man. Proceedings International Conference on Biotechnology and Agriculture Engineering di Tokyo, World Academy of Science Engineering and Technology (WASET). Japan; 2014 May 29–30. p.1740–3.
12. Nurjanah S, Gumbira-Said E, Syamsu K, Suprihatin, Riani E. Effect of sandfish (Holothuria scraba) powder on the sexual behavior and the blood testosterone level of the male mice (Mus musculus). Bandung Medical Journal. 2009 Sep; 41(3):135–41. (in Indonesia).
13. Nainggolan O, Simanjuntak JW. The effect of ethanol extract of pasakbumi (Eurycoma longifolia Jack) on behavior sexual in white mice. Cermin Dunia Kedokteran. 2005 May; 146:55–7. (in Indonesia).
14. McDonald GW. Veterinary endocrinology and reproduction. Lea and Febiger: Philadelphia; 1989.
15. Weinbauer GF, Nieschlag E. Hormonal control of spermatogenesis. In: Molecular biology of the male reproductive system. Kretser D, editor. Academic Press, Inc: San Diego; 1993. p. 99–142. CrossRef.
16. Barrat CLR. Spermatogenesis. In: Gametes and Spermatozoon. Grudzinskas JG, Yovich JL, editors. Cambrige Univ Press: New York-USA; 1995. p. 250–67.
17. Yong-Ju P, Gi-Su S, Byeong-Hoon K, Seung-Hyeon L, Se-Jae K, Takemura A, Young-Don L. Effect of photoperiod on gonadotropin (FSHβ and LHβ) regulation in the damselfish, Chromisnotata. Indian Journal of Science and Technology. 2011 Aug; 4(S8):1.
18. Fischer KA, Leyen KV, Lovercamp KW, Manandhar G, Sutovsky M, Feng D, Safranski T, Sutovsky P. 15-Lipoxygenase is a component of the mamalia sperm cytoplasmic droplet. Reproduction. 2005 Aug; 130:213–22. CrossRef.
19. Barth AD, Oko RJ. Abnormal morphology of bovine spermatozoa. Iowa State University Press L: Iowa-USA; 1989.
20. Linhart O, Alavi SMH, Psenicka M, Rodina M, Kaspar V, Li P, Hulak M, Boryshpolets S, Dzyuba B, Gela D, Flajshans M, Peknicova J, Cosson J, Ciereszko A. Spermatozoa of chondrostean fish species: Structure, motility and fertilzingability. Indian Journal of Science and Technology. 2011 Aug; 4(S8):1–2.
21. Curry MR, Watson PF. Sperm structure and function. In: Gametes and spermatozoon grudzinskas. Gedomines J, Yovich JL, editors. Cambrige Univ Press: New York-USA; 1995. p. 81–103.
22. Turner RM. Tales from the tail: What do we really know about sperm motility? Journal of Andrology. 2003 Dec; 24(6):790–803.CrossRef.
23. Toelihere M. Reproduction physiology at livestock. Angkasa Press. Bandung, Indonesia; 1981 (in Indonesia).
24. Turner CD, Bagnara JT. General endocrinology. WB Saunders Company: Philadelphia-USA; 1976.
25. Reeves JJ. Endocrinology of reproduction In: Reproduction in farm animals. Hafez ESE, editor. Lea and Febiger: Philadelphia-USA;1987. p. 85–106.
26. Jainudeen MR, Hafez ESE. Sheep and goats. In: Reproduction in farm animals. Hafez ESE , editor. Lea and Febiger: Philadelphia; 1987. p. 213–36.
27. Hidayat SS. Correlation of body weight, scrotum volume and scrotum diameter to ejaculation volume and spermatozoa concentration by applying ejaculator and artificial vagina in local sheep. Maters Thesis. Faculty of Veterinary Medicine, Bogor Agricultural University. Bogor; 2002. (In Indonesian).
28. Sufi IM. Correlation of body weight and testis volume to spermatozoa concentration in Saanen goat. Maters Thesis.

Faculty of Veterinary Medicine, Bogor Agricultural University. Bogor; 2003. (In Indonesian).

29. Roy IL, Tordjman S, Samour DM, Degrelle H, Roubertoux PL. Genetic architecture of testis and seminal vesicle weights in mice. Genetics. 2001 May; 158:333–40.
30. Fielden MR, Halgren RG, Fong CJ, Staub C, Johnson L, Cho K, Zacharewski TR. Gestational and lactational exposure of male mice to diethylstilbestrol causes long-term effects on the testis, sperm fertilizing ability in vitro, and testicular gene expression. Endocrinology. 2002 Aug; 143(8):3044–59. CrossRef.
31. Jequier AM. Clinical disorder affecting semen quality. In: Gametes and the spermatozoon. Grudzinskas JG, Yovich JL, editors. Cambridge University Press: New York-USA; 1995. p. 175–91.