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Original Article

Sandifish (*Holothuria scabra*) ameliorates aging in menopausal women by increasing estradiol hormones

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Abstract

Introduction: Sandfish (*Holothuria scabra*) is a marine species generally sold as a raw material that has been dried even though the meat contains steroid hormone with high economic value, which has the potential to become a source of safe natural steroid hormone. This study was aimed to look at the potency of sandfish as an anti-aging for menopausal women. Sandfish could become a source of natural steroid for hormone replacement therapy to replace synthetic hormone that is proven to have negative impacts on health.

Methods: This study used female rats (*Rattus norvegicus*) Sprague-Dawley Strain Variety II that were twelve weeks old and ovariectomized. In these animals, the bioassay test was conducted with the treatment of sandfish meat powder (SP) containing 30, 40, and 50 µg steroid/100 g. At the end of the treatment, the examination was carried out toward the concentration of steroid in blood serum using radioimmunoassay (RIA) and the uterine weight.

Results: The treatment with SP in 30 µg steroid/100 g could increase the estradiol hormone in blood serum of test animals that were ovariectomized and produce the highest concentration of uterine weight.

Conclusion: This study showed that a dose of SPcontaining 30 µg steroid/100 g body weight had the highest potential as an anti-aging in menopausal women.

Keywords:
Anti-aging;
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Introduction

Women are destined to always look beautiful and youthful, so generally make many efforts to achieve that goal. These efforts includes regular consumption of herbal medicines, ginseng and placenta extract, as well as supplement containing antioxidant, cosmetic surgery, nucleic acid therapy, and hormone therapy (Thompson and Maibach, 2010). Young women commonly are not worried about this point, but for the middle-aged women, it often becomes a problem. As stated by (Thompson et al., 2000) the aging is characterized by a decrease in hormone production. The hormone that has decrease in its production is estrogen hormone (Dodson and Steiner, 2002; Steinweg, 2002; Hansen, 2008). On the other hand, the estrogen plays a role in breast growth and

accumulation of fat tissue under the skin, softens the voice, inhibits the growth of mustache and hair around the face, and smooths the skin (Stevenson et al. 2005; Ruggiero et al., 2002). Estrogen also plays a role in slowing the aging process and promoting the formation of collagen tissue which serves as a supporting tissue in the body (Hansen, 2008). If estrogen is reduced, one of the consequences is the decrease in the amount of collagen-dermis resulting in thinning of the epidermis and reduction of the dermis thickness, and making the skin more susceptible to injury (Stevenson et al. 2005; Stevenson and Thornton, 2007; Thompson and Maibach, 2010). According to Stevenson and Thornton (2007) aging is associated with a decrease in skin thickness due to atrophy of the epidermis, dermis and subcutaneous fat. It's also associated with dryness, wrinkling and an increased incidence of proliferative lesions. In the epidermis, aging is associated with a decrease in epidermal thickness, flattening of the dermal papilla and a decrease in melanocyte and langerhans cell density. These conditions ultimately cause the bodies of women who enter pre-menopause and menopause phases, to be not fit anymore.

The efforts in keeping the body fit and preventing premature aging have been conducted in various ways, while the most common method is the therapy with the synthetic estrogen hormone (Thompson and Maibach, 2010), synthetic testosterone (Sherwood, 2001) or methyl testosterone combined with synthetic estrogen (Zang et al., 2008). This is understandable considering that estrogens have many important protective roles especially in skin physiology (Thornton, 2005). Estrogen protects against skin photoaging (Tsukahara et al., 2004; Stevenson and Thornton, 2007), and against the mortality rates from melanoma skin cancers (Miller and MacNeil, 1997). The hormone replacement therapy (HRT) by estrogen in menopausal women has been shown to increase the epidermal hydration, skin elasticity and skin thickness (Sator et al., 2001), reduce skin wrinkles (Phillips et al., 2001), enhance the content and quality of collagen and the level of vascularization (Brincat et al., 2005), and also stimulate proliferation and DNA synthesis of human epidermal keratinocytes (Kanda and Watanabe, 2004; Verdier-Sevrain et al., 2004), all leading to decrease in skin aging (Verdier-Sevrain et al., 2006). This therapy (HRT) is proven to treat the symptoms of premature aging and keep the bodies of middle-aged women stay fit, but it often brings side effects that cause problems of its own (negative side effects). According to literature (Riani et al., 2005; Riani et al., 2006; Riani et al., 2007; Riani et al., 2010b) the meat and waste of sandfish (*Holothuria scabra*) contain testosterone with the potential to be an aphrodisiac as well as sex reversal substance, safe consuming and environmentally friendly. These materials have the potential to be utilized as part of HRT in menopause and post-menopause stages, and can be aromatized to estradiol (Riani et al, 2010a and 2010c). Therefore, it is necessary to conduct a study regarding whether the sandfish meat has a potential as an anti-aging material.

Based on prior studies, such as Stampfer et al. (1991), Sauerbronn et al. (2000), Phillips et al. (2001), Sator et al. (2001), Sator et al. (2004), Haapasaari et al. (1997), Durvasula et al. (2002), Grady et al. (2002), Patriarca et al. (2007) and Stevenson and Thornton (2007), one way to improve the fitness and prevent the premature aging is generally in the form of patented medicine derived from synthetic hormones. It is generally known that these patented medicines can lead to various side effects. The use of synthetic hormones can cause several negative effects as proven by the rise of cancer, multiple organs disabilities, and genetic changes in chicken, livestock, mice, aquatic animals (giant prawn and ornamental fishes) as well as human (Lu, 1995; Wylie-Rosett, 2005; Sylvia and Lorraine, 2005; Riani et al., 2007; Riani, 2010d). These synthetic hormones will stimulate the formation of carcinogen adduct that lead to the formation of neoplasm or transforming the neoplasm into cancer (neoplasia) and cause cardiovascular diseases (Grady et al., 2002). Therefore, the utilization of sandfish as an anti-aging product is the latest innovation in the field of agro-industry, expected to have a quite high commercialization prospect in this era of globalization. This study is aimed to utilize active ingredients in sandfish meat and to initiate the production of endogenous estradiol hormone as an anti-aging agent in menopausal women.

Materials and methods

The protocol was approved by the Standing

Institutional Review Board,Local Ethic Committee at the Bogor Agricultural University.

The production of stock material of sandfish powder, bioassay test, and analysis of estrogen hormone concentration by using radioimmunoassay (RIA) method were conducted in this research. Sandfish obtained from Lampung Province, Indonesia were the material used in this research. The sandfish meat was processed into sandfish meat powder (SP). The synthetic hormone used in bioassay test was estradiol.

Animals used in this research were female white rats (*Rattus norvegicus*) Sprague-Dawley Strain Variety II, twelve weeks old, with body weight of 200 g, and total of 18 rats. Animals were placed in plastic cages with a lid made from ram wire and covered by brans. The environment of the cages was created to be not in humid condition with enough ventilation. The light duration was 14 hours whereas the dark duration was 10 hours. The rats were fed pellets and water ad libitum. Rats were kept in the experimental cages for 10 hours. Once adapted, 15 rats were ovariectomized and the recovery was held for 21 days before the experiments started. Each rat was placed in individual cage. The ovariectomized (OVX) rats then treated with hormone supply; 3 rats were treated with estradiol hormone, 9 rats were treated with steroid hormone of sandfish meat powder, and the rest 3 rats were untreated (Table 1).

Treatments were conducted for 20 days or 4 estrous cycles. The feeding with SP was carried out daily. After completion of the treatment period, the body weight of rats, uterus weight, and the ratio of uterus weight to body weight were determined. However, before the rats were killed, in the last phase of diestrous, blood was taken from each rat intercardially (approximately 1 ml). The blood was collected in storing tube, then were centrifuged at 2000 rpm for 15 minutes to obtain serum that was used for determination of estrogen concentration. The concentration of estradiol was also determined to represent the overall estrogen by using radioimmunoassay (RIA) method with solid phase technique using kit estrogen coat-a-count containing estradiol labeled 125I.

In order to determine the effect of the treatment on the anti-aging process, based on the body weight, the uterine weight, the ratio of uterine weight to the body weight as well as the content of estradiol hormone in the blood serum, the statistical calculation was performed using a completely randomized design with three replications. Furthermore, the results that were significantly different continued into the honestly significant difference (HSD) test.

Results

In this study, all the control rats and the OVX rats treated with different doses of steroid in sandfish meat: 30 µg/100 g body weight (bw), 40 µg/100 g bw and 50 µg/100 g bw, as well as those treated with synthetic estradiol at the dose of 30 µg/100 g bw, were alive until the end of the study. However OVX rats that were not given any treatment, of three rats, two died, so their survival rate was only 33.33%. The deaths occurred just a day before the study ended, so only one rat that was left alive at the end of study was sampled (Table 2).

The content of serum estradiol hormone in control rats and rats given treatments, is shown in Table 3.

Table 1: Experimental treatments and the dosage given to each treatment

No	Experimental animal	Type of treatment	Dosage
1	OVX[a] rats	SP[b]	30 µg/100 g bw
2	OVX rats	SP	40 µg/100 g bw
3	OVX rats	SP	50 µg/100 g bw
4	OVX rats	Estradiol 17β	30 µg/100 g bw
5	OVX rats	-	-
6	Normal rats (unovariectomized)	-	-

[a]Ovariectomized
[b]Sandfish meat powder

Table 2: Uterine weight and ratio of uterus weight to body weight in each treatment

Type of treatment	Animal number	Body weight (g) [A]	Uterus weight (g) [B]	Ratio of Uterus / Body (%) [(B:A)x100]
Control	1	258	0.7466	0.289379845
	2	248	0.6807	0.274475806
	3	234	0.3488	0.149059829
	Average	246.666667	0.59203	0.240013514
OVX	1	-	-	-
	2	298	0.2004	0.067248322
	3	-	-	-
	Average	298	0.2004	0.067248322
SP 30 µg/100 g bw	1	267	0.4896	0.183370787
	2	250	0.5128	0.20512
	3	250	0.6006	0.24024
	Average	255.666667	0.53433	0.208996089
SP 40 µg/100 g bw	1	284	0.4078	0.143591549
	2	240	0.406	0.169166667
	3	260	0.396	0.152307692
	Average	261.333333	0.40327	0.154311224
SP 50 µg/100 g bw	1	269	0.3805	0.141449814
	2	288	0.4462	0.154930556
	3	270	0.3946	0.146148148
	Average	275.666667	0.4071	0.147678356
Estrogen	1	264	0.4524	0.171363636
	2	272	0.3992	0.146764706
	3	268.2	0.4142	0.154436987
	Average	268.066667	0.42193	0.157398657

Discussion

ANOVA test results of the treatment effect on the body weight and the uterine weight, indicated that there was no significant difference between the treatment effects toward the body weight. However, the treatment effect toward the uterine weight showed a significant difference. The result of further HSD test showed that the normal rats (control) had the highest uterine weight, followed by the OVX rats treated with SP contained steroid with a concentration of 30

Table 3: Concentration of estradiol hormone in blood serum of test rats given treatments

Type of treatment	Animal number	Estradiol concentration (pg/ml)
Control	1	126
	2	128.3
	3	118.48
	Average	124.26
OVX	-	-
	2	46.6
	-	-
	Average	46.6
Estrogen	1	86
	2	83.68
	3	70.46
	Average	80.0466667
SP 30 µg/100 g bw	1	90.86
	2	89.24
	3	89.42
	Average	89.84
SP 40 µg/100 g bw	1	76.74
	2	71.02
	3	76
	Average	74.5866667
SP 50 µg/100 g bw	1	78.02
	2	74.48
	3	79.15
	Average	77.2166667

μg/100 g bw (SP 30 μg/100 g bw), the OVX rats treated by the synthetic estrogen with a dose of 30 μg/100 g bw, the OVX rats treated by the SP with a dose of 40 μg/100 g bw and with a dose of 50 μg/100 g bw (there was no significant difference between SP 40 μg/100 g bw and SP 50 μg/100 g bw). The lowest uterine weight was observed in OVX rats. These data proved that treatment with SP at the dose of 30 μg/100 g bw increases the uterine weight in test animals even better that the treatment with synthetic estrogen.

The effect of treatment on the ratio of uterine weight to body weight (%) showed a significant difference. The result of further HSD test also had a sequence that was not too much different from the sequence of the result in uterine weight. In this case, the normal rats (control) had the highest ratio, followed by SP 30 μg/100 g bw, then the groups with synthetic estrogen treatment, SP 40 μg/100 g bw and SP 50 μg/100 g bw (these three were not significantly different).

It is shown in this study that the OVX rats which were not given any treatment showed rudimentation in their uteri. This occurred because there was no more ovary in OVX animals. On the other hand, the ovaries played a role in producing estradiol hormone, as stated by Turner and Bagnara (1976) as well as Manson and Bassuk (2007) that the estrogen is produced from the ovaries, corpus luteum, placenta, and adrenal cortex of females. The absence of ovaries in these OVX animals resulted in the absence of estrogen performance in uterus, so that the uterus shrank, as happened in OVX animals that were not given any treatment. This is in line with the statement of Tietz (1995), as well as Manson and Bassuk (2007), that one of the factors cause decline in estradiol hormone is ovarian dysfunction. According to (Berga and Parry, 2000) when the ovaries removal is performed, the menopause will occur.

As stated by Guyton (1994), Manson and Bassuk (2007) as well as (Gao et al., 2015) that the main function of hormones belong to the group of estrogen is to induce the cell proliferation and the growth of tissues in genital organs and other tissues related to the reproduction, so that the estrogen may act as an anti-aging. This is consistent with the results of studies done by Manson and Bassuk (2007), Knowlton and Lee (2012) and Lee et al. (2013) that the estrogen hormone will prevent the aging, as well as preventing heart disease. However, according to Eisenlohr-Moula (2015) the cycle of estrogen fluctuation itself already can change the emotions, cognitive processes, and behaviors relevant to Borderline Personality Disorder. Therefore, the presence of estrogen hormone in the bodies of menopausal women derived from natural ingredients, that is from sandfish, is not only beneficial for anti-aging process, but also to prevent a various deadly diseases such as heart disease, which in this study was proved by the OVX animals that were untreated, with the survival degree of only 33.33%.

In this study, it can be seen that treatment with SP could help to increase the uterine weight (Table 2). This occurred because SP contained the steroid hormone in a form of testosterone (Riani et al., 2006; Riani et al., 2007 and Riani et al.; 2010a). According to Simpson et al. (1994), Craig and Stitzel (1997) and Manson and Bassuk (2007) as well as Kocoska-Maras et al. (2013) the testosterone in women will be converted into estrogen by aromatase enzyme. This aromatase enzyme is found in ovaries, brain, adipose tissue, placenta, blood, skin, bones and endometrium, and the activity of this enzyme can be seen in ovaries, adipose tissue, placenta, brain, muscle, fibroblast, osteoblast, liver and breast (Holzer et al., 2006). This enzyme will catalyze the process of estrogen formation that is through a hydroxylation of androstenedione into estrone form, and testosterone into estradiol-17β. This estrogen hormone then will stimulate the proliferation and the growth of the cells of uterine tissue. Thus, treatment with SP as well as estrogen leads to the increase of estrogen hormone in the rats serums, so in the treated OVX rats, their uteri grow back as in normal rats that are not ovariectomized.

The data analysis using ANOVA in this bioassay research showed that treatment with SP at the dose of 30 μg/100 g bw has the highest efficacy to increase uterine weight and the uterine weight to body weight ratio in test animals, compared with other doses. This indicated that the dose of 30 μg/100 g bw may be also an effective anti-aging dose because it could stimulate the growth of uterus in test OVX animals. It was shown by the occurrence of uterine growth based on the value of the uterine weight to the body weight ratio that was statistically similar to that in OVX animals treated with estradiol 30 μg/ 100 g bw. This research shows that the estradiol in SP increases uterine weight because

estradiol is able to induce proliferation of cells (Verdier-Sevrain et al., 2006) and increase extracellular matrix protein synthesis (Son et al., 2005; Hansen, 2008) in uterus.

The results of ANOVA and HSD test on uterine weight data, and serum concentration of estrogen, shows that SP with a steroid dose of 30 µg/ 100 g bw results in the highest uterine weight and the highest ratio of uterine weight to body weight. Besides, it is also capable to generate the highest concentration of endogenous estradiol in the blood serum of OVX test animals (Table 3). This indicates that a dose of 30 µg/ 100 g body weight is expected to be the effective dose to stimulate the uterine growth and increase the estradiol hormone in blood serum.

Menopausal women generally face various problems as a result of the absence of the estrogen hormone in their bodies, so that the synthetic hormone therapy is commonly used to treat. Although the hormone therapy using the synthetic estrogen hormone has been proven to provide a variety of health problems (Simpson et al., 2004), but it is still being done, since the problem faced by menopausal women is quite lots, it even can cause death. It is evident in this study that the test animals treated with SP showed no morphological abnormalities in their body organs, and not resulting in death, as happened in OVX animals which were untreated. In this case, of the three replications in this study, the death occurred in two OVX rats that untreated. In these two rats that died, there were signs of swelling (inflammation), so that both rats apparently looked fatter, and at the time of the death bleedings in eyes, ears, and nose were found. According to Lee et al. (2013) the occurrence of swelling is a key parameter of heart disease. Furthermore Lee et al. (2013) and Stampfer et al. (1991), stated that this condition happens as a result of the absence of estrogen hormone that is common in menopausal and postmenopausal women.

Although in this study no visible morphological changes in the body organs of test animals was observed, but the provision of synthetic hormone still must get a very serious attention. According to Nakajima et al. (2005), Diorio et al. (2012) as well as Shah and Frazer (2014) the estrogen replacement therapy may overcome the menopausal symptoms however, synthetic estrogen even at low doses or combined with the progesterone, causes side effects such as cancer, vaginal bleeding, breast pain, uterine cramps, liver damage, and so on. Therefore, the therapy using a natural hormone like found in SP is an option to effectively stimulate the proliferation of uterine cells, and also the other cells such as skin cells and collagen. Thus, the estrogen hormone in SP can be an anti-aging agent in animals similar to menopausal women. This study result shows that steroid in SP at the dose of 30 µg/100 g bw is better than other doses, even relatively better than the synthetic estrogen hormone.

Conclusion

In this paper, the efficacy of sandfish as an anti-aging treatment for menopausal women has been presented. The main conclusions are as follows.

1. SP contains a natural steroid that may act as an anti-aging agent in menopausal women and with relatively minimal adverse side-effects.
2. The best dose of SP for anti-aging effects in OVX rats is 30 µg steroid per 100 g body weight.

Future works

In order to develop this innovation into the products that can be marketed, ideally more detailed efficacy studies are required, particularly focused on the body concentration of estrogen hormone. Further toxicity and safety tests, preclinical test, and clinical test in humans, should be also conducted.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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