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Administration of Ulva lactuca increased the expression of AR and ER mRNA receptors in ovaries after Monosodium Glutamate induction in Ratts

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ABSTRACT

Excessive consumption of foods containing monosodium glutamate (MSG) can trigger ovarian disorders, especially in hormonal regulation, leading to adverse effects on DNA activation for mRNA transcription and protein synthesis. Ulva lactuca, a type of seaweed, is a natural therapy for treating this condition due to its various nutrients, antioxidants, and sterols. The aim of this study was to investigate whether the administration of Ulva lactuca could increase ovarian AR and ER mRNA receptors after exposure to MSG, a toxic agent. A true experiment was conducted using 30 females Rattus norvegicus divided into five groups. Statistical analysis using the Kruskal Wallis test revealed that AR mRNA showed a P value of 0.007, ERα showed a P value of 0.084, and ERβ showed a P value of 0.018. This suggests that there was a significant difference in AR and ERβ mRNA expression between the control group and the Ulva lactuca treatment group, which tended to increase after initial exposure to MSG. These findings indicate that the administration of Ulva lactuca can improve ovarian function after exposure to MSG-induced organ dysfunction.

Keywords: *Ulva lactuca, mRNA AR and ER, Monosodium glutamate, Ovarian disorders, Hormonal regulation*

INTRODUCTION

Unhealthy lifestyles are often associated with smoking, infrequent physical exercise, frequent consumption of alcohol, and foods that contain lots of added sugar, fat, preservatives, dyes, and additional flavorings such as monosodium glutamate (MSG), which is a food additive (Zerasky et al., 2010; Ashakiran & Deepthi, 2012). MSG is commonly used in food as a flavor enhancer, especially in instant and frozen foods (Bojanic et al., 2009).

However, MSG is not only used in ready-to-eat food but is also often added to cooking ingredients for home cooking which is currently known to be strongly suspected as a dietary obesogen (Holtcamp, 2012). Studies related to the effects of MSG in food on glucose balance in humans show that plasma levels of GLP-1 (glucagon-like peptide-1) significantly increase (Hosaka et al., 2012).

This condition indicates that exposure to MSG can have an effect on the regulation of hormone secretion, glucose balance, and body weight, so it is strongly suspected to have the potential as an endocrine disruptor (Shannon et al., 2016). The cytotoxic response was evaluated in subtypes of epithelial cells lining the gut and collectively constituting the largest endocrine system in the body. These cells are used by the STC-1 cell line to secrete GLP-1 (Jafri et al., 2016). Besides that, the negative effects of MSG, especially on the female reproductive system, are known to be very sensitive to dangerous environmental agents (Bojanic et al., 2009).

In this regard, the effect of MSG on the endocrine system is shown by how it affects the decrease in receptors for the gonadotropin hormone (GnRH). These receptors increase during sexual maturation for the development of the reproductive system, including the gonads, which are controlled by FSH and LH from the pituitary gland (Schafer-somi et al., 2018; Wang et al., 2021). GnRH is synthesized by the hypothalamus, including the arcuate nucleus. MSG causes lesions of the arcuate nucleus in experimental rats, thereby reducing GnRH. FSH and LH production are inhibited, and gonadal development is disrupted. Throughout sexual maturation, there are changes in plasma levels of gonadotrophins and gonadal steroids, and finally changes in the function of the HPG (hypothalamus-pituitary-gonad) axis (Fattah et al., 2016).

In vitro research on MSG as a dietary obesogen shows that it can negatively affect binding in nuclear receptors and steroidogenesis. This shows that MSG exposure has a profound effect on sex steroid hormone levels and receptors, which have an important role in energy metabolism. However, the exact mechanism of how MSG gives its effect is not yet clear (Shannon et al., 2019). The potential endocrine disruption by MSG showed an antagonistic response in cells responsive to androgens and progestogens, with a reduced cell response to androgens by 33%, 36.9%, and 50.6% (compared to the solvent control) at MSG concentrations of 50, 250, and 500 g/ml (Shannon et al., 2019).

Therefore, MSG is said to be a potential agent for endocrine disrupting chemicals (EDCs). EDCs are exogenous chemicals that can affect hormone action and increase health risks, including cancer, reproductive disorders, cognitive decline, and obesity (Merrill et al., 2020). The target of EDCs is the female reproductive system, especially the ovaries, which are the main organs responsible for reproduction and endocrine functions. EDC exposure is known to cause many problems in reproductive health, such as infertility, failure of ovarian function, and abnormal levels of sex steroid hormones (Patel et al., 2015). Several EDCs can affect adult ovarian function, including folliculogenesis and steroidogenesis.

In vivo studies have shown that the administration of MSG can inhibit the expression of steroid receptors and cause changes in hormone levels, such as estrogen, testosterone, and progesterone (Zia et al., 2014). In young rats that were orally given MSG, hypothalamic function and estrogen receptor expression decreased, and as adult rats, they tended to have decreased levels of estradiol and testosterone in their serum (Husarova & Ostatnikova, 2013). The working system of sex steroid hormones in maintaining reproductive organs does not function alone but forms a complex with specific nuclear receptors, such as androgens with AR and estrogen with ER, for the proliferation and differentiation of cells in the gonads (Contro et al., 2015).

The mechanism of action of steroids in cells can be hindered by MSG exposure, causing failure of hormonal production which results in deficiency conditions and failure to form hormone-receptor complexes. Additionally, MSG toxicity can cause intracellular oxidative stress, leading to DNA damage (Hajihasani et al., 2020). This interference can affect the process of DNA activation required for cell development in the ovary. Oxidative stress can lead to impaired oocyte maturation, impaired steroidogenesis in the ovaries, impaired ovulation to implantation, disrupted blastocyst formation and luteal maintenance during pregnancy, as well as primary and secondary infertility (Agarwal, 2012).

Natural products derived from nature that are often used for prevention and health therapy are those that are proven to be safe to use and have minimal side effects. Ulva lactuca is one of the green macroalgae that is empirically used as food by Indonesian people living along the coast. Qualitative testing on sea lettuce showed that it contains primary and secondary metabolites such as carbohydrates, proteins, alkaloids, flavonoids, mono and sesquiterpenoids. Sea lettuce has antioxidant activity and has the potential to be developed as a source of natural antioxidants (Widyasari et al., 2019).

Ulva lactuca is a seaweed species of Chlorophyta, commonly known as sea lettuce, which is used as a food ingredient in Vietnam (White & Wilson, 2015) and traditional medicine in China. The ethanol extract of Ulva lactuca contains a lot of antioxidants, protein around 10-21 g/100 g dry weight, as well as antibacterial, antifungal, and antitumor properties (Erniati et al., 2016; Widyaningsih et al., 2016). Ulva lactuca contains a variety of nutrients, including macronutrients, micronutrients, secondary metabolites, and antioxidants, and has been designated by the Food and Drug Supervisory Agency (BPOM, 2018) as a plant-based food (Mulyati et al., 2019). It has very low toxicity, making it safe to use as a food or pharmaceutical ingredient (Safitri, 2014 in Dewi et al., 2016).

Each type of green macroalgae (Ulva sp, Entermorpha sp, Chaulerpha sp, Codium sp, Halimeda sp 2, Halimeda sp 3, Halimeda sp 1 and Halimeda sp 6) contains secondary metabolites such as Alkaloids, Saponins, Flavanoids, Tannins, Terpenoids and Steroids (Nome et al., 2019). The Sterol products contained in green algae are: Isofucosterol, β-sitosterol, Cholesterol, Demosterol, Fucosterol, 24-Methylenecholesterol, Dihydrobrassicasterol, 22-E-dihydrocholesterol and Poriferasterol (Aknin et al., 1992 in Jati et al., 2018). The results of the phytochemical test, especially the green algae of the Ulva latuca species, were positive for Steroid and Saponin compound and showed that all green algae contain natural Sterols (Jati et al., 2018). The bioactive sterol compound in Ulva lactuca algae was also found through phytochemical analysis with GC/LC-MS like the study by Kapetanovic et al. (2005) and Andrade et al. (2012) showed that the Sterol compound obtained was β-sitosterol and Stigmasterol. Study by Basir et al. (2017), found that green algae extract contains antioxidants (Flavonoids and Phenols) as well as Sterols (β-sitosterol and Stigmasterol).

Sterols can act as Sterol Regulatory Element Binding Protein-1 (SREBP 1), and through Sterol Regulatory Elements (SRE) (Johnson et al., 2002), regulate steroidogenesis in the ovaries and provide cholesterol as the main precursor in the synthesis of sex steroid hormones (Murray et al., 2014). This enables hormone synthesis to increase to normal levels and overcome disturbances in the regulation of steroidogenesis in the ovary caused by toxic agents such as MSG, as previously described. Ulva lactuca is also able to fight oxidative stress, as shown by previous studies demonstrating its chemoprotective effect on the toxicity of benzo-α-pyrene by inhibiting the effects of carcinogen bioactivation (Roche et al., 2019). Ulva lactuca also has activity as a free radical scavenger, as indicated by its ES50 value (Salamah et al., 2014).

The increased expression of ERα2 mRNA in the ovary occurs during the estrous phase when estradiol-17β levels are at their peak, characterized by an increase in oocyte diameter, gonadosomatic index, and vitellogenin levels (James et al., 2012). These conditions indicate that the concentration of the hormone estradiol during the mid-menstrual cycle causes an increase in the expression of its receptors in target organ cells such as in the ovaries, leading to cell proliferation. Ulva lactuca can cause this effect through its Sterol compound. Previous studies also found that Ulva lactuca caused damage to the gonadal tissue of male rats, indicating its influence on the hormonal regulation of sex steroids and their receptors, affecting cell development. Therefore, the aim of this study is to determine the effect of Ulva lactuca on increasing steroid receptors, as indicated by the expression of AR and ER mRNA in the ovary after MSG induction in adult female rats.

MATERIAL AND METHODS

Animals

This study used 30 adults female wistar rats (Rattus norvegicus), aged 18-20 weeks and weighing 150-200 grams. Mice were kept in the pharmacology laboratory of the Faculty of Medicine, Airlangga University, Surabaya. The research was conducted in several laboratories, namely the Pharmacy Faculty testing service unit, the Pharmacology Laboratory, the Faculty of Medicine, the Embryo Laboratory, the Faculty of Veterinary Medicine and the Institute for Tropical Diseases Airlangga University. The rats were fed ad libitum and then acclimatized for 7 days, after which the rats were divided into 2 groups, namely the control group (P0) 6 mice (2 ml of distilled water) and the treatment group 24 mice (MSG induced). Giving MSG induction for + 40 days at a dose of 400 mg/kgBW. The post-MSG induction group (PA) was divided into 4 groups randomly obtained 6 rats per group each. PA group received aquadest plus a suspender (Carboxymethylcellulosum Sodium 1%), the Ulva lactuca algae extract treatment group (ULT1) at a dose of 100mg/kgBW, the algae extract treatment group (ULT2) at a dose of 200mg/kgBW and the algae extract treatment group (ULT3) at a dose of 400mg/kgBW. Treatment was given using a gastric tube for + 40 days. After all the treatments ended, the rats were terminated and anesthetized first with 10% ketamine, to take the right and left ovary organs.

Monosodium Glutamate Preparation

Monosodium glutamate used in this research is pure MSG 99.0% FCC Grade/E621 Made in China Multi Chemical Indotrading. Formula C5H8NNaO4 CAS No 142-47-2, molecular weight 169.11 g/mol. MSG is in the form of white crystalline powder which is dissolved in 2 ml of distilled water, then given to experimental animals according to the dose obtained.

Preparation of Ulva lactuca algae ethanol extract

Green algae obtained from the north coast of Singaraja Bali were taken around January, then washed thoroughly with running water. Clean algae are dried and put in the oven for 3 days. The dried algae were then crushed into coarse powder, then extracted using the maceration method. Algae that had been soaked in 96% ethanol were left for 5 days. After 5 days the solution was filtered using filter paper to separate the filtrate and debris. The filtrate was evaporated with a rotary evaporator to evaporate the ethanol at 40°C until a thick extract was obtained and no solvent dripped. The evaporated extract is again evaporated using a water bath, so that a viscous extract is obtained that is dark green to bluish green which indicates the presence of steroid bioactive compounds. The material extracted from algae is prepared as a preparation according to a predetermined dosage, which is obtained by weighing the extract first using a digital balance, then dissolving it with a suspender (Carboxymethylcellulose Sodium 1%) plus distilled water.

Sample preparation

After the rats were dissected, samples of the right and left ovarian organs were taken, then they were put into a sample pot containing distilled water. Organ samples were cut with a thickness of 5 mm and put into an Eppendorf tube, then immersed in RNAlater solution with a ratio of 1:20 for 24 hours at 4°C. Samples were taken from the solution and then put into a new Eppendorf tube and stored at -200°C.

RNA extraction

Samples of 30 tissues were taken and weighed, about 20-25 mg were crushed and homogenized. The mashed sample was put into a 1.5 ml Eppendorf tube and added 350 µl RLT+ Betamercapto Buffer, then vortexed for 15 seconds and spindown. Incubate at room temperature for 30 minutes. Next, 150 µl 70% ethanol was added to the sample tube, then mixed with a pipette. After that the sample mixture was put into the Rneasy Mini spin column, centrifuged at 8000 rpm for 15 seconds. Then put 700 µl Buffer RW1 into the Rneasy Mini spin column. Centrifuge 8000 rpm for 15 seconds. Replace new column. Put 500 µl of RPE Buffer into the Rneasy Mini spin column. Centrifuge

8000 rpm for 15 seconds. Put 500 µl of RPE Buffer into the Rneasy Mini spin column. Centrifuge 8000 rpm for 2 minutes. Replace new column. Centrifuge again at 13000 rpm for 1 minute to dry the membrane on the spin column. Replace the column with a sterile 1.5 ml tube. Add 35 µl RNAse free sterile water, incubate at room temperature for 15 minutes, centrifuge 8000 rpm for 1 minute. Store at -20°C before proceeding to the next step. cDNA synthesis was carried out using the Go-Taq Revers Transcriptase System which was carried out in two stages. The first stage was carried out with specifications, namely 1 µl random primer, 1 µl Nuclease Free Water, 1 µl RNA template (as many as 30 templates) which were incubated at 70°C for 5 minutes. The second stage was carried out 5 times with 4 µl of Go Script Buffer, 6 µl of MgCl2 which was incubated at 25°C for 5 minutes, 1 µl of PCR Nucleotide Mix incubated at 42°C for 60 minutes, 1 µl of Enzym RT incubated at 70°C for 15 minutes and Nuclease Free Water as much as 3 µl.

Real Time PCR Relative Quantitative Method

Relative RT-PCR quantification was performed using primer: Forward Gene Androgen receptor GGA GAA CTC TTC AGA GCA AG. Reverse Gen androgen receptor AGC TGA GTC ATC CTG ATC TG. Forward Estrogen Receptor Gene alpha 5-TAA GAA CCG GAG GAA GAG TTG. Reverse estrogen receptor gene alpha 5-TCA TGC GGA ATC GAC TTG. Forward Estrogen Receptor beta 5-GAG gene CTC AGC CTG TTG GAC C. Reverse estrogen receptor beta 5-GGC gene CTT CAC ACA GAG ATA CTC C. Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) gene was used as a house kipping gene for normalization. Amplification was carried out in a total volume of 20 µl, consisting of 140 cDNA templates from the sample, Kapa Sybr Fast 2X, Kapa RT mix (50X), distilled water to 20 ul. Amplification was carried out on a thermal cycler machine for 40 cycles using the following protocol: 42°C for 5 minutes, 95°C for 5 minutes, 95°C for 3 seconds, and 600 for 20 seconds.

Data analysis

Analysis of research data used the normality test with the Shapiro-Wilk test and homogeneity using the Levene's test, showing that the data in each sample group for the expression of mRNA AR, ERα and ERβ were not normally distributed and homogeneous, so the statistical test used was a nonparametric test with the Kruskal Wallis test with a significance level ($\alpha = 0.05$) which was statistically analyzed using the SPSS 21 program.

RESULT

Examination of the relative expression of mRNA genes in the ovarian organs was carried out by the PCR amplification method using a real-time PCR (Polymerase Chain Reaction) machine, which involves gene primers in mouse samples and gene house kipping which will act as an internal control in gene expression analysis. Genes tested such as AR and ER alpha and ER beta and GAPDH (glyceraldehyde-3-phosphate dehydrogenase).

TABLE 1: "Results of differences in AR mRNA gene expression"

No	Sample	n	Mean	SD	Min	Max	C	P
1	P0	6	3,30	5,26	0,02	12,04	13,938	0,007
2	PA	6	0,007	0,01	0,00	0,03		
3	ULT 1	6	3,14	7,54	0,04	18,55		
4	ULT 2	6	12,65	22,84	0,03	56,62		
5	ULT 3	6	4,19	10,18	0,02	24,99		

Based on table 3-1, it shows that the relative expression of AR mRNA in each group through the Kruskal Wallis test, because in the normality test with Shapiro-Wilk the data is not normally distributed and, in the homogeneity, test using the Levene's test the data is not homogeneous. Based on the test results, the value of C = 13.938 was obtained with a value of P = 0.007 at a 95%

confidence level (P <0.05), indicating that the relative expression of AR mRNA between groups was significantly different. The mean value of AR mRNA expression for each group is shown in the graph below:

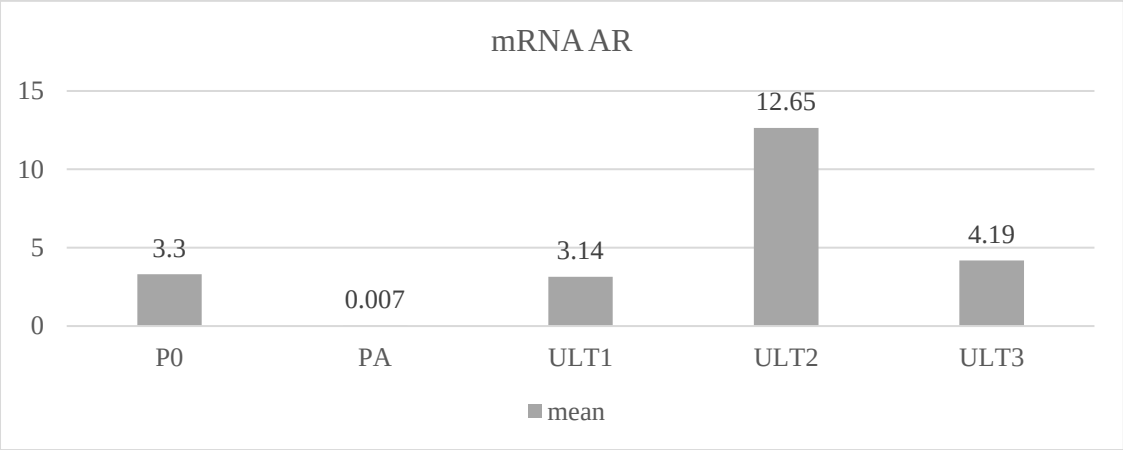

FIGURE 1: "AR mRNA Expression Mean"

Based on Figure 3-1, the P0 group and ULT groups 1, 2, 3 had a higher average AR mRNA expression than the post-MSG induction group. In the ULT 1 it appeared slightly lower than the P0, then in the ULT 2 the average expression began to increase significantly higher than the other groups, but decreased again in the ULT 3.

TABLE 2: "Results of differences in ERα mRNA gene expression"

No	Sample	n	Mean	SD	Min	Max	C	P
1	P0	6	5,05	9,29	0,00	23,10	8,212	0,084
2	PA	6	0,015	0,017	0,00	0,04		
3	ULT 1	6	1,93	4,61	0,02	11,35		
4	ULT 2	6	19,11	29,67	0,01	61,61		
5	ULT 3	6	6,91	16,80	0,02	41,21		

Based on table 3-2. It shows that the relative expression of ERα mRNA in each group through the Kruskal Wallis test, because in the normality test with Shapiro-Wilk the data is not normally distributed and, in the homogeneity, test using the Levene's test the data is not homogeneous. Based on the test results, the value of C = 8.212 was obtained with a value of P = 0.084, the relative expression of ERα mRNA between groups did not differ significantly at the 95% confidence level (P <0.05). The average value of each group is shown in the graph below:

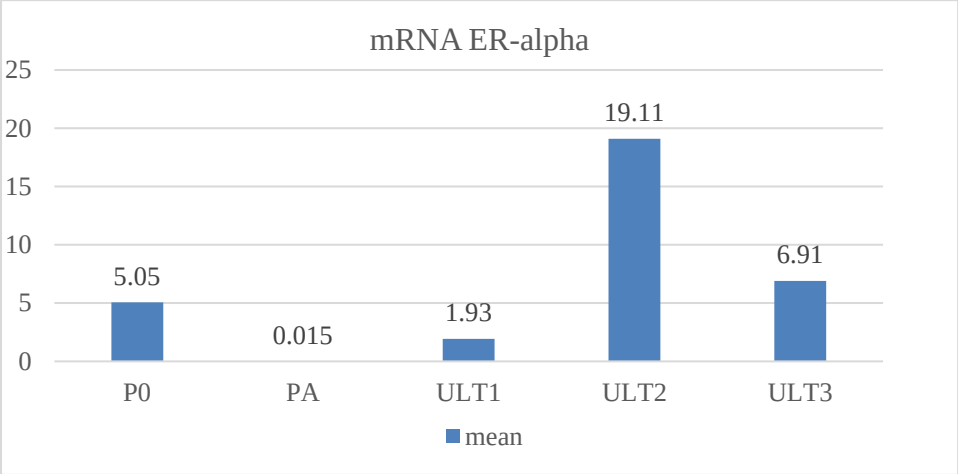

FIGURE 2: "ERα mRNA Expression Mean"

Figure 3-2, it shows that the P0 and ULT 1, 2, 3 groups had a higher mean ERα mRNA expression than the PA group. However, in the ULT 1 it was lower than the P0. In ULT 2 the average expression began to increase significantly higher than the other groups, but decreased in ULT 3.

TABLE 3: "Results of differences in ERβ mRNA gene expression"

No	Sample	n	Mean	SD	Min	Max	C	P
1	P0	6	8,17	14,02	0,00	34,07	11,959	0,018
2	PA	6	0,015	0,016	0,00	0,04		
3	ULT 1	6	2,53	3,61	0,10	8,40		
4	ULT 2	6	20,57	33,65	0,03	79,33		
5	ULT 3	6	7,80	19,02	0,02	46,63		

Based on table 3-3, the relative expression of ERβ mRNA in each group through the Kruskal Wallis test, because in the normality test with Shapiro-Wilk the data was not normally distributed and, in the homogeneity, test using the Levene's test the data was not homogeneous. Based on the test results, the value C = 11.959 was obtained with a value P=0.018, relative expression of ERβ mRNA between groups differed significantly at 95% confidence level (P<0.05). The mean value of each group and the comparison of the mean values of the three gene expressions in each sample group are shown in the graph below:

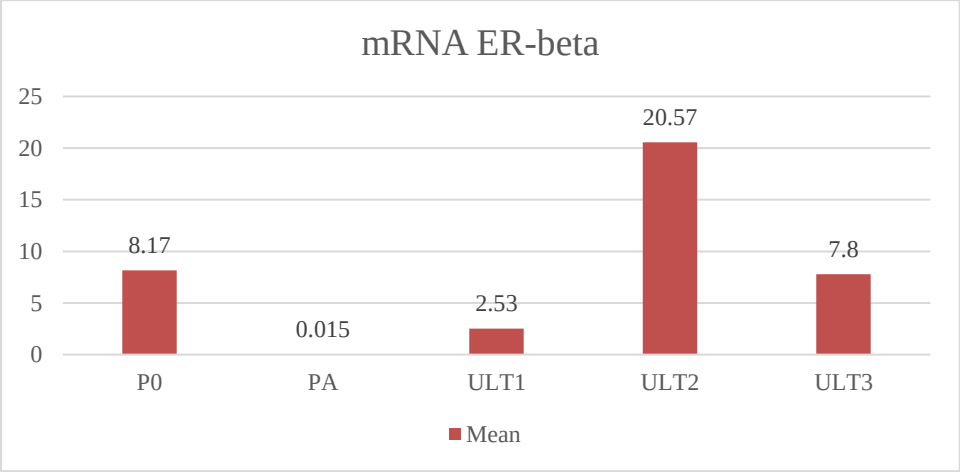

FIGURE 3: "ERβ mRNA Expression Mean"

Based on Figure 3-3, it shows that the P0 and ULT 1,2,3 groups had a higher mean ERα mRNA expression than the PA group. However, in the ULT 1 it was lower than the P0. In ULT 2 the average expression began to increase significantly higher than the other groups, but decreased in ULT 3.

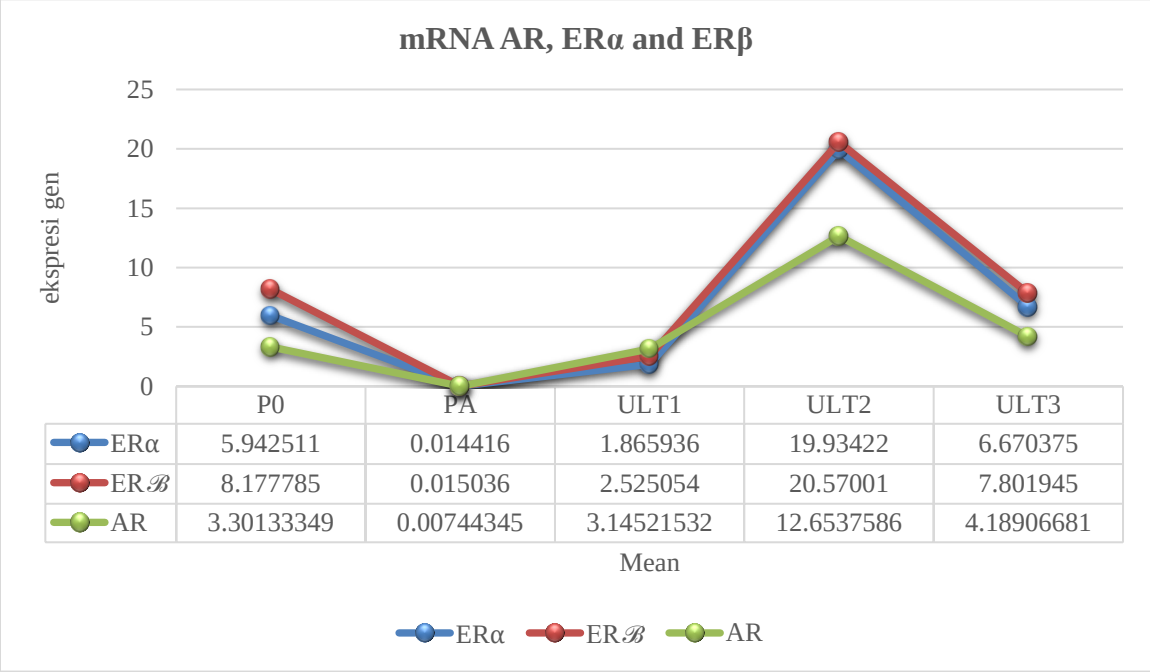

FIGURE 4: "Trends in mean gene expression of mRNA AR, ERα and ERβ values"

Based on Figure 3-4, it shows that the average value of mRNA gene expression experienced a decrease in the average in the PA group and a significant increase in the second treatment group compared to the control. The trend of decreasing or increasing mean expression in each group was almost the same for the three gene mRNA expressions tested, but for AR mRNA expression, the mean for each group was lower than ER mRNA expression.

DISCUSSION

Effect of Ulva lactuca ethanol extract on AR mRNA expression in Rattus norvegicus ovaries after exposure to MSG

Based on the laboratory tests via real-time PCR, the relative expression of the AR mRNA gene in the ovaries was found to be highest in the group treated with 200 mg/kg BW of Ulva lactuca algae extract, with a mean and standard deviation of relative expression of 12.65 + 22.84. In contrast, the lowest relative expression was observed in the PA group with a mean and standard deviation of 0.007 + 0.01, compared to the ULT and P0 groups. These findings indicate that there was a decrease in the relative expression of AR mRNA genes after MSG induction from healthy controls, which increased after the administration of Ulva lactuca algae extract. Additionally, the gene expression levels differed significantly between all groups, as evidenced by the Kruskal Wallis test results with C = 13.938 and P = 0.007 ($P<0.05$). The average increase in gene expression after MSG induction was observed in the treatment with a dose of 100 mg/kg BW and was found to be optimal at a dose of 200 mg/Kg BW. However, the gene expression decreased at a dose of 400 mg/Kg BW, falling below the second dose.

The condition of decreased gene expression observed at a dose of 400 mg/kg BW may be attributed to a negative feedback response in the body after administering a dose that exceeded the body's capacity to respond positively to the extract. This negative feedback response is consistent with the physiology of the endocrine system, which helps maintain homeostasis by regulating hormone levels in appropriate concentrations. In a negative feedback mechanism, high concentrations of hormones will signal back through a regulatory system to stop the production of these hormones by inhibiting the secretion of hormones that stimulate their release (Kleine & Rossmanith, 2016). Administering high doses that exceed the body's capacity to accept exogenous steroid-containing extracts can result in high concentrations of steroid compounds in the body, which may signal the inhibition of the release of endogenous steroids such as testosterone through a certain mechanism. The deficiency of this hormone can affect the AR receptor, leading to its inactivity, as indicated by a decrease in the expression of the AR mRNA gene. This is because the number of receptors in cells can increase or decrease depending on the response to a specific stimulus.

However, the administration of Ulva lactuca algae extract has led to an increase in gene expression, which had initially decreased after MSG induction compared to the control. The decrease in receptor mRNA gene expression after MSG induction compared to the control was caused by the negative effect of MSG on the ovarian organs. According to previous studies, excessive MSG consumption, especially in women, can cause damage to the hypothalamus and pituitary glands, reduce the activity of antioxidant enzymes in the body, and affect the female gonads, namely the ovaries. This, in turn, can lead to a decrease in ovarian function, specifically folliculogenesis for follicle growth and development, and steroidogenesis for the synthesis of sex steroid hormones (Wang et al., 2020; Mostafa et al., 2021).

Hormones that fail to be synthesized will have an effect on the specific receptor gene expression of the hormone. As a result of hormone deficiency, the receptor becomes inactive and binds to heat shock protein (HSP) (Smith and Toft, 2008), making it difficult or even undetectable as a hormone-specific receptor. This, in turn, leads to a decrease in the expression of sex steroid receptors (Traish & Kim, 2006) because the concentration of the hormone in the target organ cells affects the cell's response in activating its specific receptors. In the treatment group with the administration of Ulva lactuca algae extract, there was an improvement in ovarian function, especially in the regulation of steroidogenesis, leading to hormone synthesis restoration. The presence of hormones will stimulate the cell nucleus to activate specific receptors, leading to the formation of bond complexes between hormone-receptors in the cell nucleus, which allows for cell activity to take place

If the hormone receptors have been reactivated and many hormones trigger the activation of these receptors, then many specific receptors will

be detected in cells, indicating increased gene expression. The formation of sex steroid hormones in the process of steroidogenesis can affect target organs through a mechanism of action with their receptors (Baldassare et al., 2013). The mechanism of action of steroids begins with the entry of sex steroid hormones into the nucleus of the target cell through the plasma membrane, then binding to the receptor protein to form a hormone-receptor complex in the hormone response element (HRE) area (an area related to the structure of the hormone receptor). The hormone-receptor complex activates the target cell's DNA as a transcription factor, which undergoes mRNA transcription. The formed mRNA undergoes translation to produce new proteins that exit the nucleus towards the cytoplasm. The protein begins as a collection of several amino acids and undergoes modification to become a more complex protein, ready for use by the body in cell development (Belfiore, 2018).

Based on the AR structure which consists of several domains such as NTD (N-terminal domain/amino terminal domain), DBD (DNA Binding Domain) and LBD (Ligan Binding Domain). NTDs are unique and long sequences of amino acid sequences, which contain important sequences for gene regulation, exhibiting structural plasticity and contributing to the specificity of the steroid hormone-receptor response (Betney & McEwan, 2003; Schaufele et al., 2005; McEwan et al., 2007). Thus, in NTD there are several amino acids to compose complex polypeptides and proteins. The compound of Ulva lactuca which contains amino acids can interact and gather in NTD that it triggers processes in the DBD domain, including the hormone testosterone which is bound to the LBD area. Regarding the amino acids that match the structure in AR, it has been analyzed using the molecular docking method on compounds in Ulva lactuca and adjusted for AR (specific hormone) controls, namely Tryptophan, Arginine, Glycine and Proline which are present in compounds of Myristic Acid, Hexadecanoid, Palmitic Acid and Isofucosterol.

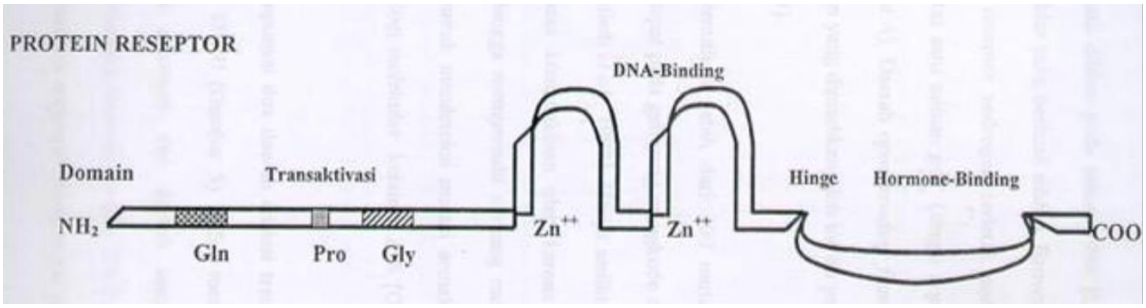

FIGURE 5: "Structure of AR genes and proteins"

(Quigley et al., 1995 in Davey and Grossmann, 2016)

Based on this, it is shown that the increase in mRNA gene expression, which initially decreased after MSG induction, can also be caused by the binding process of several essential amino acids contained in Ulva lactuca. These amino acids correspond to the AR structure in the N-terminal domain and trigger the process of DNA transactivation to mRNA transcription, in addition to the role of bonding with its ligand, namely the hormone testosterone in the hormone binding area/LBD.

Effect of Ulva lactuca ethanol extract on the expression of ERα and ERβ mRNA in Rattus norvegicus ovary after exposure to MSG

Based on the results of laboratory tests via real-time PCR for the relative expression of the ERα mRNA gene in the ovaries of the sample group, it was found that the group treated with 200 mg/kg BW of Ulva lactuca algae extract had the highest mean and standard deviation of ERα

mRNA gene expression of 19.11 ± 29.67 compared to the other groups. Similarly, the relative expression of the ERβ mRNA gene at the same treatment dose of Ulva lactuca extract had an average of 20.57 ± 33.65. In the PA group, the lowest relative expression was 0.015 ± 0.017 compared to the ULT and P0 groups. Regarding the relative expression of the ERβ mRNA gene, the PA group had a mean expression of 0.015 ± 0.016.

This shows that there was a decrease in the relative expression of the ERα and ERβ mRNA genes after MSG induction compared to healthy control conditions, and an increase again after administration of Ulva lactuca algae extract. However, the expression of the ERα mRNA gene did not differ significantly between the groups, as indicated by the Kruskal-Wallis's test with a value of C=8.212 and P=0.084 (P>0.05). Meanwhile, the expression of the ERβ mRNA gene differed significantly between the groups with C=11.959 and P=0.018 (P<0.05). The increase in the average gene expression of both ERα and ERβ was observed after MSG induction, with the highest increase seen at a dose of 200 mg/kgBW. However, at a dose of 400 mg/kgBW, the gene expression decreased below the second dose, possibly due to a negative response in the body after administration of a dose that exceeded its capacity to respond positively.

The decrease in ER mRNA gene expression is similar to what occurs in AR mRNA at high doses. This decrease may be due to negative feedback, which is the body's response to extracts given in large amounts. Such extracts can inhibit the mechanism of action of sex steroid hormones and their receptors, leading to a decrease in the expression of the ER mRNA gene. The optimal increase in gene expression that occurs after the administration of Ulva lactuca algae extract treatment, especially at a dose of 200 mg/Kg BW compared to the PA and P0 groups, can be attributed to the presence of Steroids and Sterols such as Isofucosterol. These compounds can affect the ovaries and re-regulate the synthesis of sex steroid hormones, especially estrogen with the largest type, namely estradiol. Estradiol production, triggered by the re-regulation process, activates its specific receptors, namely ER alpha and beta. Estradiol that enters the Nucleus cells forms a complex with ER in the ERE (estrogen response element) area to carry out cell biological activities in proliferation and differentiation (Yasar et al., 2016). Whereas in conditions of estradiol hormone deficiency due to the influence of MSG exposure causes the ER to become inactive and bind to HSP, which is shown in the decrease in mRNA from ER.

In the free state the receptor protein is inactive, then it forms bonds with the HSP 90 chaperoning machine, which is a multiprotein chaperone complex with large molecular weights or macromolecules that are easily denatured by increasing temperature. The function of the HSP is to bind to the receptor in the absence of stimulation. Connection between the receptor and this protein chaperone occurs in an orderly, gradual manner and is necessary for the maintenance of the unliganded receptor in a state ready to bind and respond to hormones. HSPs also modulate how receptors respond to hormones and activate target genes. When the receptor binds to a specific hormone or ligand, the ligand induces a change in formation that the receptor separates from the HSP and becomes active. This active complex promotes interaction with chromatin and other regulatory proteins thereby inducing specific gene expression and target cell activity (Smith and Toft, 2008). After the sex steroid hormone binds to a specific receptor to form a complex, the steroid hormone will cause the receptor to undergo a structural change (conformation) such that the inactive receptor becomes active. The activated receptor then binds to specific sites on the chromosomal DNA, namely regulatory sites and genes and activates these genes to modulate gene transcription. This is followed by a translation process, in which the genetic codes carried by the gene are translated into a series of amino acids to form polypeptides to certain proteins (Belfiore, 2018).

The compound of Flavonoids and Phenols in Ulva lactuca can also play a role in the recovery of ovarian organs due to exposure to free radicals/toxic agents derived from food such as MSG. This source of antioxidants in Ulva lactuca can inhibit the effects of free radicals by

capturing free radicals. The results of the last study showed that the ethanol extract of Ulva lactuca algae had antioxidant activity with a percentage value of 51.63% at a concentration of 100 mg/L (Ulaan et al., 2019). The ability to capture free radicals of the ethanol extract of Ulva lactuca is known to be better than Vitamin C seen from the IC50 value that the higher the concentration of Ulva lactuca extract, the higher its ability to capture free radicals (Emelda and Annisa, 2019). Free radicals are atoms that have one or more unpaired electrons, that they have a pair of free radicals that will bind to the electrons around them by attacking the cells or tissues around them which cause damage. Degenerative diseases caused by cell damage due to free radicals such as cancer, autoimmune and inflammatory diseases. The human body can naturally produce antioxidants, however, if the number of free radicals exceeds the number of antioxidants in the body, free radicals will attack proteins or DNA, that in this condition the body needs antioxidants from outside (Winarsih, 2007).

Phenol compounds, Flavonoids and carotene compounds contained in Ulva lactuca can function as antioxidants (Tamat et al., 2007), and have Phytomelatonin compounds which are active substances as strong antioxidant activity that can counteract free radicals (Julyasih et al., 2009). The Ulva lactuca ethanol extract from Gunung Kidul Beach has a better ability to inhibit free radicals than ascorbic acid with IC50 values of 17.25 μg/ml and 28.9 μg/mL respectively (Emelda and Annisa, 2019). Thus, the effect of free radicals or ROS caused by exposure to MSG which has a negative effect on the ovaries can be minimized by antioxidant compounds in Ulva lactuca algae extract, either through their influence on the hypothalamus-pituitary or directly on the ovaries. Based on this, the ovaries can carry out their functions optimally again as before.

Besides that, the essential amino acid compound in Ulva lactuca can also interact with the ER in the n-terminal region of the ER structure so as to trigger a process of DNA activation and subsequent ER mRNA transcription, in addition to activation of specific receptors in response to the hormone estradiol. Based on the ER structure which consists of several domains such as the NTD/N-terminal activation function-1 (AF-1), DBD and LBD/activation function-2 (AF-2) domains (Weatherman et al., 2001). The compound of Ulva lactuca which contains amino acids can interact and gather in the n-terminal region that it triggers the process of activating DNA in the DBD domain, including estradiol which is bound to LBD. Some of the amino acids in Ulva lactuca that match the structure of the ER have been analyzed using the molecular docking method and adjusted for ERα (specific hormone) control, namely Lysine, Arginine, Isoleucine, Alanine and ERβ namely Phenylalanine, Leusine, Methionine, Histidine which are present in compounds of Miristic Acid, Hexadecanoaid, Palmitic Acid and Isofucosterol.

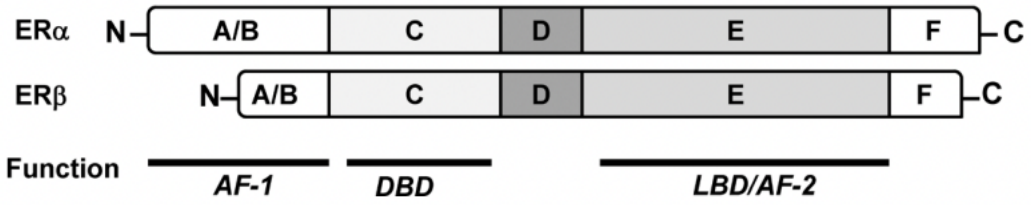

FIGURE 6: "The structure of the ER" (Kleine and Rossmanith, 2016).

Based on this, it shows that the increase in gene mRNA expression which initially decreased after MSG induction, can also be caused by the binding process with several essential amino acids contained in Ulva lactuca which corresponds to the ER structure in the N-terminal domain, then triggers the process of DNA transactivation to mRNA transcription, in

addition to the role of binding with its ligand, namely the hormone estradiol in the hormone binding area /LBD. Ulva lactuca is also known to have the amino acid Methionine but in small concentrations. Methionine plays a major role in the normal function of cells and is also a Cysteine precursor which is used to form several important molecules such as Glutathione which is the body's natural antioxidant (Sena et al., 2013). Lysine compound in Ulva lactuca has an important function in normal cell growth and metabolism (Yang et al., 2016). In addition, Tyrosine was also found to act as a high antioxidant against peroxide radicals (Matsui et al. 2018).

After exposure to or induction of MSG, rats experienced a decrease in ovarian sex steroid hormone receptor mRNA gene expression when compared to controls. This condition is caused by pure MSG which is an oxidant which, if consumed for a long and excessive period of time, will cause excessive activity of glutamate receptors in the brain and central nervous system, this will cause an increase in free radicals such as reactive oxygen species (ROS) and reduce antioxidant enzymes in the body (Glutathione peroxidase, Glutathione S-transferase and Superoxide dismutase) thereby triggering cell oxidative stress including causing damage to the hypothalamic arcuate nucleus (Cervantes et al., 2014). Disorders of the hypothalamus will also affect the pituitary gland, eventually reducing the secretion of gonadotropins (FSH and LH) and further inhibiting the work of the gonads in the female reproductive system, namely the ovaries, especially in the process of synthesizing sex steroid hormones (Shannon et al., 2019; Wang et al., 2020).

Several past studies have proven that MSG causes endocrine disorders through the hypothalamus-pituitary-axis (HPA) mechanism. The neurotoxic effect of MSG causes excitotoxicity in the brain which causes disturbances in the HPA axis mechanism (Hanipah et al., 2018). Glutamate is an excitatory neurotransmitter and intracellular calcium influx in nerve cells, so high levels of glutamate can cause an increase in uncontrolled intracellular calcium influx and cause nerve cell death. Disruption of the HPA mechanism reduces levels of hormones including FSH and LH as well as gonadal steroids, these disturbances ultimately have a negative effect on reproductive tissue (Hamza et al., 2014). The toxic and metabolic effects of MSG are reported to induce oxidative stress events in tissues (Diniz et al., 2004; Onyema et al., 2006). Oxidative stress on cells that is not handled properly, simultaneously over time will cause damage and even death to organ cells.

Monosodium glutamate can also cause toxicity to the reproductive system through the influence of free radicals which induce oxidative stress. Organs of the reproductive system are targets of reactive oxygen species (ROS) because there is adipose tissue in these organs (Hanipah et al., 2018). An increase in lipid peroxidation such as Malondialdehyde and a decrease in the activity of antioxidant enzymes such as Glutathione will affect the increase in oxidative stress of reproductive organs, and a decrease in the activity of antioxidant enzymes after administration of MSG (Hamza et al., 2014; El-Sawy et al., 2018; Hanipah et al., 2018). Increased production of free radicals due to MSG can trigger lipid peroxidation and dysfunction of spermatozoa membranes, damage to cell DNA, and failure of motility (Khaled et al., 2016).

The results of a study by Maidawilis (2010), MSG administration to mice lowered gonadotropin hormone levels, causing disruption in the process of oogenesis in the ovaries. This toxic effect of MSG is also known as an oxidizing effect, which is a chemical reaction that can produce free radicals, thus triggering a chain reaction that can damage cells to the genetic stage which in turn affects the female reproductive organs. Some of the effects caused by oxidative stress on the reproductive cycle are impaired oocyte maturation, impaired steroidogenesis in the ovaries, impaired ovulation to implantation, blastocyst formation so that luteal maintenance in pregnancy is disrupted, as well as primary and secondary infertility (Agarwal, 2012). Monosodium glutamate is also said to have great potential as an endocrine disruptors chemical (EDC) that can affect organs and the reproductive system. These endocrine disrupting chemicals

are exogenous chemicals that interfere with hormones, thereby increasing adverse health risks including cancer, reproductive disorders, cognitive deficits and obesity (Merrill et al., 2020). The target of EDC is the female reproductive system, especially the ovarian organs. The ovaries are the main organs responsible for reproduction and endocrine functions. EDC exposure is known to cause many problems in reproductive health such as infertility, failure of ovarian function, and abnormal levels of sex steroid hormones (Patel et al., 2015).

Several EDCs and their effects on adult ovarian function are folliculogenesis and steroidogenesis. In steroidogenesis, EDC can affect the expression of protein levels or enzyme activity, namely steroidogenic enzymes, so that it can produce changes in sex steroid hormones. At the stage of folliculogenesis EDC can affect the development of primordial follicles since a woman is born and the maturity of the follicles at a later stage, namely from primary, preantral and antral follicles. Antral follicles are the mature follicle type and can ovulate and produce sex steroid hormones. The effect caused by EDC is to affect primordial follicles and lead to conditions of early and persistent infertility. The effect of EDC on antral follicles can result in infertility and changes in hormonal levels (Patel et al., 2015).

Disturbances in steroidogenesis in the ovaries can inhibit the production of hormones such as testosterone and estradiol, which are sex steroid hormones and the end products in steroidogenesis. These hormones are produced by the theca cells and granulosa cells of the ovaries. Hormone deficiency can cause disturbances in the working system of steroid hormones in cells, as the action of testosterone and estradiol in cells is mediated by specific receptors, such as androgen receptors for testosterone and estrogen receptors for estradiol. If there is a decrease in hormones, cells will give a negative response by deactivating specific receptors, leading to a decrease in the expression of hormone receptors as observed through the expression of receptor mRNA. This can eventually lead to the failure of the formation of interactions between hormones and specific receptors that are necessary for cell growth and development. As a result, the failure in the process of transcription and translation of new proteins can cause follicular cell degeneration.

In line with research by Arini (2016) related to the mechanism of action of sex steroid hormones in the reproductive system along with their receptors, it shows that hypogonadism in post-castration male rats causes a drastic decrease in androgen hormones. However, the administration of exogenous androgens is able to restore the condition of target organ cells, including hormonal regulation with its receptor, which was observed through an increase in hormone receptor messenger RNA in the target organ cells studied. Research by Liao et al. (2006) showed that the androgen-AR complex is strongly associated with RNA, such as Cytoplasmic ribonucleoproteins and nuclear forms. The interaction between AR-RNA is a physiological event such as processing, transport and formation of RNA. Androgen deficiency will eventually lead to the loss of the AR-RNA linkage and its function as previously described. Thus, the administration of exogenous androgens causes DNA repair in target organs that initially experience cell damage, so that the transcription process can take place.

The increased expression of the sex steroid hormone receptor mRNA gene in the ovaries occurred due to the administration of green algae Ulva lactuca extract, which initially decreased after exposure or induction of MSG when compared to controls. This effect is attributed to the Sterol compound in Ulva lactuca, such as Isofucosterol, which has been identified by the GC-MS method. Isofucosterol acts as a sterol regulating element binding protein-1 (SREBP1) with an essential role in providing cholesterol in mammals, which is a precursor for the synthesis of sex steroids. This regulation of cholesterol in turn re-regulates the process of steroidogenesis in the ovaries. Sterol regulatory element binding protein can also regulate steroidogenesis through the sterol response element (SRE) region in the promoter region of the gene that encodes the steroid acute regulatory protein (StAR) in mammals. Then, it binds directly to the hormone response element (HRE) (Lopez et al., 2002). In

other words, sterols play a vital role in steroidogenesis in mammals (Johnson et al., 2002). With an increase in the hormones testosterone and estradiol as the end products of steroidogenesis, the nucleus of target organ cells such as the ovary responds by the appearance of specific receptors such as AR and ER in a quantity that corresponds to the increase in hormones, marked by increased mRNA from AR and ER.

To carry out the biological activity of cells in cell growth and development, there must be an interaction between the hormone and its receptor to form a bond in the element response area (Speroff & Fritz, 2005). Thus, the hormone receptors will respond to any stimulus given by sex steroid hormones according to the type, location, and amount of hormone. Therefore, the re-increase in hormone receptors is most likely preceded by an increase in the hormone as a ligand of the receptor, as a form of response from the target cell. The estrogen hormone can only enter cells containing estrogen receptors, and these cells will respond to the hormone (Hammes & Levin, 2011). When a receptor binds to estrogen, there will be a change in receptor formation that allows coactivator binding and activates transcription factors. The activation of gene transcription will direct the synthesis of certain proteins that affect various cell functions, depending on the type and target (Yazawa et al., 2019). Deficiency of sex steroid hormones causes the receptors to become inactive and remain in the target cell nucleus, where they bind to heat shock proteins, which prevents the mechanism of action of sex steroid hormones for cell proliferation from occurring and vice versa (Busillo et al., 2014).

The hormones estrogen and androgen are involved in the development and maintenance of normal reproductive function. These hormones exert their biological effects through interactions with nuclear steroid receptors namely AR, ERα and ERβ (Dostalova et al., 2017; Farzaneh et al., 2016). The hormone receptor complex binds to the hormone responsive element/HRE region on DNA and undergoes activation for the transcription process of m-RNA (Messenger ribonucleic acid) as a template for the translation of new proteins (Federman et al., 2006; Speroff & Fritz, 2005), which is useful for cell regeneration. When the hormones estrogen and androgen bind to their receptors, they work to regulate cell growth and development in the female reproductive system. The mechanism of action of hormones in cells is influenced by the concentration of hormones and the response of target cells to the presence of hormones. If a receptor binds to its hormone, there will be a change in receptor formation that allows coactivator binding and activates transcription factors.

Hormone receptors will be activated by bonding with hormones to form complexes so that if there is an increase in hormone levels, receptor expression will also increase as a response of target cells to hormones. Estrogen and androgen hormones in cells will function if they form complexes with their receptors (Speroff & Fritz, 2005), namely estrogen-receptors (ERα and ERβ) and androgen-receptors (AR) (Baldassare et al., 2013). The interaction that has been formed between testosterone and AR in the ARE (androgen response element) area and estradiol and ER in the ERE (estrogen response element) area will trigger the activation of transcription factors to carry out the transcription process. Activation of gene transcription will direct the synthesis of certain proteins which then affect various cell functions depending on the type and target (Baldassare et al., 2013).

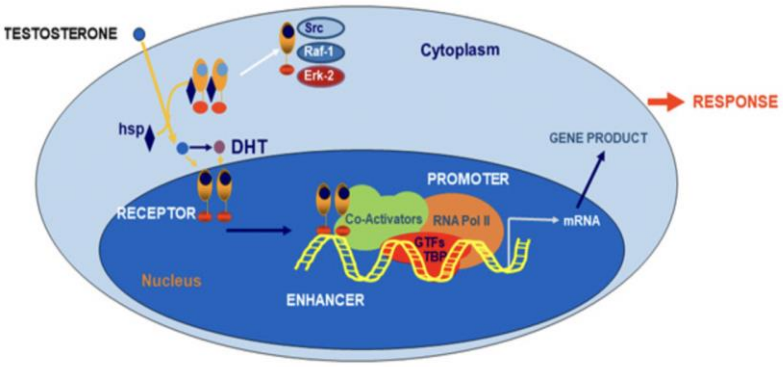

FIGURE 7: "Schematic of AR Activation" (Gruber et al., 2002)

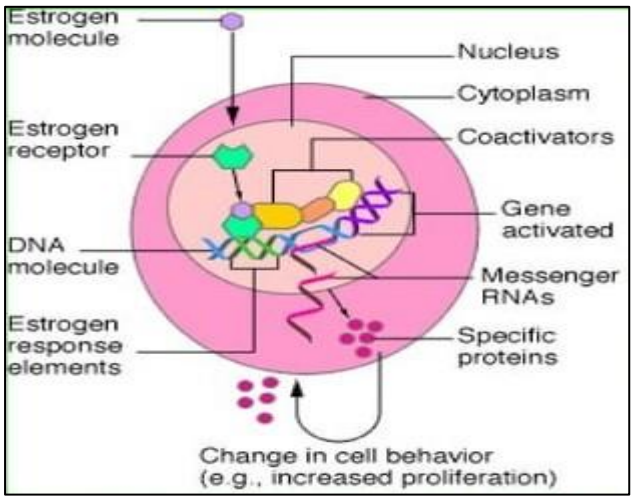

FIGURE 8: "Schematic of ER Activation" (Gruber et al., 2002); (Lewis et al., 2010)

The sex steroid hormone receptor is inactive and associated with HSP (heat shock protein) when it is free. It becomes active when it binds to a ligand, such as the appropriate sex steroid hormone, and is separated from HSP. In a free state, the mechanism of action of sex steroid hormones for cell proliferation is inhibited. However, it can take place again in conditions where receptors and hormones form bonds/complexes (McDonnel & Norris, 2002). Based on this, the receptor is activated by bonding with the hormone to form a complex. If there is an increase in hormone levels, receptor expression will also increase as a response of target cells to hormones. A study by James et al. (2012) proved that there is an increase in ovarian ER mRNA when entering the menstrual period, where there are fluctuations in the hormone estrogen. This indicates that if there is an increase in sex steroid hormones in the blood, there will also be an increase in their receptors on target organ cells. Increased expression of this hormone receptor mRNA is useful for the proliferation and differentiation of target organ cells.

Sterols in Ulva lactuca can also exert their influence on cells through the help of a mediator, namely the CYP51 enzyme, by activating the meiotic process in the ovaries, namely as meiosis activating sterols (MAS) which can induce oocyte maturation as well as the development of its follicles induced by FSH (Byskov et al., 2002; Jin et al., 2006). Through Meiosis activating sterol, it will affect the follicular fluid-MAS to bind to ovarian intracellular receptors in the HRE area, activate transcription and increase gene expression, causing oocyte maturation (Byskov et al., 2002; Hao et al., 2014). In addition, Sterols

can also act as sterol regulating element binding protein-2 (SREBP2) which will directly bind to transcription factors to carry out transcriptional activity in cells. The entire mechanism of the role of Sterols in Ulva lactuca will eventually produce new proteins and cause follicles to experience cell regeneration which is marked by an increase in de Graaf follicles, a decrease in atresia follicles and an increase in the corpus luteum. The action of Sterols is known to resemble the action of endogenous estrogens, namely as an estrogen antagonist under normal conditions, changing the pattern of synthesis and metabolism of endogenous hormones and modifying hormone receptor levels and one of the benefits is having an estrogenic effect on women who experience estrogen hormone deficiency (Wu et al., 2005).

Apart from the sterol pathway, Ulva lactuca algae also plays a role through the compounds of flavonoids and phenols, which act as antioxidants by inhibiting the harmful effects of free radicals that are dispersed in cells. Exposure to free radicals caused by MSG initially affects the hypothalamus-pituitary, leading to disturbances in ovarian function. However, administering Ulva lactuca can lead to cell recovery, thereby restoring normal cell function and biological activity. Furthermore, flavonoids and phenols can directly affect the ovaries.

Past studies have shown that the bioactive compounds that act as antioxidants in seaweed are compounds from the phenol and flavonoid groups, which are also found in many higher plants (Farasat et al., 2014). The class of flavonoids, phenolic acids, tannins, and lignans is a group of natural antioxidants. Flavonoids act as antioxidants by capturing free radicals, thereby preventing oxidative stress. The activity as an antioxidant possessed by most flavonoids is contributed by the phenolic hydroxyl groups in their molecular structure and also through their ability to capture free radicals and their activity as a metal chelating agent. Some research results also report that sterol group steroids actually have the potential to serve as a source of antioxidants (Pramana & Saleh, 2013 in Gazali et al., 2018).

The increase in sex steroid hormone receptor mRNA genes can also occur due to the compound of Ulva lactuca algae which contain lots of amino acids, which, if associated with the structure of hormone receptors such as AR and ER. The structure of the AR consists of three main functional domains, including: the N-terminal transcription regulatory domain, the DNA binding domain (DBD) and the ligand binding domain (LBD). The N-terminal domain is the most variable region, while DBD is the most stable region among steroid hormone receptors. This leads to the binding of selective androgen response elements (AREs) allowing AR activation. DBD is connected to a ligand-binding domain which also has a similar structure, functions to mediate interactions between AR and chaperone proteins and interacts with the N-terminus of AR to stabilize androgen bonds (McEwan et al., 2007).

Androgen receptors and other steroid hormones function as transcription factors that are inactive or in a free state, and become active when they bind to their specific ligand, namely the hormone testosterone. Therefore, if more and more amino acids are collected in the n-terminal, it is possible to trigger an increase in the hormone receptor mRNA gene in addition to an increase in the hormone-receptor complex in the LBD area due to an increase in hormones. The estrogen receptor consists of 6 functional domains, namely: 1) The A/B domain is the part whose activation is not dependent on the ligand or is called the transactivation function 1 (AF 1). 2) Domain C is the binding site for DNA (DNA binding domain). This region has an amino acid similarity of 99% in both ERs. 3) Domain D, is the part that contains the signal with the nucleus and is associated with domain C. 4) Domain EF is the terminal part which is the part that binds to the ligand, dimerization occurs or the process of combining two similar molecules into one larger molecule and the transactivation function that is ligand dependent (AF2). This section has an amino acid similarity of approximately 58% (Nilsson and Gustafsson, 2011).

Marine algae are known to contain Phenols, enzymes, nucleic acids, amino acids, vitamins (A, B, C, D, E and K) and macro minerals such as nitrogen, oxygen, calcium and selenium as well as micro minerals such as iron, magnesium and sodium (Anggadiredja et al., 2009). The

compound of amino acids, vitamins and minerals in macroalgae reaches 10-20 times compared to land plants (Sulistyowati, 2003). Ulva lactuca can be a source of essential amino acids, some of which such as Histidine are found in levels similar to those found in nuts and eggs (Lordan et al., 2011). Many traditional foods use Ulva lactuca algae as well as in the agricultural industry. Foods made from Ulva lactuca algae are known to be safe and have good nutritional compound such as protein compound (up to 30%) and high Fe, unsaturated fatty acids and have essential amino acids (Li et al., 2018). Ulva lactuca was identified as having 9 essential amino acids and 6 non-essential amino acids, with a total of 883 ± 120.42 amino acids (Pratiwi et al. 2021).

On the other hand, there are differences in expression between the ERα and ERβ mRNA genes, it appears that the average expression of ERβ mRNA is higher than that of ERα. Besides that, in the average expression of ERα mRNA, it was found that the difference was not significantly different from ERβ. Both of these can be caused because the ovarian tissue is predominantly ERβ and only a small portion is ERα, in which ERα is more dominant in vaginal tissue than in the ovary. Hence, the effect of the compounds of Ulva lactuca is more detected in the ERβ mRNA gene due to the location of the test in ovarian tissue.

The location of ERα is mainly found in the mammary glands, uterus, ovaries (theca cells), bones, male reproductive organs (testes and epididymis), prostate (stroma), liver, and adipose tissue. ERβ is found primarily in the prostate (epithelium), bladder, ovaries (granulosa cells), colon, adipose tissue, and the immune system. The two receptor subtypes act differently in some target cells and tissues than estrogen (Dahlman-Wright et al., 2006; Haris, 2007). This subtype of ERα has a more prominent role in mammary and uterine gland proliferation and in homeostatic and metabolic balance (Dahlman-Wright et al., 2006; Heldring et al., 2007). ERα also plays a greater role in cases of ovarian and breast cancer, ERα binds to estrogen and proliferates abnormally (Tang et al., 2019). ERβ is mainly found in the nervous system, ovaries, cardiovascular system, and male reproductive system (Jia et al., 2015).

ERα and ERβ form bonds with estrogen to carry out cell biological activities in target cells such as cell development, because the effect of estrogen on cells is mediated by these two ERs (Andreinei et al., 2019). Meanwhile, new estradiol is synthesized from the aromatization process of the hormone testosterone in granulosa cells with the help of the aromatase enzyme (Kleine and Rossmanith, 2016). Thus, ERα which is expressed in the ovarian theca cells cannot form a complex with estradiol, in the end it is very likely that ERα will be slightly expressed and its receptors will be much inactive compared to ERα. Expression of the alpha isoform of ER is an important receptor for mediating the specific response of the vagina to estrogen (Gebhart et al., 2001 in Armayanti et al., 2016). Meanwhile, beta estrogen receptors are very dominant ERs in normal ovaries (Andreinei et al., 2019). This suggests that there is a possibility that there are differences in the location of the majority of the estrogen receptors of these two subtypes which lead to differences in gene expression from the ovaries. The ERβ mRNA gene is known to be localized mainly to granulosa cells, developing follicles and preovulatory follicles. Immunohistochemical examination showed that the levels of ERα and ERβ proteins had different specificities for tissues and cell types (Andreinei et al., 2019).

The study revealed differences in gene expression between ER and AR mRNA, with the latter showing a lower average expression in each group. This may be attributed to the fact that estrogen receptors dominate expression in female ovaries more than androgens (Tang et al., 2019). Androgens and their receptors (AR) play a significant role in reproductive function, particularly in normal ovarian function under physiological and pathophysiological conditions. In female rats with neuroendocrine and ovarian disorders, infertility has been observed. AR is expressed in three components of ovarian follicles: theca interstitial cells, which are responsible for androgen production; granulosa cells, which convert testosterone to estrogen; and germ cells, i.e., oocytes (Sen A et al., 2014). AR

is essential in maintaining follicle growth during the early stages of folliculogenesis and preventing follicular atresia (Sen A et al., 2010 in Ma et al., 2016). Ovarian granulosa cells have the highest abundance of AR RNA genes and proteins (Franks and Hardy, 2018). This could be the reason for the low expression of AR mRNA in the study, as granulosa cells convert testosterone to estrogen, and the abundance of estrogen levels in these cells may result in only a few ARs becoming active due to testosterone deficiency.

The regulation of the female reproductive system was initially influenced by androgens; Thus, concentration of hormone testosterone was higher than that of estradiol, this condition occurred when the experimental animals were in the proestrus-estrus phase. This could be due to testosterone in the early stages of the cycle acting as a substrate for the aromatase enzyme for the production of estradiol in circulation so that estradiol levels will increase (Hammes and Levin, 2019). Thus, at the beginning of the estrus cycle testosterone will increase first compared to estradiol then in the middle of the cycle testosterone will slightly decrease. Ovarian follicles that have matured under the influence of FSH will release the hormone estrogen and begin to be regulated under the control of LH, causing proliferation of the cells that make up the endometrial wall and causing an increase in vaginal mucus. The increased concentration of estrogen during follicular growth eventually influences the cervix to secrete mucus as occurs during the proestrus-estrus phase (Aritonang et al., 2017).

There is a different opinion which states that the Flavonoids synthesized by almost all of the plant world can inhibit the aromatase enzyme. The disruption of the aromatase enzyme, which functions to catalyze the conversion of androgens to estrogens, causes the number of androgens to remain high. However, when compared with the results of this study, the higher expression of ER mRNA compared to AR mRNA proves that estradiol levels had increased previously. This increase in estrogen is made possible by the influence of the aromatase enzyme, which converts the hormone testosterone into estradiol (Yazawa et al., 2019), from which testosterone levels were initially higher than estradiol. It was previously known that hormone availability stimulates the ovarian nucleus to activate specific receptors as a form of cellular response to hormones. The system of steroid hormones in cells depends on the availability of hormones in cells and the response of target organ cells to hormones (Nugroho, 2016).

The low expression of AR mRNA gene compared to ER mRNA gene is related to hormone levels. Testosterone undergoes an aromatization process during steroidogenesis to become estradiol, leading to an increase in estradiol levels and a corresponding increase in ER expression as a cell response to increased hormone levels. The second dose of Ulva lactuca treatment resulted in the most significant increase in gene expression compared to the other groups. This increase in gene expression is in line with the increase in hormone levels after Ulva lactuca treatment, which had initially decreased due to MSG exposure compared to the control group. Thus, the increase in the expression of mRNA genes of sex steroid hormone receptors observed in the ovaries after exposure to MSG, which had initially decreased in the post-induction group, proved that there was an effect of giving ethanol extract of Ulva lactuca algae in increasing the expression of these genes. This increase in gene expression is useful for the proliferation and differentiation of cells in the ovaries.

The mechanism of the observed increase may have started with an increase in levels of the hormones estradiol and testosterone that occurred in the Ulva lactuca algae extract treatment group, which initially decreased after exposure to MSG compared to the control group. This working system occurs during steroidogenesis in the ovary, and involves the influence of Sterol and Flavonoids metabolite compounds from Ulva lactuca in neutralizing and inhibiting ROS caused by toxic agents such as MSG. The process of steroidogenesis is re-regulated after being disrupted by MSG, and Isofucosterol plays an important role in the synthesis of the hormone testosterone as well as estradiol. These two hormones are the result of the production of steroidogenesis, which then stimulates the

ovaries to carry out their biological activities in the cells. In the presence of these hormones, the cell will respond, forming an interaction between the hormone and a specific receptor in the cell nucleus. DNA activation occurs followed by the process of mRNA transcription, leading to the translation of a new protein.

The advantages of this Ulva lactuca algae as material for the reproductive system, especially hormonal regulation compared to direct administration of Hormone Replacement Therapy (HRT) or exogenous steroid hormones, the presence of Flavonoid and Phenolic compounds in green algae can inhibit the adverse effects of ROS by capturing free radicals caused by compounds that are toxic, so that the oxidation process is hampered (Farasat et al., 2014). The formation of ROS plays an important role in cell damage, ROS will continue to be controlled by endogenous antioxidant systems in the form of enzymatic or non-enzymatic antioxidants. If there is an excessive increase in ROS and a decrease in the number of antioxidants produced by the body due to the influence of exogenous and endogenous factors, an imbalance occurs between antioxidants and ROS (Thiele, 2001; Valko et al., 2007). This causes pathological conditions and oxidative stress. The protective effect of natural antioxidants such as flavonoids plays an important role in protection against oxidative stress (Wong et al., 2016).

The mechanism of the observed increase may have started with an increase in levels of the hormones estradiol and testosterone that occurred in the Ulva lactuca algae extract treatment group, which initially decreased after exposure to MSG compared to the control group. This working system occurs during steroidogenesis in the ovary, and involves the influence of Sterol and Flavonoids metabolite compounds from Ulva lactuca in neutralizing and inhibiting ROS caused by toxic agents such as MSG. The process of steroidogenesis is re-regulated after being disrupted by MSG, and Isofucosterol plays an important role in the synthesis of the hormone testosterone as well as estradiol. These two hormones are the result of the production of steroidogenesis, which then stimulates the ovaries to carry out their biological activities in the cells. In the presence of these hormones, the cell will respond, forming an interaction between the hormone and a specific receptor in the cell nucleus. DNA activation occurs followed by the process of mRNA transcription, leading to the translation of a new protein.

The antioxidant activity of Phenols is related several mechanisms such as free radical scavenging, hydrogen atom donation to free radical compounds for stabilization, quenching single oxygen, chelating metal ions, and acting as substrates for radicals such as superoxide and hydroxyl (Sonani et al., 2017; Adwas et al., 2019). Therefore, Ulva lactuca algae has several advantages, such as containing steroids or sterols that play a role in hormonal regulation in the reproductive system, as well as several types of antioxidants such as flavonoids and phenols, which can inhibit free radical compounds and contribute to the recovery of female reproductive organs, in addition to the role of sterols. This is considered better than the direct administration of exogenous steroid hormones, which are riskier and have more side effects.

CONCLUSION

Based on the results of the study as a whole, it shows that the effect of giving Ulva lactuca algae extract can lead to an increase in the expression of mRNA genes AR, ER (α,β). This condition can be seen from the mean expression of the tested mRNA genes in the post-MSG induction group which initially decreased compared to the control, then increased slowly in the administration of Ulva lactuca algae with an optimal increase in the second dose compared to the first and third doses. Based on this, it suggests that Ulva lactuca algae can lead to improvements in the function of ovarian organs after exposure to MSG, resulting in an increase in sex steroid hormone receptors in the cell nucleus after decreasing.

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CONFLICT OF INTERESTS

The authors confirm that the content of this article has no conflict of interest

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