Effect of Brown Algae Extract Sargassum sp on Malondialdehyde Levels in White Rats (Rattus Norvegicus) Pregnant Wistar Strains

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Abstract

Pregnancy is an inflammatory condition that produces unstable oxidative stress and can damage macroeconomic tissue including DNA and protein which disrupt the placentation process. Increased levels of malondialdehyde as an indicator of lipid peroxidation and decreased levels of antioxidants. This research was conducted experimentally with pretest-posttest Control Group Design. Using three treatment groups for the white rat (Rattus Norvegicus) test for Pregnant Wistar Strains. Twenty-one samples consisted of three groups (Group 1 negative control, Group 2: sea algae extract Sargassum sp dose 300 mg/kg bb/day, Group 3: sea algae extract Sargassum sp dose 600 mg/kg bb/day. Malondialdehyde levels were determined by examination Eliza sandwich The results showed that Sargassum Sp brown algae extract had a significant effect on decreasing levels of malondialdehyde (300mg p: 0.008 dose; 600mg p: 0.001 dose) in white rats (Rattus norvegicus) Pregnant Wistar Strains.

Keywords: *Sargassum sp, Malondialdehyde, Rattus norvegicus.*

Introduction

In pregnancy will increase, increase, increase. Oxidative stress that occurs can occur because antioxidants do not compensate. Therefore, under certain conditions triggered by oxidative stress, endogenous antioxidants become insufficient and require exogenous antioxidants to maintain optimal cellular function.

Malondialdehyde (MDA) levels in pregnant women are higher than in nonpregnant women. Increasing MDA levels increase with increasing gestational age from the first, second and third trimesters, in this case, there has been an increase in peroxidation which is a marker (marker) to increase free radicals in the blood[1]. Oxidative stress will disrupt the placentation process. Abnormalities associated with certain diseases for example preeclampsia, mola hydatidosis, and abortion. Increased risk of failure associated with increased free radicals that increase in the development of placental function and effect on the fetus[2]. The increase in oxidative stress corresponds to an increase in lipid peroxidation formation. Oxidative stress will cause damage to trophoblast cells which will start to become abortion (miscarriage).

Reactive oxygen species (ROS) can be obtained endogenously or exogenously but both can affect oocytes and embryos and can also cause oxidative DNA damage which increases the speed of mutagenesis and chromosomal instability[3]. Mitochondrial dysfunction by stress or improper mitochondrial integrity control causes an increase in electron leakage from the respiratory chain. Very reactive superoxide causes itself to be very unstable. This can damage the different macromolecules in the mitochondria including fats, proteins, and DNA that can affect mitochondrial function and stimulate extensive electron leakage and ROS production[4].

Brown algae Sargassum sp has the highest antioxidant activity compared to red and green seaweed and the main antioxidant component found in Sargassum sp is a polyphenol compound[5]. Most compounds in the brown algae Sargassum sp are carotenoids which act as antioxidant activities. The ability of polyphenols as antioxidants is based on the presence of hydroxyl groups which are aromatic compounds as contributors to hydrogen atoms for free radicals and the ability of electron delocalization which has a double bond for conjugation, as well as the stability of the resonance structure[6].

Materials and Method

This study used a simple randomized design method using three groups of test animals of white rat (Rattus norvegicus) strains of pregnant Wistar and were treated as follows:

a. Negative Control Group (Group 1): mice were only given distilled water on days 7-19 of pregnancy with a frequency of 1 time per day.

b. Treatment group 1 (Group 2): rats were given seaweed extract Sargassum sp on days 7-19 of pregnancy with a dose of 300 mg/kg rat frequency 1 time per day

c. Treatment group 2 (Group 3): rats were given seaweed extract Sargassum sp on days 7-19 of pregnancy with a dose of 600 mg/kg rat frequency 1 time per day.

In this study, the sample was adjusted to the inclusion criteria, while the prospective white rat (Rattus norvegicus) strain of female Wistar with a bodyweight of 200-250 grams with age 3-4 months. Female mice were mated with male rats during the estrous phase with a system of one female and one male partner for 24 hours which had previously been examined by vaginal smears to confirm the lust phase of the estrus period(7). The next day the female rat was examined to ensure marriage by checking vaginal smears containing much sperm and examined signs of pregnancy such as signs of plugs (vaginal redness, swelling and thick lumps in the vaginal introitus) which were stated as the first day of pregnancy. The cage is placed at room temperature using 12 hours of bright lighting and 12 hours of darkness, as well as maintaining the humidity of the room. Each rat was given once a day according to the treatment set.

The three groups of test animals, each consisting of seven white rats (Rattus norvegicus) strains of pregnant Wistar, were examined for Malondialdehyde levels on the 7th day of pregnancy as a pre-test examination, and given the intervention of brown algae extract Sargassum sp in both treatment groups according to the dose of treatment predetermined and negative control groups are only given equates once a day. The treatment began on the 7th day until the 19th day. Furthermore, on the 19th day, the level of malondialdehyde was examined as a post-test.

All conditions and handling of test animals are carried out by following a protocol approved by the medical research ethics committee of the Faculty of Medicine, University of Hasanuddin, number: No.199 / UN4.6.4.5.31 / PP36 / 2019.

Manufacture of Sargassum sp brown algae extract

The tools needed for the manufacture of Sargassum sp brown algae extracts are trash, blender, 30 mesh sieve, scales, stirrer, 1-liter tube, filter paper, Erlenmeyer flask, measuring flask and rotary evaporator, while the material for extracting is Sargassum sp. water, and ethanol 96%. Sargassum sp brown algae powder which has been mashed in maceration with 96% ethanol solvent, then allowed to stand for 3 days but still stirring every day. The ethanol extract is then stored in the Ellen Meyer flask to be evaporated with a Rotary vacuum evaporator at 40°C to produce a familiar extract (concentrated) with a constant weight.

Measurement of malondialdehyde levels using an enzyme-linked immunosorbent assay (ELISA) sandwich technique.

Prepare all reagents (standard, control) and samples. Prepare a strip well that is conditioned at room temperature for ± 30 minutes. Add 50 µl of standard solution to well A1-A6. Add 40 µl of serum to the well and then add 10 µl of anti-MDA antibody to the wells. Add 50 µl of streptavidin-HRP each to the sample well and standard well. Cover well with seal. Incubate at 37°C for 60 minutes. Remove the seal and wash the plate with buffer washing 5 times. Add 50 µl of substrate A solution to each well then add 50 µl of substrate B solution to the well (standard and sample). Cover the plate with a seal. Incubate at 37°C for 10 minutes. Add 50 µl stop solution

into the well (standard and sample), there will be a color change from blue to yellow. Measure the optical density (OD value) of each well using a microplate reader using a wavelength of 450 nm within 10 minutes after stopping adding solutions.

Results

a. Malondialdehyde (MDA) levels in white rats (Rattus norvegicus) pre and post-test strains of pregnant women by a group.

MDA levels at the lowest pre-test were found in the negative control group (0.26 ± 0.11 nmol / ml) compared to the Sargassum sp extract group with a dose of 300 mg / kgBB / day (0.43 ± 0.10 nmol / mL) and Sargassum sp extract group dose 600 mg / kgBB / day (0.41 ± 0.07 nmol / mL). At the post test, the lowest MDA level was found in the Sargassum sp extract group at a dose of 600 mg / kgBB / day (0.25 ± 0.10)) nmol / mL) compared to the Sargassum sp extract group at a dose of 300 mg / kgBB / day (0, 40 ± 0.12 nmol / mL) and negative control group (0.41 ± 0.06 nmol / mL) (Table. 1).

Table 1. Malondialdehyde (MDA) levels in white rats (Rattus norvegicus) Pregnant Wistar Strains Pre and Post Test by Group

Group	n	MDA levels (nmol / mL)					
		Pre Test		Post Test		Change	
		Mean (SD)	Median (Min-Max)	Mean (SD)	Median (Min-Max)	Mean (SD)	Median (Min-Max)
Negative Control	7	0,26 (0,11)	0,27 (0,10–0,38)	0,41 (0,06)	0,43 (0,28–0,45)	0,14 (0,11)	0,11 0,03 – 0,35)
Sargassum sp 300 mg dose	7	0,43 (0,10)	0,45 (0,25 –0,60)	0,40 (0,12)	0,43 (0,15–0,51)	-0,03 (0,10)	0,02 (-0,1 – 0,08)
Sargassum sp 600 mg dose	7	0,41 (0,07)	0,41 (0,32 –0,50)	0,25 (0,10)	0,22 (0,11 –0,42)	-0,15 (0,12)	-0,2 (-0,2 –0,1)

b. Differences in levels of malondialdehyde (MDA) in white rats (Rattus norvegicus) strains of pre and post-test pregnant Wistar by group

MDA levels in the negative control group increased 0.14 ± 0.11 nmol / mL and based on the results of the Pairet T-Test statistic, there was a significant difference in the increase in MDA levels in white rats (Rattus norvegicus) strains of pregnant Wistar between pre-test and post-test (p: 0.012).The MDA levels in the Sargassum sp extract group at a dose of 300 mg/kg bb/ day decreased 0.03 ± 0.10 nmol/mL.While the MDA levels in the Sargassum sp extract group dose 600 mg/ kg bb/day there was a decrease of 0.15 ± 0.12 nmol / mL and based on the results of the Pairet T-Test statistic it was found that there was a significant difference in the decrease in MDA levels in white rats (Rattus norvegicus) strains of pregnant Wistar between pre-test and post-test (p: 0.019) (table.2)

Table 2. Differences in levels of Malondialdehyde (MDA) in White Rats

(Rattus norvegicus) Pregnant Wistar Pre and Post Test Strains Based on group

Group	Time	N	MDA levels (nmol / mL)		p-Value
			Mean (SD)		
			Score	Change	
Negative Control	Pre Test Post Test	7 7	0,26 (0,11) 0,41 (0,06)	0,14 (0,11)	0,012
Sargassum sp 300 mg dose	Pre Test Post Test	7 7	0,43 (0,10) 0,40 (0,12)	-0,03 (0,10)	0,425
Sargassum sp 600 mg dose	Pre Test Post Test	7 7	0,41 (0,07) 0,25 (0,10)	-0,15 (0,12)	0,019

c. Differences in levels of malondialdehyde (MDA) in white rats (Rattus norvegicus) strains of pregnant Wistar between groups

Based on the results of the Independent statistical test sample t-test, there was a significant difference in changes in MDA levels between the negative control group and the Sargassum sp extract group dose 300 mg/kg bb/day (p: 0.008), there was a significant difference in changes in MDA levels between the negative control group and the extract group Sargassum sp dose 600 mg/kg bb/day (p: 0.001), and there was no difference in changes in MDA levels between the Sargassum sp dose 300 mg/kg bb/day and Sargassum sp extract group dose 600 mg/kg bb/day (p: 0.072) (table.3)

Table 3. The difference in levels of malondialdehyde (MDA) in White Rats

(Rattus norvegicus) Intergroup Pregnant Wistar Strains

Group	n	Change in MDA levels (nmol / mL)		p-Value*
		Mean (SD)		
		Score	Change	
Negative Control Sargassum sp 300 mg dose	7 7	0,14 (0,11) -0,03 (0,10)	0,18 (0,05)	0,008
Negative Control Sargassum sp 600 mg dose	7 7	0,14 (0,11) -0,15 (0,12)	0,30 (0,06)	0,001
Sargassum sp 300 mg dose Sargassum sp 600 mg dose	7 7	-0,03 (0,10) -0,15 (0,12)	0,12 (0,06)	0,072

Discussion

The results of this study found that administration of Sargassum Sp brown algae extract had a significant effect on decreasing levels of malondialdehyde (MDA) in white rats (Rattus norvegicus) strains of pregnant Wistar at a dose of 300 mg/kg bb/day (p: 0.008) and a dose of 600 mg/kg bb/day (p: 0.001) compared to the negative control that had increased. This indicates that the brown algae Sargassum sp at a dose of 300 mg/kg bb and 600 mg/kg bb gave a positive effect on the decrease in levels of malondialdehyde in pregnant Wistar strain white rats.

The content of bioactive compounds than brown algae Sargassum sp is a flavonoid compound that directly reacts with free radicals by capturing unpaired electrons to free radicals without producing other free radicals as a result of the reaction[8]. Flavonoids can inhibit initiation by capturing major radicals such as superoxide. The effects of flavonoids on ROS are through two mechanisms by increasing endogenous antioxidants and capturing free radicals or neutralizing free radicals[9].

The flavonoid groups identified in the Sargassum sp extract are catechins and quercetin[10]. These two groups of flavonoids are thought to have a role to ward off free radicals so that they can reduce levels of malondialdehyde in the white rat group of pregnant Wistar strains that have been treated. Quercetin is a flavonoid compound that has the most powerful antiradical properties against hydroxyl radicals, peroxyl and superoxide anions[11].Flavonoids protect cells from attack by reactive oxygen compounds such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals, and peroxynitrite. Lipid damage occurs through three phases, namely the initiation, propagation and the termination stage which is the final stage by binding a free radical with other free radicals so that they are no longer reactive[12].When a hydrogen atom is removed by a molecular lipid some compounds will react with a hydrogen atom that forms hydroxyl radicals (●OH),alkoxy (RO),peroxyl (ROO) and possibly also with HO2 but not including H2O2.Membrane lipids are phospholipids consisting of unsaturated fatty acids that facilitate peroxidation due to the presence of hydrogen atoms containing only one electron, in this case, there is a carbon atom without electron pairs. The double bonds in fatty acids will weaken the CH bonds in carbon atoms adjacent to the double bonds which makes it easy for hydrogen atoms to transfer. Likewise, if there are sufficient oxygen concentration of lipid radicals, it will react with oxygen to form peroxyl radicals (ROO●),this stage occurs in propagation. For the termination stage, peroxyl radicals (ROO●) will attack other hydrogen atoms originating from other lipid molecules that are close and produce lipid peroxides and peroxyl radicals or interact with other antioxidants. Therefore this process causes the cessation of the oxidation process by neutralizing free radicals that are formed during oxidation.

This study shows that in test animals with control groups there was a significant increase in malondialdehyde levels between pre-test and post-test (p: 0.012).This illustrates that in pregnancy two phenomenological oxidative stress phenomena are found, which occur in the trimester in the peripheral part of the placenta.Therefore there is an increase in local oxygen concentration at a stage of pregnancy so that the trophoblast has a concentration and activity of major or endogenous antioxidants such as superoxide dismutase (SOD) is low[13]. The presence of major trophoblastic oxidative damage and progressive degeneration of the villi will trigger the formation of the fetal membrane which is an important developmental step for vaginal delivery.

Conclusion

Based on research conducted found Sargassum Sp brown algae extract gives a significant effect on decreasing levels of malondialdehyde (MDA) in white rats (Rattus norvegicus) pregnant Wistar strains at a dose of 300 mg/kg bb/day and a dose of 600 mg/kg bb/day.

Ethical Clearance- Taken from Medical Faculty ethical committee

Source of Funding- Self

Conflict of Interest - Nil

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