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POTENTIAL OF SEAWEED (Eucheuma cottonii) EXTRACT AS A HEPATOPROTECTOR IN RABBIT (Oryctolagus *cuniculus*) INDUCED ORGANOPHOSPHATE PESTICIDES

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Abstract

The use of organophosphate pesticides in developing countries is already widespread and can contaminate animal feed or human food ingredients which has an impact on liver damage and other organs. The use of seaweed containing polyphenols as a hepatoprotector for organophosphate poisoning has not been widely used by the public. The purpose of this study was to determine the potential of seaweed extract as a hepatoprotector in organophosphate-induced rabbits as seen from serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) levels. This study used a complete randomized design with 20 male rabbits as experimental animals which were divided into 4 treatments with 5 repetitions, namely: P0 was the control group which was only given distilled water, P1 was the group given seaweed extract at a dose of 200 mg/Kg BW, P2 is the group given seaweed extract at a dose of 400 mg/KgBB and P3 is the positive control group which is not given seaweed extract and distilled water. On the 15th day the P1, P2, and P3 groups were given profenofos pesticide at a dose of 50 mg/kg BW. The results showed a very significant difference (p-value = 0.02, α = 0.05) in SGOT levels between the P0 control group (135.4 ± 67.00 U/L) and the P1 treatment group (41.8 ± 17.45 U/L), P2 (25.8 ± 8.75 U/L), and the positive control group P3 (70 ± 29.04). The results of the study also found that there was no significant difference in SGPT levels in the P0 control group and the P1, P2, and P3 control groups. Giving seaweed extract with different doses provides a hepatoprotector effect by reducing the release of SGOT into the blood.

***Keywords**: 3-5 keywords arranged in alphabetical order (TNW 10)*

Abstrak

Penggunaan pestisida organofosfat pada negara berkembang sudah meluas dan dapat mengkontaminasi pakan ternak atau bahan pangan manusia yang berdampak pada kerusakan hati dan organ yang lain. Pemanfaatan rumput laut yang mengandung polifenol sebagai hepatoprotektor untuk keracunan organofosfat belum banyak digunakan oleh masyarakat. Tujuan penelitian ini adalah untuk mengetahui potensi ekstrak rumput laut sebagai hepatoprotektor pada kelinci yang diinduksi organophospat dilihat dari kadar serum glutamate oxaloacetate transaminase (SGOT) dan serum glutamate pyruvate transaminase (SGPT). Penelitian ini menggunakan Rancang Acak Langkap dengan 20 ekor kelinci jantan sebagi hewan coba yang dibagi menjadi 4 perlakuan dengan 5 kali ulangan yaitu: P0 adalah kelompok kontrol yang hanya diberikan akuades, P1 adalah kelompok yang diberikan ekstrak rumput laut dengan dosis 200 mg/KgBB, P2 adalah kelompok yang diberikan ekstrak rumput laut dengan dosis 400 mg/KgBB dan P3 adalah kelompok kontrol positif yang tidak diberikan ekstrak rumput laut dan aquades. Pada hari ke-15 kelompok P1, P2 dan P3 diberikan pestisida profenofos dengan dosis 50 mg/kgBB. Hasil penelitian mendapatkan perbedaan yang sangat nyata (p-value =0,02, α = 0,05) terhadap kadar SGOT pantara kelompok kontrol P0 (135,4±67,00 U/L) dengan kelompok perlakuan P1 (41,8±17,45 U/L), P2 (25,8±8,75 U/L), dan kelompok kontrol positif P3 (70±29,04). Hasil penelitian juga mendapatkan bahwa kadar SGPT tidak terdapat perbedaan yang nyata kelompok kontrol P0 dan kelompok perlakuan P1, P2 dan kontrol kontrol positing P3. Pemberian ekstrak rumput laut dengan dosis berbeda memberikan efek hepatoprotektor dengan menurunkan pelepasan SGOT ke dalam darah.

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***Kata Kunci**: Hepatoprotektor, rumput laut, pestisida. SGOT, SGPT*

1. INTRODUCTION

The use of organophosphate pesticides in Indonesia is still very common among farmers to repel and eradicate pests. Improper use of pesticides in controlling plant pests (OPT) can have undesirable consequences. The use of pesticides must be done as well as possible by minimizing the negative impacts that arise (Ministry of Agriculture, 2020). Thannos et al (2016) stated that the signs and symptoms of organophosphate intoxication in rabbits can be described in DUMBELS: Diarrhoea, Urination, Miosis, Bronchospasm, Emesis, Lacrimation, and Salivation.

Based on data from the Directorate General of Agricultural Facilities and Infrastructure in 2016 as many as 3,930 pesticide formulations have been registered for their use. The number of these pesticides has increased every year, from this data the most frequently registered were insecticide-type pesticides with 1,342, and herbicide formulations with 1,037 formulations. The use of pesticides can be used to reduce the population of plant-disturbing organisms (OPT) to a balance limit but must be selective and not kill non-target insects such as natural enemies (Ministry of Agriculture, 2017). Toxicity or toxicity is the innate nature of a pesticide that describes the potential for a pesticide to cause immediate death or other harm to higher animals, including humans (Runia, 2008).

Seaweed or algae has long been a product that is widely consumed by the world community. Seaweed in Indonesia has been consumed by people, especially in coastal areas (Waryono, 2001). Advances in science and technology for the use of seaweed are very diverse, both for food and non-food products. Broadly speaking, seaweed derivative products can be grouped into 5P, namely Food, Feed, Fertilizer, Cosmetic Products, and Pharmaceutical Products (KKP, 2016). The use of seaweed herbs to minimize the occurrence of organophosphate pesticide poisoning in rabbits has not been widely used by the public.

Seaweed has a source of nutrients including antioxidants in capturing free radicals and regenerating vitamin E (Soo et al, 2005). In general, the main natural antioxidants are polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotene), and vitamins (vitamins E and C).Manach et al. 2004;).Zenginet al. (2011) stated that natural antioxidants, both in the form of crude extracts of their chemical content, are very effective in preventing destructive processes caused by oxidative stress. These ingredients will make plants such as seaweed become hepatoprotectors.

Hepatoprotector is a compound that can protect the liver from liver damage (Yusuf et al, 2018). Antioxidants are one of the targets of the hepatoprotector mechanism, therefore antioxidants are needed to convert free radicals into unreactive compounds (Hanifa and Hendriani, 2016). Seaweed also contains flavonoids which are derived from secondary metabolite compounds that contain antioxidants. Polyphenols contained in seaweed have antioxidant activity so that they can prevent various degenerative diseases and diseases due to oxidative stress, including cancer, aging, and narrowing of blood vessels (Suparni and Sahri, 2009). The high content of polyphenols is very potential as a hepatoprotector because it can reduce levels of liver enzymes in the blood,

The research will discuss potential extract potential seaweed (Eucheuma cottonii) containing fophenol as a hepatoprotector by measuring serum levels of glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) against organophosphate poisoning contained in pesticides. The use of less controlled

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organophosphate pesticides, especially in developing countries, can contaminate animal feed or human food ingredients which has an impact on liver damage and other organs as animal feed. Seaweed extract is expected as an alternative in preventing liver damage (hepatoprotector) caused by organophosphate pesticides in both livestock and humans.

2. RESEARCH METHOD

This research was conducted in July 2019. The seaweed extraction site was carried out at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram. Administration of seaweed extract and organophosphate pesticides was carried out at the Teaching Animal Hospital (RSHP) of the Faculty of Veterinary Medicine, Mandalika University of Education. SGOT and SGPT examinations were carried out at the West Nusa Tenggara Testing and Calibration Health Laboratory Center.

2.1 Research Design

This research is a laboratory experimental study with a randomized post-test only control group design. This study used male rabbits (Oryctolagus cuniculus) as experimental animals.

This study used a complete randomized design (CRD) with 20 male rabbits which were divided into 4 treatments with 5 repetitions, namely: P0 was the control group which was only given distilled water, P1 was the group given seaweed extract at a dose of 200 mg/Kg BW, P2 is the group given seaweed extract at a dose of 400 mg/KgBB and P3 is the positive control group which is not given seaweed extract and distilled water.

On the 15th day the P1, P2, and P3 groups were given profenofos pesticide at a dose of 50 mg/kg BW. The number of repetitions for each treatment is calculated using the Kusriningrum formula (2008), with the following calculations:

t (n-1) ≥ 15	4n≥19
4 (n-1) ≥ 15	n≥4.75

Description:
t=treatment; n = repetition

In consideration of this formula, t is the number of treatments to be given and n is the number of repetitions so that in this study 5 rabbits were used, and each treatment

2.2.Research Tools and Materials

The tools used to extract seaweed (Eucheuma cottonii) are a macerator, shaker, rotary evaporator, analytical balance, Erlenmeyer, and stirrer. Tools for treatment were gastric sonde, cage, 1 ml syringe, test tube rack, centrifuge, and Eppendorf for feed and drink. Tools for examining SGOT and SGPT using a spectrophotometer. Belongs to the West Nusa Tenggara Testing and Calibration Health Laboratory.

The materials for extracting seaweed are distilled water, 70% alcohol, 96% ethanol, sodium thiosulfate and sodium nitrite, sterile cotton, whattman paper no.41, absorbent plastic, and thick cotton cloth. Materials for treatment are seaweed extract, organophosphate pesticides, distilled water, drinking water, and food. Materials for examining SGOT and SGPT are rabbit serum, SGOT, and SGPT reagents.

The experimental animals used in this study were male rabbits aged 1-2 months with an average weight of 1-2 kilograms (kg). The rabbits were obtained from breeders who are located in the manggar district, Central Lombok regency, West Nusa Tenggara province. Experimental animal treatment was carried out at the Educational Animal Hospital (RSHP), Faculty of Veterinary Medicine, Mandalika University of Education. Images of male

rabbits (Oryctolagus cuniculus) used in this study can be seen in Figure 1.

Figure 1. Rabbit (Oryctolagus cuniculus) (Personal Documentation, 2019)

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2.3 Seaweed Extraction

The seaweed used comes from Jerowaru, East Lombok Regency. Seaweed is washed with water, drained, cut, and then dried without direct sunlight until dry. The dried seaweed was crushed using a blender and sieved using a 40-mesh sieve (Septiana and Asnani, 2013). Seaweed image(Eucheuma cottonii) originating from Jerowaru, East Lombok Regency, West Nusa Tenggara Province, can be seen in Figure 2.

Figure 2.*Eucheuma cottonii* (Personal Documentation, 2019)

328 g of dried seaweed powder was dissolved in 150 ml of 96% ethanol and then stirred once a day for 4 days and on the 5th day, it was filtered using filter paper to obtain extract and dregs. The resulting extract was filtered again with Whatman No. 41 and separated from the solvent by evaporation using a rotary evaporator. The remaining solvent was removed with nitrogen gas to obtain a concentrated extract in liquid form of as much as 40 ml (Septiana and Asnani, 2013).

2.4 Administration of Seaweed Extract

Administration of seaweed extract in the form of liquid and distilled water using a stomach tube. Aquades were given to the treatment group (P0) until day 14. Seaweed extract (Eucheuma cottonii) was given at a dose of 200 mg/KgBW because the weight of 1 ml = 1000 mg, treatment group 2 (P2) was given seaweed extract 400 mg/KgBW the administration was carried out 1 (one) time a day and the treatment group 3 (P3) was not given aquadest or seaweed extract for 14 days. On the 15th day, Profenofos pesticides were given which were purchased from UD SINTA Mataram at a dose of 50 mg/Kg BW in the treatment group (P1 and

P2). The process of giving grass extract to rabbits can be seen in Figure 4.

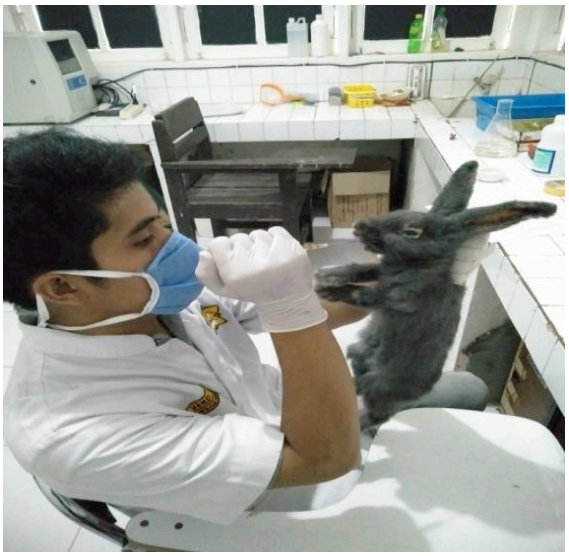

Figure 4. Giving grass extract to rabbits (Personal Documentation, 2019)

2.5 Administration of Organophosphate Pesticide

The organophosphate given was a type of profenofos LD50 400 mg/Kg BW (Dreisbach, 1983) using a gastric sonde with a dose of 50mg/KgBW due to high toxicity in the use of organophosphate pesticides as much as <50mg/Kg (Runia, 2008)in the treatment group (P1, P2 and P3) on the 15th day after the treatment was given 1 (one) time. The seaweed extraction process can be seen in Figure 3.

Figure 3. Seaweed Extraction Process (Personal Documentation, 2019)

2.6 Collection of Rabbit Blood Serum

According to Prabu (2008), symptoms of organophosphate and carbamate pesticide poisoning often develop after 4 hours of exposure, although they can happen after 12 hours. Blood was taken from a rabbit using a 1 ml syringe in the auricular vein on the 17th day following treatment. The blood sample was placed in a test tube, and after waiting for the blood to coagulate, it was centrifuged at 300 rpm. After the blood serum had been centrifuged, it was labelled with a distinct color and placed in an Eppendorf tube for laboratory analysis.

2.7 Examination of SGOT and SGPT

SGOT examination was carried out by inserting 20 µl of a sample (rabbit blood serum) into Eppendorf, added with 1000 µl of SGOT reagent, mixed, and incubated at 37°C for 1 minute. The absorbent on the photometer was read with a wavelength of 340, factor 1745, program K 20. SGPT examination was carried out by inserting 20 µl of a sample (rabbit blood serum) into Eppendorf and adding 1000 µl of SGPT reagent. Incubate at 37°C for 1 minute. The absorbent was read after exactly 1 minute, 2 minutes, and 3 minutes at a wavelength of

340, factor 1745 and on the K 20 program (Haribi et al., 2009).

2.8 Data analysis

Data on serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) levels obtained with a spectrophotometer were analyzed using the Anova test (Analysis of Variance) with α = 0.05, if there is a difference between the treatments, continue the Duncan test to find out the highest and lowest results. Data analysis using the application SPSS (Statistical Program for Social Science) for windows.

3. RESEARCH RESULTS

Rabbits as experimental animals in each treatment consisting of control group (P0) which was only given distilled water, group (P1) which was given seaweed extract at a dose of 200 mg/KgBB, group (P2) which was given seaweed extract at a dose of 400 mg/KgBB and group (P3) which was not given the extract. seaweed and distilled water, then on the 15th-day groups P1, P2 and P3 were given profenofos pesticide at a dose of 50 mg/kgBW. On the 17th day, rabbit blood was taken using a 1 ml syringe in the auricular vein to measure SGOT and SGPT levels.

This study has carried out measurements of serum levels *glutamic oxaloacetic transaminase*(SGOT) and serum glutamic pyruvate transaminase (SGPT) in the blood serum of 20 male rabbits using a spectrophotometer. The results of measuring SGOT and SGPT levels in rabbit blood in each treatment (P0, P1, P2, and P3) can be seen can be seen in Table 1, for SGOT and Table 3 for SGPT.

Table 1. Rabbit Blood Serum SGOT Levels

Treatment	SGOT U/liter level $\overline{X}$ ±SD	P-Value
P0	135.40±67.00*	
P1	41.8±17.45	0.02
P2	25.8±8.75	
P3	70±29.04	

$\overline{X}$ = Average; SD = Standard Deviation; Values with different symbols in the same column are significantly different at the 0.05 level (*p<0.05)

Table 2 states that the results of measuring serum glutamic oxaloacetic transaminase (SGOT) levels in rabbit blood serum in each treatment included: P0 = 135.40 ± 67.00 U/liter; P1 = 41.80 ± 17.45 U/liter; P2 = 25.80 ± 8.75 U/liter, and P3 = 70.00 ± 29.04 U/liter. The results of the analysis using the Anova test (Analysis of Variance) with α = 0.05, the SGOT value in rabbit blood serum with SPSS for windows, found significant differences between the treatments with p≤0.05 (p-value = 0.02).

The results of the ANOVA test obtained significant differences between treatments for AST levels in rabbit blood serum, then continued with the Duncan test to see which treatment produced the highest and lowest AST levels. This study uses Duncan's test as a follow-up test because it is desired to compare the range of the sample mean subset with the smallest significant range that is calculated. Bewicket al. (2014) stated that the procedure of Duncan's multiple-range test is based on a comparison of the range of a subset of the sample mean to the calculated least significant range.

The results of Duncan's test for AST levels in rabbit blood serum in each treatment using SPSS for windows can be seen in Table 2.

Table 2. Rabbit Blood Serum Duncan test results

Treatment	AST levels (U/liter)	
	Subset for alpha = 0.05	
P2	25.80	
P1	41.80	
P3	70.00	
P4		135,40
significance	0.098	1.00

Note: SGOT levels in the column in different subsets, there is a significant difference (p≤0.05)

The results of the Duncan test in Table 2 stated that the levels of AST in rabbits that had the highest values were found at P0 which was significantly different from P1, P2, and P3. The lowest AST levels were found in P2 which was not significantly different from P1 and P3 and significantly different from P0. This shows that the administration of Eucheuma cottonii extract in different treatments with control or without administration of Eucheuma cottonii on SGOT values, with the lowest value found in P2 with seaweed extract 400 mg/Kg BW.

Table 3. Rabbit Blood Serum SGPT Levels

Treatment	**SGOT U/liter level** $\overline{X}$ **±SD**	**P-Value**
P0	37.2±14.20	
P1	29.2±5.26	0.490
P2	31.2±4.32	
P3	28.4±11.26	

$\overline{X}$ = Average; SD = Standard Deviation; Values with different symbols in the same column are significantly different at the 0.05 level (*p<0.05)

Table 3 states that the results of measuring serum glutamic pyruvate transaminase (SGPT) levels in rabbit blood serum in each treatment included: P0 = 37.2 ± 14.20 U/liter; P1 = 29.2 ± 5.26 U/liter; P2 = 31.2±4.32 U/liter, and P3 = 28.4±11.26 U/liter. The results of the analysis using the Anova test (Analysis of Variance) with α = 0.05 SGPT value in rabbit blood serum with SPSS for windows found significant differences between the treatments with p≤0.05 (p-value = 0.49).

4. DISCUSSION

The results of this study indicated that seaweed extracts affected SGOT and SGPT levels in rabbits induced by organophosphate pesticides as seen from the average results of SGOT and SGPT levels. SGOT enzyme activity in normal rabbits according to Malole (1989) is 42.5-98.0 U/liter and SGPT enzyme activity in normal conditions in rabbits is 48.5-78.9 U/liter. A decrease in SGOT and SGPT enzyme levels is an indication of the healing of damaged liver cells caused by induction of organophosphate pesticides. The decrease in SGOT and SGPT enzyme levels in this study occurred because seaweed extract contains antioxidants that act as an inhibitor of the spread of free radicals in the body.

High AST levels in the control group (P0) which was only given distilled water were significantly different from the group (P1) which was given seaweed extract at a dose of 200 mg/KgBB, group (P2) which was given seaweed extract at a dose of 400 mg/KgBB and group (P3) which was not given seaweed extract and distilled water. Panjaitan et al (2007) stated that increased levels of AST in the blood were caused by severe liver damage accompanied by necrosis so that enzymes from mitochondria also came out of cells.

The lowest AST level of rabbits in this study was group (P2) which was given seaweed extract at a dose of 400 mg/KgBB but was not statistically significantly different from the group (P1) which was given seaweed extract at a dose of 200 mg/KgBB and group (P3) which was not given seaweed extract and distilled water. In general, SGPT levels are lower than the normal SGPT value in rabbits. The lowest SGPT levels were found in P3 but not significantly different from P0, P1, and P2.

Based on the mechanism of decreasing liver damage according to Muthusamy et al. (2012) by preventing the formation of Reactive oxygen species (ROS) by the flavonoid compounds contained in seaweed extracts. Flavonoids can carry out the scavenger mechanism by directly capturing radical compounds, namely superoxide and peroxynitrite. Based on these data, the liver function of all experimental groups has decreased, this can also occur due to

negligence in handling when storing serum in the Eppendorf tube which should be tested immediately using a spectrophotometer in the laboratory or stored at 2-8°C. Kaplan (1989) states that the SGPT enzyme can be stable for up to 7 days if cooled in a refrigerator with a temperature of 2-8°C. This explains why the activity of the SGPT enzyme was not damaged even though the analysis was carried out two days after the blood collection. Serum samples that are stored at room temperature for too long can affect the activity values of the SGOT and SGPT enzymes.

Administration of seaweed extract at a dose of 200 mg/KgBW and a dose of 400 mg/KgBW was able to protect the liver (hepatoprotector) as shown by a decrease in liver SGOT and SGPT activity in all groups compared to the control group. This shows that administration of seaweed extract with different doses has a hepatoprotective effect by reducing the release of SGOT and SGPT into the blood. Research conducted by Wardani et al (2017) reported that administration of seaweed extract at doses of 200, 400, and 800 mg/KgBW can reduce SGOT and SGPT levels induced by lead acetate. Dewi et al (2020) stated that giving seaweed extract at a dose of 150 mg/KgBW significantly reduced liver damage in mice as seen from SGOT and SGPT levels induced by sodium nitrite (NaNO2). Decreased release of SGOT and SGPT and liver damage showed minimal necrosis in liver cells (hepatocytes). Wiranatha et al. (2019) stated that necrosis in hepatocyte cells would cause the release of SGOT and SGPT into the blood circulation.

The decrease in serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) was also caused by seaweed extract containing flavonoids, polyphenols, tannins, and alkaloids. These compounds are known to have activity as antioxidants (Mu'awwanah and Ulfah, 2017). This antioxidant activity is allegedly related to the hepatoprotector activity of seaweed extract. Reactive free radicals cause cell damage through two main mechanisms, namely covalent bonds, and lipid peroxidase. The lipid peroxidase process has been shown to increase collagen synthesis and fibrosis. Therefore, antioxidants may play a role in inhibiting liver damage induced during cell damage (Slater, 1988).

5. CONCLUSIONS

Based on the discussion above, it can be concluded that the administration of seaweed extract with different doses has a hepatoprotective effect in rabbits by reducing the release of SGOT and SGPT into the blood.

Seaweed extract has the potential to be an alternative therapy in preventing liver damage (hepatoprotector) caused by organophosphates which need to be strengthened by histopathological studies in experimental animals. Information can be given to farmers to be more careful when using these pesticides.

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