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The Hepatoprotective Effect Of Sea Cucumber (*Holothuria scabra*) Extract Originating From Gorontalo District Using SGOT And SGPT Parameters On Mice Induced By Hepatotoxic Dose Of Paracetamol

Widy Susanti Abdulkadir^{1*}, Robert Tungadi²

^{1,2}Pharmaceutical Department, Faculty of Sport and Health
Gorontalo State University, Indonesia

Abstract: Sea cucumber is one of marine animals which can be consumed as a food and also as a drug. The one of sea cucumber features is it has a high ability to regenerate cells. This research aims to observe the hepatoprotective effects of sea cucumber (*Holothuria scabra*) extract on mice that had been given hepatotoxic doses of paracetamol by using certain parameters such as SGOT and SGPT. This research utilized a pure experimental design with the randomized design used control group of pretest-posttest. The research method used mice which divided into 6 treatment groups: positive control, negative control, treatment group of 1%, 1.5%, 2%, and 2.5%.

The results of this research showed that SGOT value after treatments gave a significant differences between positive and negative control (sig. 0.000), group 1% (sig. 0.000), group 1.5% (sig. 0.000), group 2% (sig. 0.000), group 2.5% (sig. 0.000) and there were no significant difference between negative control and treatment groups: group 1% (sig. 0.925), group 1.5% (sig. 1.000), group 2% (sig. 0.925) and group 2.5% (sig. 0.975). On the other hand, SGPT value after treatments stated that there were significant differences between positive and other groups: negative control group (sig. 0.000), group 1% (sig. 0.000), group 1.5% (sig. 0.000), group 2% (sig. 0.000), group 2.5% (sig. 0.000), and no significant difference between negative control group and the other groups: group 1% (sig. 0.812), group 1.5% (sig. 0.069), group 2% (sig. 0.272), and group 2.5% (sig. 0.110).

Keywords: *Holothuria scabra*, hepatoprotective, paracetamol.

Introduction

The liver is the intermediate organ between the digestive and blood system. One of the important functions of the liver is to protect the body against the buildup of harmful substances that enter from outside such as drugs. Many of the drugs are fat soluble and not easily excreted in urine, so the enzyme system in the liver microsome will bio-transform in such a way so that the metabolite which is more soluble in water and can be purified through urine can be formed. With such fact, it is not surprising that the liver has considerable possibilities to be destroyed by the drug.

Hepatitis due to drugs generally does not cause permanent damage, but sometimes it is persistent and can be fatal. Hepatotoxins are chemical compounds that have toxic effects on liver cells, and excessive dosing (toxic dosage) or long-term seizure of the compound can cause acute or chronic liver damage. Common used

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drugs can cause toxic effects of the liver and about 2% of all cases of jaundice in hospitalized patients are due to the side effects of drugs.

Hepatotoxic paracetamol in humans can occur in a single dose of 10-15 grams. Symptoms on the first day of acute paracetamol toxicity do not reflect threatening dangers, anorexia and nausea and vomiting followed by abdominal pain occurs in 24 hours and lasts for a week or more. Liver dysfunction occurs within 24 hours and reaches the climax approximately 4 days after administration of paracetamol. Chronic hepatitis occurs in long-term exposure (about 1 year) to paracetamol (within 4 grams of therapy per day). The description above reveals that paracetamol in the therapeutic dose used in the long term needs to be considered because chronic hepatitis can occur.

Utilization of natural materials as a traditional medicine began to be developed and tested to obtain more satisfactory results in terms of treatment and side effects caused. Sea cucumbers are marine biota which is widely used as a traditional medicine. Sea cucumbers contain antibacterial, antifungal, antitumor and anticoagulant ingredients¹. Zancan and Mourao's research has shown that in the wound healing, the sea cucumber extract contains anticoagulant and antithrombotic compounds². Sea cucumbers also contain compounds that can reduce cholesterol and lipids, anticancer and antitumor compounds³, as well as antibacterial compounds⁵. Based on the above results it is necessary to do research on the effect of hepatoprotector of sea cucumber extract on giving hepatotoxic doses of paracetamol with SGOT / SGPT parameter in experimental animals.

Materials and Methods

Material Collection and Processing

The material used in this research was the sea cucumber (*Holothuriscabra*) originating from Wajo Village, Tilamuta District, Boalemo District, Gorontalo Province. The sea cucumber samples were cleaned and washed with clean water until the inherent dirt was gone.

The Making of Viscous Extract

The sea cucumber samples were processed by splitting and removing the stomach contents and then washed with clean water and free of dirt, then cut into small pieces and put into a container of maceration and soaked using methanol until all of sea cucumber surface was completely immersed. The marine sea cucumber samples were stirred 3 times daily and replaced its solvent every 3 days with the same treatment. This immersion was carried out until the solvent of the immersion did not give any color anymore or the solution of the immersion appears clear. The extracted solution from the first day until the last day (15 days) was mixed to be evaporated to produce the sea cucumber extract. Sea cucumber extract was evaporated again at room temperature until it dry and then the result was ready to be tested.

The Making of Na-CMC 1% Suspension

The Na-CMC suspension was prepared by dissolving approximately 1.0 grams of Na-CMC which has been carefully weighed into 100 ml of distilled water (which has been heated at 70°C) gradually and stirred with an electric stirrer until homogenous suspension was formed.

The Making of The Suspension of Paracetamol Hepatotoxic Dose 250 mg/kg

Paracetamol was suspended into 1% Na CMC prepared by diluting measured 625 mg paracetamol into 100 mL Na-CMC 1% (paracetamol hepatotoxic dose 6.25 mg/25 gram of mice weight) carefully.

The Making of Dried Extract of Sea Cucumber in Suspension of Na-CMC 1%

Dried extract of sea cucumber was suspended into Na-CMC 1% suspension that made by dissolving the sea cucumber dried extract which has been weighed according to the desired concentration of 1%; 1.5%; 2%; 2.5%

The Treatment of The Experimental Animal for The Sea Cucumber Dried Extract Test

The methods of animal treatment have been approved by the health ethics committee, The Faculty of Medicine, Hasanuddin University.

The experimental animals used were healthy male mice, 2-3 months old with weight 20-30 grams, consisting of 6 treatment groups with sea cucumber extract concentration of 1%; 1.5%; 2%; 2.5% and 1 negative control group (1% Na-CMC control) and 1 positive control group (Paracetamol hepatotoxic dose control).

Testing of TheHepatoprotector Effect of The Sea Cucumber Dried Extract

Positive control group: the solvent of Na-CMC 1% carrier with 1 ml/25 g W was given to mice orally for 7 consecutive days and on the 8th day, it given paracetamol of hepatotoxic dose at the dose of 6.25 mg / 25 gram, on day 9th the mice was rested and on the 10th day, its blood was taken for SGOT and SGPT examination.

Negative control group: the solvent of Na-CMC 1% carrier with 1 ml/25 g W was given to mice orally for 7-8 consecutive days and on the 8th day, it given paracetamol of hepatotoxic dose at the dose of 6.25 mg / 25 gram, on day 9th the mice was rested and on the 10th day, its blood was taken for SGOT and SGPT examination.

Group 1: the dried extract of sea cucumber (1%) with 1 ml/25 g was given to mice orally for 7 consecutive days and on the 8th day, it given paracetamol of hepatotoxic dose at the dose of 6.25 mg / 25 gram, on day 9th the mice was rested and on the 10th day, its blood was taken for SGOT and SGPT examination.

Group 2: the dried extract of sea cucumber (1.5%) with 1 ml/25 g was given to mice orally for 7 consecutive days and on the 8th day, it given paracetamol of hepatotoxic dose at the dose of 6.25 mg / 25 gram, on day 9th the mice was rested and on the 10th day, its blood was taken for SGOT and SGPT examination.

Group 3: the dried extract of sea cucumber (2%) with 1 ml/25 gr W was given to mice orally for 7 consecutive days and on the 8th day, it given paracetamol of hepatotoxic dose at the dose of 6.25 mg / 25 gram, on day 9th the mice was rested and on the 10th day, its blood was taken for SGOT and SGPT examination.

Group 4: the dried extract of sea cucumber (2.5%) with 1 ml/25 gr W was given to mice orally for 7 consecutive days and on the 8th day, it given paracetamol of hepatotoxic dose at the dose of 6.25 mg / 25 gram, on day 9th the mice was rested and on the 10th day, its blood was taken for SGOT and SGPT examination.

Measurement of SGOT and SGPT Blood of Experimental Animal

For SGOT and SGPT testing, the blood of experimental animal was taken at the vein of the tail (the vein tail was dilated with warm water), then the blood serum sample was added with dried heparin and the SGOT and SGPT test reagent was then measured.

Statistic analysis

Statistical analysis was performed using Anova Test (one way anova) and then tested using Post Hoc LSD test.

Results and Discussions

Processing of sea cucumber samples was done by drying in the outdoors without direct sun and avoiding boiling as people do to avoid the occurrence of protein denaturation that causes damaged protein. The maceration extraction was carried out by respecting the things above, so that the sample that had been processed into powder then extracted directly until the sample is perfectly extracted. Initial research was conducted by testing the characteristics of secondary metabolite compounds on dried extract of sea cucumber because it needed to do the introduction identify of the content of secondary metabolite compounds contained in sea cucumber extract type *Holothuria scabra*. The next step was doing research to see the effect of sea cucumber extract hepatoprotector which interspersed with paracetamol hepatotoxic dose by using SGOT and SGPT parameter.

Instead, the use of paracetamol induces liver damage because many paracetamol drugs are used as an

antipyretic analgesic in large areas, especially in children and elderly who are used for long periods of time. Paracetamol will normally be metabolized by the liver, as much as 90% is converted into sulphate and glucuronic metabolites (by conjugate sulfate and conjugate glucuronate), so that the compound is not toxic and will be excreted through urine. Approximately 4% is metabolized by the cytochrome P450 to the active metabolite compound of N-acetyl-p-aminobenzoquinonimine (NABQI). Normally, NABQI is detoxified by the conjugate reaction with glutathione and subsequently excreted. But the existing glutathione can be spent by a large amount of paracetamol so that NABQI is accumulated and binds to the liver cell macromolecule irreversibly so the liver undergoes necrosis. Giving of paracetamol was done on 8th of day after giving extract of sea cucumber on first day to 7th. This is done with the aim of protecting the liver of the mice first before it is damaged by paracetamol, on day 9th. This research is more focused on the preventive concept to see the potential of *Holothuriascabra* as a hepatoprotector agent.

SGOT and SGPT enzymes were used as an indicator of liver damage. When liver damage occurs, this enzyme is released from the cytoplasm and mitochondria so that the levels increase in the blood. Transaminase is a group of enzymes which are a catalyst in the process of transferring amino groups between an amino acid with a keto, so that the amino acid is formed one of 2-keto acid, and from the actual keto acids formed one amino acid. In a process that relies on energy, the liver takes the amino acid. Amino acids help the liver in protein formation. The primary examination in the clinic for serum transaminase examination is to determine the presence of liver inflammation (but does not represent liver cell regeneration). This is because serum transaminases have some beneficial properties such as increase in transaminase levels occurs early in the course of the disease before the change of any other value occur, the serum transaminase level is the last level that turn into normal in the course of liver disease and the serum transaminase level can be used as a guide to the recurrence of a liver disease.

In the hepatoprotector study, all mice were given sea cucumber extract on 2nd of the day to 8th. For SGOT values, some mice had values below the SGOT standard value (23.2-48,4 U/l), and this indicating that there was no liver damage. SGOT and SGPT counted under the normal level indicated they were healthy, and only can be a problem if the result is twice above the reference value⁵.

In **Table 1**, the increase in SGOT levels of positive control group was not very high (2 times the normal value) compared to the normal values. Increased levels of SGOT higher than SGPT occur in severe or chronic liver damage. Non-specific SGOT elevations suggest severe or chronic liver damage. Increased SGOT does not specifically indicate the presence of liver cell damage as present in liver, SGOT is also present in heart, skeleton, brain and kidney cells⁶. The value of SGPT in the positive group had doubled from normal levels so it illustrates the inflammation in mice liver because SGPT is a characteristic enzyme found only in the liver so that an increase in the enzyme indicates inflammation in liver cells.

Table 1. Measurement of SGOT and SGPT Level Before and After Treatment

Treatment Grouping	Mice	SGOT (U/l) (23,2-48,4 U/l)		SGPT (2,1-23,8 U/l)	
		Before Treatment	After Treatment	Before Treatment	After Treatment
Positive Control	1	19	36	20	25
	2	11	52	16	83
	3	13	57	16	97
	4	24	97	17	84
	5	21	40	18	89
Negative Control	1	11	8	14	8
	2	9	6	11	7
	3	15	8	11	6
	4	13	7	10	8
	5	20	9	13	7
ETP 1%	1	11	12	12	10
	2	14	8	10	9
	3	9	6	11	8

	4	21	7	12	9
	5	25	8	11	9
ETP 1,5%	1	20	9	22	23
	2	21	6	18	21
	3	16	8	19	20
	4	11	7	18	22
	5	17	8	21	21
ETP 2%	1	11	8	20	17
	2	9	9	19	15
	3	21	11	18	16
	4	20	7	17	15
	5	23	6	18	15
ETP 2,5%	1	9	6	18	21
	2	11	6	15	19
	3	14	8	16	18
	4	21	9	17	19
	5	23	10	15	21

From the table above can be seen that there is a decline in SGOT and SGPT Values after treatment.

Hepatoprotector testing was performed by giving hepatotoxic doses of paracetamol on the last day. This method was a preventive method so that after the treatment of sea cucumber extract, SGOT decreased below normal levels (SGOT values below normal levels indicated that the liver was in good health⁵) and decreased levels of SGPT in normal range caused by liver cells was protected by sea cucumber extract.

Based on statistical results of one way anova on hepatoprotector test showed that the ratio of SGOT and SGPT for all groups after treatment was significantly different with the value of sig = 0.000. For the value of SGOT after the treatment (Table 2.) of sea cucumber extract, it was found that there was a significant difference (sig = 0.000) between positive control and concentration of 1% ; 1.5%; 2% and 2.5% (sig.0.000). At concentration of 1% there was no significant difference (sig = 0.925) between the negative control.

Table 2. The Results of Post Hoc LSD Test of SGOT Level After Treatment

	Negative Control	Positive Control	Group 1%	Group 1,5%	Group 2%	Group 2,5%
Negative Group		Sig. 0.000*	Sig. 0.925	Sig. 1.000	Sig. 0.925	Sig. 0.975
Positive Group	Sig. 0.000*		Sig. 0.000*	Sig. 0.000*	Sig. 0.000*	Sig. 0.000*
Group 1%	Sig. 0.925	Sig. 0.000*		Sig. 0.925	Sig. 1.000	Sig. 0.950
Group 1,5%	Sig. 1.000	Sig. 0.000*	Sig. 0.925		Sig. 0.925	Sig. 0.975
Group 2%	Sig. 0.925	Sig. 0.000*	Sig. 1.000	Sig. 0.925		Sig. 0.950
Group 2,5%	Sig. 0.975	Sig. 0.000*	Sig. 0.950	Sig. 0.975	Sig. 0.950	

Notes: The sign (*) shows significant differences between the groups

This means that SGOT values at concentrations of 1% are close to normal values such as negative controls; The concentration of 1.5% did not differ significantly (sig = 1,000) with a negative control which means that the SGOT value at 1.5% concentration was close to normal values such as negative control. At concentration of 2% there was no significant difference (sig = 0.925) between the negative control. This means that SGOT values at concentrations of 2% are close to normal values such as negative controls; The concentration of 2.5% did not differ significantly (sig = 0.975) with a negative control which means that the SGOT value at 2.5% concentration was close to normal values as negative control. From the results above

illustrate that dried extract of sea cucumber of *Holothuria scabra* at concentration of 1% had been able to decrease SGOT value into normal as negative control.

In Table 3., the value of SGPT after the treatment of sea cucumber extract, it was found that there was a significant difference (sig = 0.000) between positive control and concentration of 1% ; 1.5% ; 2% and 2.5% (sig.0.000). At concentration of 1% there was no significant difference (sig = 0.812) between the negative control. This means that SGPT values at concentrations of 1% are close to normal values such as negative controls; The concentration of 1.5% did not differ significantly (sig = 0.069) with a negative control which means that the SGPT value at 1.5% concentration was close to normal values such as negative control. At concentration of 2% there was no significant difference (sig = 0.272) between the negative control.

Table 3. The Results of Post Hoc LSD Test of SGPT Level After Treatment

	Negative Control	Positive Control	Group 1%	Group 1,5%	Group 2%	Group 2,5%
Negative Group		Sig. 0.000*	Sig. 0.812	Sig. 0.069	Sig. 0.272	Sig. 0.110
Positive Group	Sig. 0.000*		Sig. 0.000*	Sig. 0.000*	Sig. 0.000*	Sig. 0.000*
Group 1%	Sig. 0.812	Sig. 0.000*		Sig. 0.110	Sig. 0.385	Sig. 0.612
Group 1,5%	Sig. 0.069	Sig. 0.000*	Sig. 0.110		Sig. 0.445	Sig. 0.812
Group 2%	Sig. 0.272	Sig. 0.000*	Sig. 0.385	Sig. 0.445		Sig. 0.597
Group 2,5%	Sig. 0.110	Sig. 0.000*	Sig. 0.168	Sig. 0.812	Sig. 0.597	

Notes: The sign (*) shows significant differences between the groups

This means that SGPT values at concentrations of 2% are close to normal values such as negative controls; The concentration of 2.5% did not differ significantly (sig = 0.110) with a negative control which means that the SGPT value at 2.5% concentration was close to normal values as negative control. From the results above illustrate that dried extract of sea cucumber of *Holothuria scabra* at concentration of 1% had been able to decrease SGPT value into normal as negative control. Meanwhile, a study conducted by Widysusanti (2009) using a sea cucumber made by suspension depicts at a concentration of 35% provides necrotic improvement in hepatic mice cells induced by hepatotoxic doses of paracetamol. Research by the Faculty of medicine of Airlangga State University revealed that the sea cucumbers *Holothuria scabra* type can prevent hepatocyte damage induced by CC14 at doses of 50mg / 200 gra, experimental animal.

Conclusion

From the results of this study it can be concluded that sea cucumber extract (*Holothurias scabra*) at 1% concentration has been able to give hepatoprotector effect in experimental animals which given paracetamol dose of hepatotoxic with SGOT and SGPT parameters.

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