

ACUTE TOXICITY STUDY OF SEPAT RAWA (*Trichopodus trichopterus*) FLOUR OF SOUTH KALIMANTAN ON THE WHITE MICE

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Abstract

Sepat Rawa (*Trichopodus trichopterus*) can be used in the health field. In the context of its development as a medicinal ingredient, it is necessary to conduct a reasearch of its safety. The purpose of this study was to determine the symptoms of acute toxicity and LD50 values using the OECD 425 (Up and Down Procedure) method using 425 Pgm Stat software . in this study used seven female white mice test animals which were divided into two groups, the first groups was the treatment group consisting of five test animals and the second groups was negative control consisting of two test animals. The treatment group was given a dose of 2000 m/ kgbw and observed for 14 days, after being given a test animal treatment showed no symptoms of acute toxicity. Then proced with a dose of 5000 mg/ kgbw, test animals do not show symptoms of acute toxicity as observed in previos doses. Test animal body weight was analyzed using a statistical test with the result ($P>0,05$) meaning that there was no significant difference between the weight of mice treated and negative controls. The value of LD50 obtained more than 5000 mg/ kgbw.

Keywords: *Acute Toxicity, Sepat Rawa (T. trichopterus), OECD 425.*

Introduction

South Kalimantan has quite large fishery potential in public (Rais et al., 2018). Mahreda & Dekayanti (2012) was Sepat Rawa (*Trichopodus trichopterus*) stated that the highest fish in South Kalimantan. The Research on the protein and amino acid of fresh sepat siam had significant differences, where it was known of fresh was 5.08% while that by 65.21% in flour of meal (Putra *et.,al*, 2017). Increase level meal of Amino acid levels are caused by the hydrolysis (Utami et al., 2016). Sepat Rawa Flour with dose of 300 mg/kgbw known have antidiabetic activity which has the potential to reduce blood glucose levels (Astuti & Fitriyanti, 2020). Lysine have known to control glucose levels in the blood by inhibiting the alpha glucosidase enzyme, this decrease in carbohydrate metabolism into glucose (Prastari et al., 2017). There is no data or scientific research that supports information about the safety

on sepat rawa (*T. trichopterus*) with OECD 425 method as an initial stage of safety testing in its use when administered certain medicinal substance.

Materials and Methods

Sample collection. The Flour were collected from Amuntai, South Kalimantan and then identified at the Research center for Biology on Indonesian Institute of Sciences. The result showed that the official name of the sample is *Trichopodus trichopterus*. The experimental animal in this research is female Balb/c mice at the age of 6-8 weeks, with minimal body weight about 20-35 grams. Approval from animals ethics committee from the Ethical committee of medical research medical faculty university of Lambung Mangkurat with No.281/KEPK-FKUNLAM/EC/VIII/2019.

Preparation of Sepat Rawa Flour. Fresh meal is carried out by removing the contents of the stomach, eyes, scales and fins and taking 4 kg of body parts. The Proses with steamed at 90°C for 20 minutes and baked at 70°C for 80 minutes. The manufacture of this flour refers to research (Astuti & Fitriyanti, 2020). Then the sample in quality test with biuret, sakaguchi, ninhidrin, milon, fohl and xanthoprotein.

Acute toxicity Methods. The Test using OECD 425 method was performed using OECD 425 according to Acute Oral Toxicity. LD50 value was obtained through counting the death and alive while performing each test for maximum 48 hours after treatment. The stages of oral acute toxicity test were as follow. The mice were fasted overnight and then the body weight was measured. In the first test, the first rat was given a dose limit of 2000 mg/kgbw of suspension flour of sepat rawa and let it rest for 48 hours. If the first animal lives, then 4 test animals were given the same dose. If 3 or more die then the dose of the main test was processed. If 3 or more live the observation was continued till 14 days. The treatment was continued to the limit test dose 5000, if the number of animals that live more was than 3 in the test of the test dose limit of 2000 mg/kgbw. In the test limit test dose 5000, if the number of animals live is more than 3 then the value of LD50 > 5000 mg/kgbw. If the number of live animals is less than 3 then it is continued to the main test test. The first observation of toxic symptoms was performed individually at 30 minutes after administration. Then the observations were performed again at the 24th hour and 14th day unless the animals die then observation was stopped.

Acute toxicity Assesment. The observation parameters of this study include LD50 values, observation of toxic symptoms, changes in body weight. Toxic symptoms were analyzed qualitatively during testing based on the criteria set by OECD 425 (OECD, 2008) including skin and hair, eyes,

lethargy convulsions, tremors, diarrhea and death. Body weight changes was quantitatively analyzed from weighing on the first day of test preparation until the 14th day of observation. The weights were statistically processed using Independent T-test with 95% confidence level ($\alpha=0.05$).

LD50 assessment. LD50 value was assessed statistically including maximum likelihood method. All the doses and animal test responses were used for input to AOT425StatPgm software to obtain the value LD50 estimation.

Results and Discussion

Sample of Sepat Rawa Flour. The flour has tested with that reagen in biuret, sakaguchi, ninhidrin, milon, fohl and xanthoprotein show positive in all sample. The biuret reaction is based on the principle of a substance containing two bonds of peptide or more that can form a purple complex (Purnama et al., 2013). In the sakaguchi obtained by the formation of a brown color, this reaction shows the presence of the typical arginine contained on the sample (Roopalatha & Vijai, 2013). The ninhydrin reaction namely the formation of a blue color that indicates the presence of not bonding on amino acids (Prastika et al., 2019). The milon reaction showed by the formation of brick red protein deposits due to tyrosine found in the sample. The fohl reaction showing the presence of amino acids sulfur on the flour. The xantoprotein reaction gives a positive result with that indicates the presence of cyclic acids

Acute toxicity test. After a single dose of Flour 2000 mg / kgBw and till doses of 5000 mg / kgBw in mice that the Observation of toxicity at the 30th minutes in 4 hours and then observed daily until the 14th day the mice show no signs of symptoms of toxicity. Mice given treatment have the same activity as negative control. Observation of toxicity symptoms till the presence of clinical symptoms that indicate toxic effects in the test group are show in table 1.

Table 1. Behavioral patterns of mice in sample treated (2000 mg/Bw p.o.and 5000 mg/kgBw)

Parameters	Doses 2000 mg/kgbw and 5000 mg/Kgbw											
	30minutes		4 hours		24 hours		28 hours		7days		14 days	
	C	F	C	F	C	F	C	F	C	F	C	F
Skin and hair	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Lethargy	-	-	-	-	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-	-	-	-	-
Tremors	-	-	-	-	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-	-	-	-	-

Death	-	-	-	-	-	-	-	-	-	-	-	-
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Key: C = Control group, F= *T. trichopterus* flour treated groups, N = Normal, (-) = Not found

Jothy et al., (2011) show changes in the weight of test animals can describe toxic effects after administration of a substance. The results of the analysis test at doses of 2000 mg / KgBB and 5000 mg / KgBB obtained sig (2-tailed) $p > 0.05$ means that there is no meaningful difference between the weight of the animal negative control test and the group treatment. The result of body weight in 14 days treath with control and test with *T. trichopterus* flour are show in figure 1 and 2. result LD50 is specified using AOT 425 StatPgm software in the guide OECD 425. So it can be that samples of *T. trichopterus* have LD50 more than 5000 mg/KgBB or have no acute toxicity.

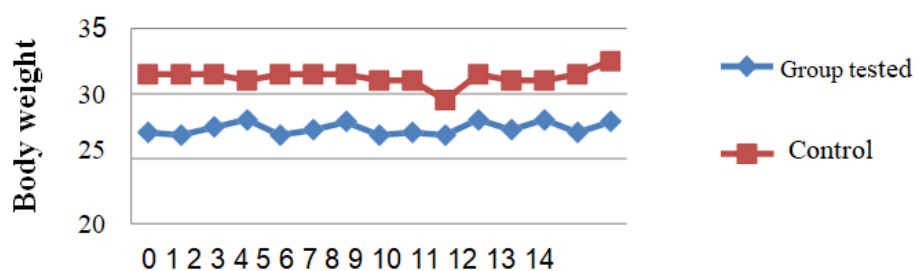


Figure1. Body Weight Changing Charts on Doses 2000 mg/kg bw in 14 days treath

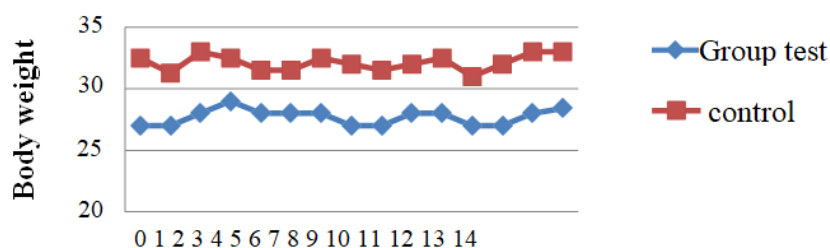


Figure 2. Body Weight Changing Charts on Doses 5000 mg/kg bw in 14 days treat

AOT425StatPgm

New Test Load Data Save Data Get Report Options About AOT425 Exit

Test / Substance: Enter test description.

Test Type: Limit

Limit Dose: 2000

Assumed values at start of the main test:
LD50: Default Sigma: 0.5

Test Seq.	Animal ID	Dose mg/kg	Short-term Outcome	Long-term Outcome	Program's Data Entry Messages
1	1	2000	0	0	
2	2	2000	0	0	
3	3	2000	0	0	
4	4	2000	0	0	
5	5	2000	0	0	

The limit test is complete.
The LD50 is greater than 2000 mg/kg.

Figure 3. limit test result in doses 2000 mg/kgBw

AOT425StatPgm

New Test Load Data Save Data Get Report Options About AOT425 Exit

Test / Substance: Enter test description.

Test Type: Limit

Limit Dose: 5000

Assumed values at start of the main test:
LD50: Default Sigma: 0.5

Test Seq.	Animal ID	Dose mg/kg	Short-term Outcome	Long-term Outcome	Program's Data Entry Messages
1	1	5000	0	0	
2	2	5000	0	0	
3	3	5000	0	0	
4	4	5000	0	0	
5	5	5000	0	0	

The limit test is complete.
The LD50 is greater than 5000 mg/kg.

Figure 4. limit test result in doses 5000 mg/kgBw

Conclusion

LD50 value of *T. trichopterus* flour found to be more than 5000 mg/kgBW. According, lethal doses of *T. trichopterus* flour considers as practically non-toxic category and that sample could be the safe therapy agent.

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