

**Acute and Subchronic Toxicity Of Temu Tis (*Curcuma purpurascens*)  
Rhizome in  
White Rat(*Rattus norvegicus*)**

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**Abstract**

Temu tis (*Curcuma purpurascens*) is one of *Curcuma* species that has not been widely studied. Although it is not very well known, temu tis is also used as a traditional medicine to treat coughs, stomach aches, and skin infections. The results of several studies proved that temu tis rhizome extract has bioactivity as an antioxidant and anti-cancer. Temu tis is also proven to contain flavonoids, terpenoids, triterpenoids, steroids, and essential oils. Because it contains bioactive substances, it is estimated that temu tis rhizome extract has the potential to be used as a medicine, therefore its safety needs to be tested. In this study, an acute and subchronic toxicity test was conducted for the ethanol extract of temu tis rhizome which was given orally to white rat (*Rattus norvegicus*) using various increased doses to see the toxic effects, both qualitative and quantitative, and subchronic effects in an increase of serum level measurements for both serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT). From the results, there was no visible toxic effect and mortality of white rat in the doses tested, namely between 1250-5000 mg/Kg body weight with the administration for 14 days. Of the calculation results, the LD50 value of temu tis rhizome extract is greater than 5000 mg/Kg body weight, so it is classified into category 5 or non-toxic according to the Globally Harmonized System of Classification (GHS). Subchronic toxicity test results also showed that the administration of temu tis extract for 28 days in a dose range of 1,250-5,000 mg /Kg body weight did not significantly affect the activity of the SGOT and SGPT enzymes in the experimental rat.

**Keywords:** *Curcuma purpurascens*, temu tis, toxicity, SGOT, SGPT, white rat

**Introduction**

Temu tis (*Curcuma purpurascens*) is a plant species that is less well known and considered unimportant. Temu tis is categorized under the ginger group (Zingiberaceae) and has long been used by Indonesians, especially as traditional medicine. This plant is known as temublenyeh or temuglenyehin Yogyakarta (Sok-Lai et al., 2014). Temu tis contains flavonoids, terpenoids, triterpenoids/steroids, and essential oils (Jalip et al., 2013; Sok-lai et al., 2014; Vibrianti, 2005). Temu tis rhizome is usually used as a traditional medicine to cure coughs and skin infections.

People in Trunyan village, Bangli district, often use the rhizome of *C. purpurascens* to treat abscess, stomach ache and itching of the skin (Sok-Lai et al., 2014; Sudirga, 2012). According to Rauhollahi et al., (2015) temu tis has activity as an anti-cancer and as a gastroprotective. On the other hand, it has been shown to have an antiproliferative effect on human carcinoma cells (Hong et al., 2014) and has the potential to be chemo-preventive (Rauhollahi et al., 2015). Jalip et al., (2013) pointed out that temu tis rhizome extract has a fairly strong antioxidant activity.

Extract of temu tis rhizome with methanol, hexane, MTC, ethyl acetate, butanol and water solvents, each of which can inhibit fungal growth (*Candida albicans*) (Vibrianti, 2005). Meanwhile, plant extracts or natural ingredients that contain high antioxidant activity have generally high therapeutic potential as well. Of several research results that have been described, it appears that the temu tis rhizome has great potential to be further developed as a drug or medicinal substance and therefore it is necessary to examine its safety and toxicity. Moreover, toxicity testing is a formal prerequisite for the safety of a drug or traditional medicine (Priyanto, 2009).

The toxicity test method can be divided into 2 groups, namely the general toxicity test and the specific toxicity test. The general toxicity test is designed to evaluate the general effect of a compound, while the specific toxicity test is designed to evaluate in detail the specific types of toxicity such as the teratogenicity test, muta-genity test, and carcinogenicity test (Donatus, 2005; Parasuraman, 2011). Furthermore, general toxicity tests consist of an acute toxicity test, a sub-chronic toxicity test, and a chronic toxicity test (Priyanto, 2009). Acute toxicity testing is carried out by giving the compound being tested once or several times within a 24 hours period, then it is observed for 14 days.

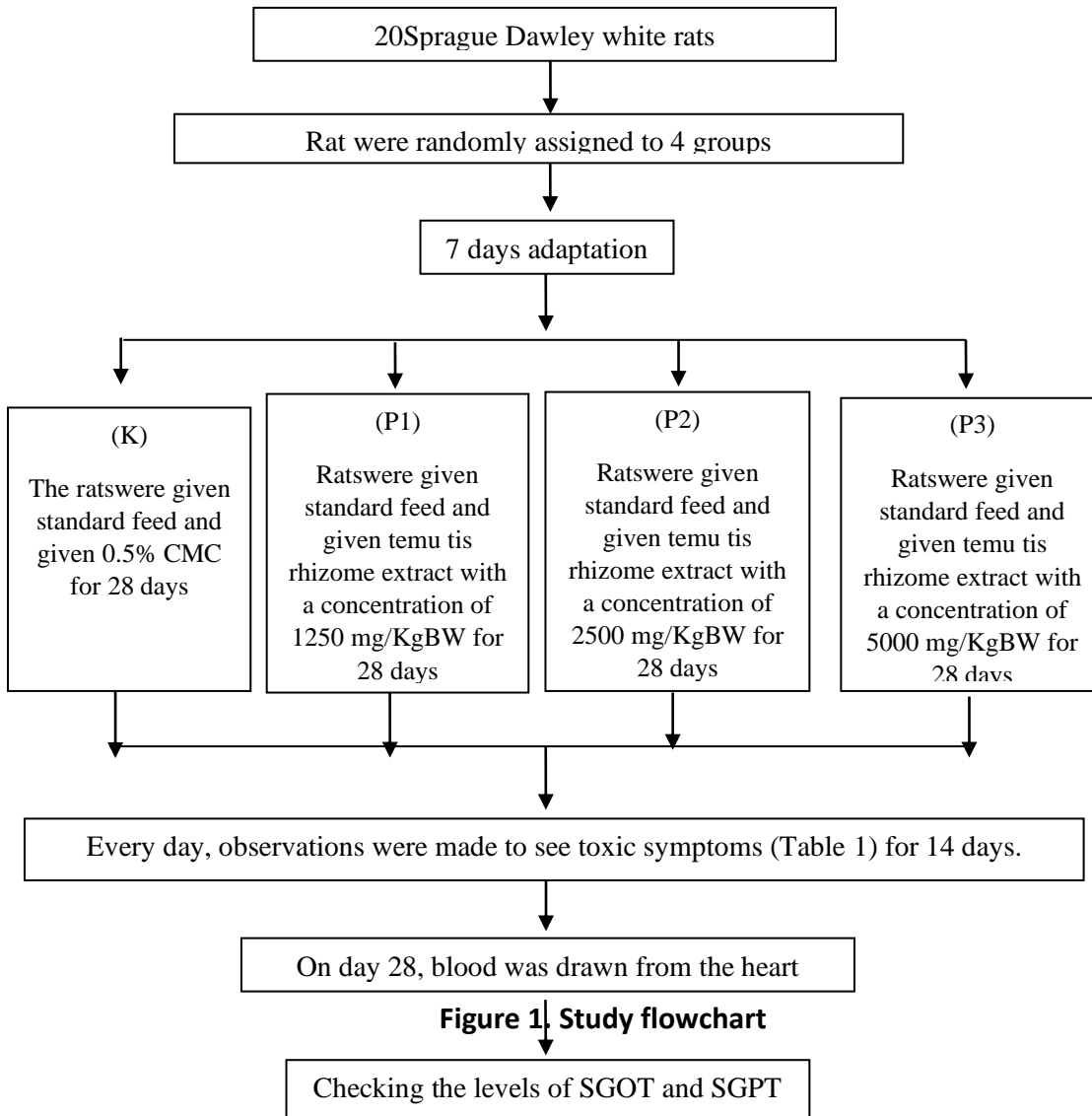
This study is designed to determine the Lethal Dose (LD<sub>50</sub>), besides that it can also show target organs that may be damaged and their specific toxic effects, and provide clues about the dosage that should be used in a longer test (OECD, 2001; Parasuraman, 2011). Subchronic toxicity tests are carried out to evaluate the effect of the compound, if it is given repeatedly to test animals. Usually, the test of compound is given every day for approximately 10% of the animal's life span, namely 1-3 months for mice. Meanwhile, the chronic toxicity test is carried out by giving the test compound repeatedly during the life of the test animal or most of its life, for example 18 months for house mouse, 24 months for rat, and 7-10 years for dogs and monkeys (Parasuraman, 2011; Priyanto, 2009; OECD; 2001).

The main objective to conduct an acute toxicity test is to obtain an overview of the potential toxicity of a toxic substance (test preparation) in a relationship between dose and response (Donatus, 2005). There are two parameters in the acute toxicity examination, namely qualitative and quantitative parameters. Qualitative observations included the presence or absence of eye damage (pupil area/red spots around the eyes), changes in the skin of the fur, behavior (stress), respiratory system (shortness of breath), excretory system (diarrhea), and coma. All changes that occur are compared to the control group (Aisyah and Sari 2013; Donatus, 2005; OECD, 2001). On the other hand, quantitative parameters are used to measure acute toxicity in the form of LD<sub>50</sub> (Lethal Dose 50), which is the amount of dose that causes death in 50% of the test animal population (Donatus, 2005).

One of the parameters used in the subchronic toxicity test is liver toxicity because the liver is a central organ in metabolism in the body (Sacher and McPherson, 2004). Since the liver functions as the main organ in drug metabolism, the liver is consequently very vulnerable or easily damaged if the drug is consumed in excess. The use of high doses and a long time can cause hepatotoxicity effects that damage of liver cells (Nafrialdi and Setawati, 2007). The parameters that are often used in examining liver damage are biochemical tests, namely the examination of the activity of Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) (Cahyono, 2014; Nafrialdi and Setawati, 2007). Changes in the activity of the SGOT and SGPT enzymes are an indication of the loss of hepatocyte cell integrity and as an accurate indicator of liver damage because these two enzymes will increase earlier and drastically compared to other enzymes, but it should be noted that the SGPT enzyme activity is a better indicator of liver damage than SGOT, because the liver is the main source of the SGPT enzyme (Cahyono, 2014). Apart from the liver, subchronic toxicity testing includes the digestive tract, lungs, kidneys and pancreas (Yuet Ping et al., 2013). In this study, an acute and subchronic toxicity test (checking levels of SGOT and SGPT) from the ethanol extract of temu tis (*C. purpurascens*) was administered orally to white rats (*R. norvegicus*) using various doses. From this research, it is hoped that information about the acute and subchronic toxicity of the temu tis rhizome ethanol extract can be obtained.

## Methods And Materials

The research was conducted at the Chemical Laboratory and Zoology Laboratory of the Universitas Nasional, Jakarta. The test of animal was a white rat (*Rattus norvegicus*) Sprague Dawley strain originating from the Laboratory Animal Management Unit of the Faculty of Veterinary Medicine, Bogor Agricultural Institute/Institut Pertanian Bogor (IPB), Darmaga, Bogor, with the inclusion criteria being male, approximately 3-4 months old and weighing 175-200g, in healthy condition. Temu tis rhizome (*C purpurascens*) was obtained from the Agricultural Science and Technology Park (TSTP) of the Agricultural Research and Development Agency at Cimanggu-Bogor. The rhizome of temu tis is thinly sliced, then dried through wind drying. The dried rhizomes are ground into a fine powder and sieved with a sieve measuring 18. The rhizome powder is soaked in 96% ethanol with a ratio of 1:3, left for 24 hours, then filtered with Whatman No. paper. 1. Soaking residue is repeated up to 3 times. The filtrate is collected and evaporated by vacuum evaporator to obtain a thick extract preparation. This research is a laboratory experimental study with a completely randomized design (CRD). The research flowchart is depicted in Figure 1.



**Figure 1.** The Research Flowchart

**Table 1. Observations of Toxic Symptoms (Donatus, 2005; Aisyah and Sari 2013)**

Observations	Toxic Symptoms
Eyes	There are reddish patches around the eyes. The animal may be in a state of severe pain and discomfort, enlarged pupils and gray eyes to blindness.
Fur and skin	Fur: The fur of the animal looks hard or strained. This could be a sign of abnormality. Skin: Bruising or lumps, bleeding or cuts above the subcutaneous
Behavior	Inactivity, including lethargy and / or reluctance to move, eat and drink.
Breath difficulty	Breathing becomes shallow quickly, and is usually accompanied by a sound.
Diarrhea	Diarrhea can be in the form of watery and bloody stools (dysentery). Increased frequency of bowel movements can indicate the severity of the digestive system. Animals that are sick, may die accompanied by weight loss, dehydration and weakness.
Death	Animals will become limp, have no appetite, dying and then die

### A. Acute Toxicity Test

The treatment of toxicity test were carried out by using the OECD 423 method (Marlina, 2017) following the OECD 423 method. Before the toxicity test was carried out, acclimatization was carried out and randomized grouping of the tested of animals, then each group consisted of 5 mice. Adaptation is carried out in advances for 7 days. Before being given the extract, the test of animals were measured of their weight and calculated the volume of the extract for each animal test with a dose of 1250-5000 mg/Kg of body weight (BW). The ethanol extract of temu tis rhizome was given using sonde with 3 ml each of each dose given to mouse for 28 days. After that, the first observation is intensively observed spelling individually at least 30 minutes to 4 hours after treatment. Then the observation is repeated for 30-60 minutes at 24 hours later. The test of animals that died in the first 24 hours were immediately subjected to surgery, while animals test that were still alive would be followed by observation for 14 days. All changes that occurred were compared with the control. If there are animals test that have died, the time of death is recorded. Until found the LD<sub>50</sub> value that has been categorized by the Globally Harmonized Classification (GHS).

### B. Subchronic Toxicity

The animal tests that were given the treatment from day 1 to day 28, on day 28 of the rat had their blood drawn from the heart organ, by performing ratsurgery. Then the SGOT and SGPT activities were analyzed based on biochemical parameters with enzymatic reactions to see the level of liver damage. The blood collection procedure (cardiac puncture) is based on a modification from Muharani's research (2016). The white rat was first sedated using chloroform

until they passed out. After the rats were unconscious, they were operated on using a scalpel/surgical scissors, starting from the skin opening of the abdomen to the diaphragm border. Surgery was carried out carefully so as not to hit the mice's heart. Then the diaphragm is opened by freeing the heart area for blood to be drawn using a 3cc syringe. The blood obtained is put into an EDTA tube. To get the plasma, the blood is centrifuged for 15 minutes at a speed of 4000 rpm. The serum obtained was checked for SGOT and **SGPT levels**. The determination of SGOT and SGPT activities using the Cobas brand tool, the IFCC kinetic method. The procedure for SGOT examination is to put 11  $\mu\text{L}$  of serum into the cuvette, add 40  $\mu\text{L}$  of 1 SGOT reagent, incubate for 5 minutes at 37°C, add 17  $\mu\text{L}$  of 2 SGOT reagent, homogenize, measure the absorbance at a wavelength of 378 nm and record the absorbance value. The SGPT examination procedure was put 11  $\mu\text{L}$  of serum into the cuvette, added 59  $\mu\text{L}$  of 1 SGPT reagent, incubated for 5 minutes at 37°C, added 17  $\mu\text{L}$  of reagent 2 SGPT and homogenized, measured the absorbance at a wavelength of 378 nm, recorded the absorbance value.

Data analysis was performed by determining the range of LD50 values from the results of acute toxicity by the OECD 423 method. Toxic symptoms can be seen from the data on behavior, body weight and AST and SGPT levels. Data analysis used the one-way ANOVA test (One-Way Anova) and if there were differences, it was continued with Least Significant Different (LSD) as a follow-up test with the help of the SPSS 18 program software.

## Results and Discussion

In this study, a test was carried out to see the effect of the ethanol extract of temu tis rhizome (*Cypripuracens*) on acute toxicity and subchronic toxicity tests. Liver as a parameter for subchronic toxicity testing is described by measuring SGOT and SGPT levels. Testing of SGOT and SGPT levels as a parameter of liver damage aims to see whether there is a hepatotoxic effect of the compounds contained in the ethanol extract of temu tis. The results of the data that have been obtained were performed the ANOVA statistical test and carried out the LSD (Least Significant Different) advanced test.

### A. Analysis of Acute Toxicity Test Data

#### 1. Observation for Signs of Acute Toxicity (Qualitative)

Observations of signs of toxicity were carried out, including behavior, eyes (pupils/around the eyes), respiratory system and mucous membranes after giving temu tis rhizome ethanol extract in each group at a dose of 1250-5000 mg/Kg BW and control (only given CMC 0, 5%) were observed every day. For the characteristics of test of animals that are affected by toxic effects, will experience poisoning, marked by several symptoms including eye damage (pupil area/red spots around the eyes), changes in fur, behavior (stress), respiratory system (shortness of breath), excretion system (diarrhea), and coma (Aisyah and Sari 2013; Donatus, 2005; OECD, 2001). Symptoms of irritation will be followed by dehydration, reduced urine volume, and death which is sometimes followed by paralysis within a few days. The effects of poisoning can be seen in a period of about 2-24 hours, even some studies of toxic effects in the form of poisoning can occur after more than 24 hours, namely 48 hours and 72 hours to 14 days (Donatus, 2005; OECD, 2001; Parasuraman, 2011). The results of this study found there

were no toxic symptoms such as the above characteristics in the test animals that had been given temu tis rhizome ethanol extract from a dose of 1250-5000 mg/Kg bodyweight after observation every day for up to 14 days. These results are supported by the results of research by Rouhollahi et al. (2014) that in the administration of ethanol extract of temu tis to mouse at a dose of 1000 mg/Kg body weight (BW) and 2000 mg/Kg BW there were no symptoms of toxicity and behavior change. The results of other studies show that giving the ethanol extract of turmeric (*Curcuma longa* Linn.) Doses of 100, 200, and 500 mg / Kg BW for 31 days did not cause toxic symptoms in white rat (Maharani and Bachri, 2015).

## 2. Rats Body Weight

The graph in Figure 2 shows the results of observing the body weight (BW) of rats that have been given temu tis rhizome extract at a dose of 1250-5000 mg/Kg BW and controls given CMC show that body weight has increased but tends to fluctuate. There was no significant increase in body weight between the test animals given 0.5% CMC and the treatment group (show the result of One Way Anova,  $p=0,05$ ). This results to indicate that temu tis rhizome extract did not interfere with the growth of the test animals. Fluctuating of body weight can be caused by several factors. The weight loss of test of animals that occurred in the control group and the treatment group was thought due to stress. Those stress occurs due to giving oral treatment using sonde to rats in a conscious state which can cause weight loss during the test period (Madinah et al., 2017). The change in body weight was able due to the growth process experienced by rats. Whilst increasing the age of the rats can be followed by the weight gain of the rats, and added with the feed intake in each group. Body weight is a general or specific indicator of toxicity (Kuncarli and Djunarko, 2016). These results are **supported by the results of** research by Rouhollahi et al (2014) that in the administration of ethanol extract of temu tis to mice at a dose of 1000 mg/Kg and 2000 mg/Kg, there is no change in body weight. The results of research by Hendrikos et al (2014) on the effect of the ethanol extract of temu mango (*Curcuma mangga* Val.) against the pancreatic  $\beta$  cells of white mice that were induced histologically by alloxan stated that body weight increased was not significant, presumably due to the effect of temu mango extract containing curcuminoids and essential oil. Curcumin compounds are efficacious as an appetite enhancer and accelerate the production of bile causing body weight to increase, but in essential oil compounds it functions to accelerate peristalsis of the small intestine and accelerate gastric emptying so that body weight decreases.

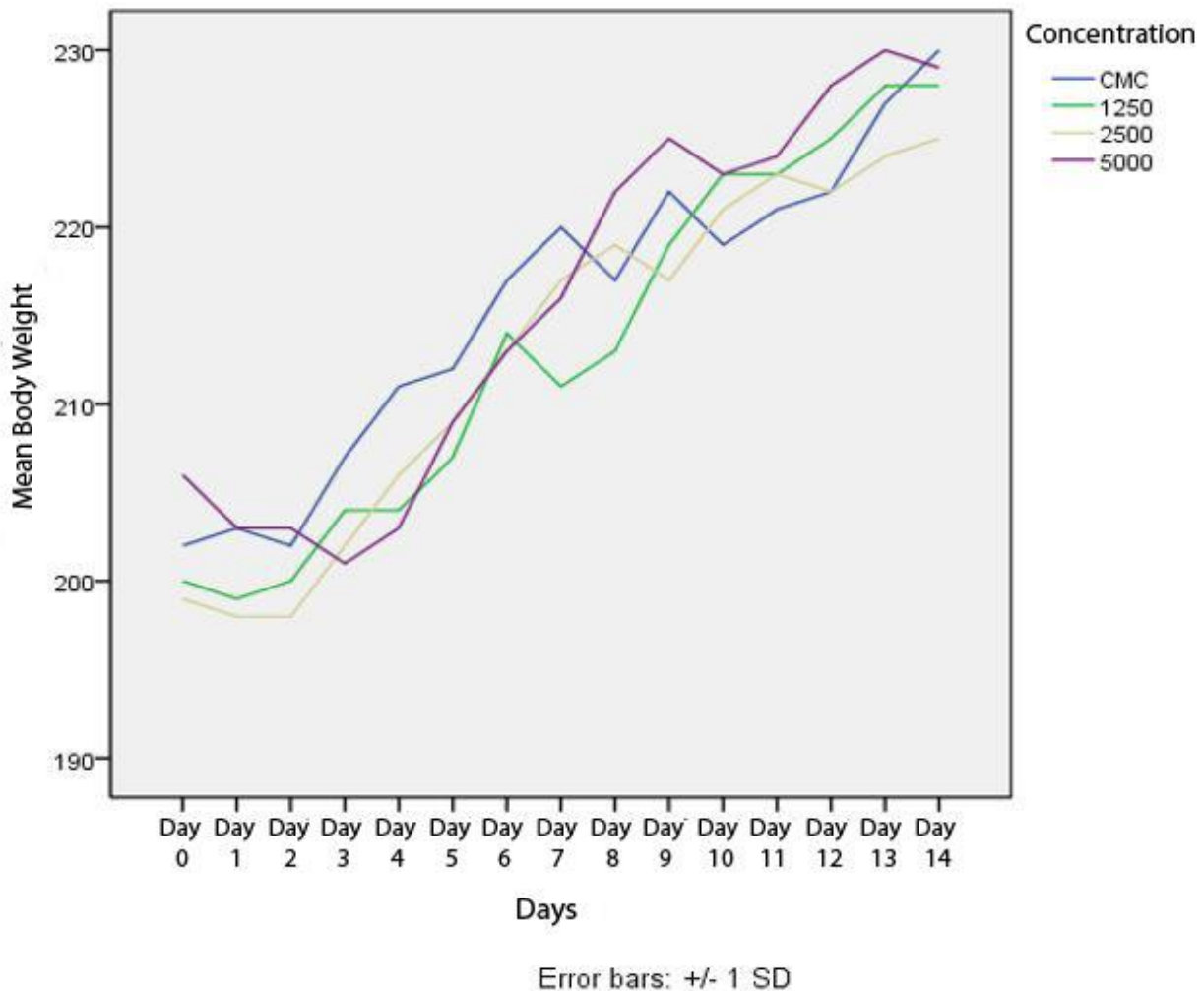


Figure 2. The results of observing the body weight (BW) of rats that have been given temu tis rhizome extract

### 3. The Mortality of LD50 (Quantitative)

Based on the observation of the number of dead test of animals, it indicates that by giving a single dose of temu tis rhizome extract orally to mice up to the maximum dose given technically to the test animals, namely 5000 mg/kg BW, it turns out that it does not cause death in the test animals from all groups, so that the potential the acute toxicity of the test extract could not be determined. These results indicate that temu tis rhizome extract can be said to be relatively safe because for 24 hours to 14 days there is no death as much as 50% of the white rat population, and this dose is included in the non-toxic category according to the Globally Harmonized Classification (GHS), the toxic dose is  $> 5000$  mg / Kg BW. The results of this study are in line with the research by Winarsih et al., (2012) that the toxic dose of turmeric rhizome extract (*Curcuma longa* Linn.) Has a toxic value  $> 15$  g/kgBW ( $> 15000$  mg/kg BW). Other research results show that the administration of ethanol extract of turmeric (*C. longa* Linn.) At



doses of 100, 200 and 500 mg/Kg BW for 31 days is not toxic to white mice (Maharani and Bachri, 2015). This is supported by the results of research by Rouhollahi et al (2014) that in the administration of ethanol extract of temu tis to mouse at a dose of 1000 mg/Kg BW and 2000 mg/Kg BW, there are no symptoms of toxicity, behavior change, weight change and no one has an LD50, indicates that the test compound is categorized as non-toxic ( $LD_{50} > 5000$  mg/kgBW).

## **B. Subchronic Toxicity**

### **1. Levels of SGOT**

The results of examining the SGOT levels of white rats fed temu tis rhizome extract with several concentrations are shown in Table 2.

Table 2. Average and standard deviation of SGOT levels

Treatment	SGOT U/L Average $\pm$ SD
(K) CMC 0,5%	124,80 $\pm$ 14,916 U/L
(P1) 1250 mg/KgBW	103,00 $\pm$ 31,432 U/L
(P2) 2500 mg/KgBW	111,20 $\pm$ 16,331 U/L
(P3) 5000 mg/KgBW	168,80 $\pm$ 62,010 U/L

Based on Table 2, it is known that the highest average SGOT level is in the treatment group of 5000 mg/Kg BW of  $168.80 \pm 62.010$  U/L, while the lowest average AST levels were in the treatment group 1250 mg / Kg BW at  $103.00 \pm 31.432$  U/L. In the treatment group 2500 mg/Kg BW had an average SGOT level of  $111.20 \pm 16.331$  U/L, while the CMC 0.5% treatment group had an average SGOT level of  $124.80 \pm 14.916$  U/L. The distribution of data in each group can be presented in the form of a bar chart in Figure 2.

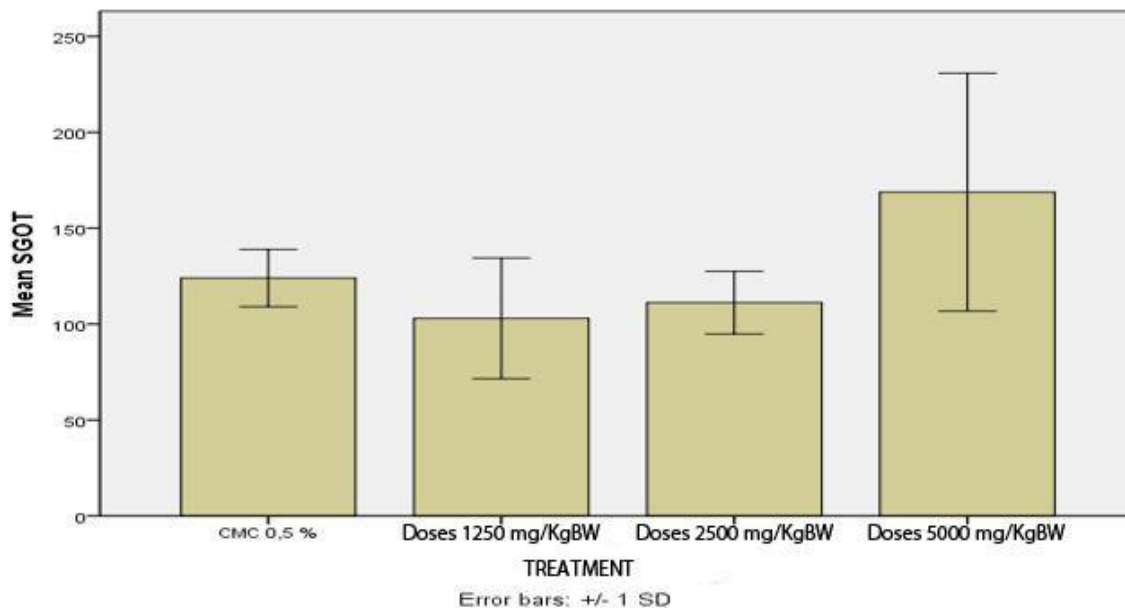


Figure 3. Bar diagram of SGOT levels

The bar chart in Figure 3 shows that giving temu tis extract tends to increase of SGOT levels in the treatment group of 5000 mg / Kg BW with the highest yield, but at concentrations of 1250 and 2500 mg / Kg BW, the results of SGOT levels are lower than the control. The factor in increasing of AST levels is the stress factor. The accumulation of metabolites in the body will cause oxidative stress, which is a condition of balance disturbance between the production of free radicals and antioxidants that have the potential to cause cell damage, resulting in an increase in SGOT levels (Jawi and Sutirtayasa, 2007). Unbalanced free radical production will cause damage to macromolecules including proteins, lipids and DNA (Atessahin et al., 2005). The destruction of cells by reactive free radicals is preceded by damage to the cell membrane, among others, changing the fluidity, structure and function of the cell membrane. The administration of temu tis extract to increase of SGOT levels of white rats can also be influenced by the amount of dose and the length of treatment. Based on the bar chart (Figure 3) shows that in the treatment group of 5000 mg / Kg BW which was given temu tis extract, it was able to increase of SGOT levels by a difference of approximately 44.00 U / L compared to the control group which was only given 0.5% CMC. The insignificant decrease in AST levels in the treatment group of 1250 mg / Kg BW and 2500 mg / Kg BW was thought to be because the body was doing recovery or repair so that AST levels tended to remain or decrease compared to the control group.

SGOT is an enzyme that is distributed in various tissues of the heart, kidneys and brain. SGOT is present in mitochondria and to a lesser extent in the cytosol. The enzyme of SGOT is less sensitive as indicators of liver damage, because this enzyme also binds to damage to other organs (Alwi et al., 2009). This is in line with the statement of Bigoniya et al. (2009) stated that SGOT enzyme levels are less specific to describe the level of liver damage, but increased of AST

levels can occur in acute necrosis or abnormalities in other parts such as mitochondria. Increased of AST levels cannot all be ascertained from the liver. SGOT is an enzyme found mostly in muscle, heart and liver. While in moderate concentrations found in the skeletal muscles, kidneys and pancreas. At low concentrations it is present in the blood, unless there is cellular injury (Alwi et al 2009; Cahyono, 2014). Statistically test showed that there was no significant difference in SGOT levels between the negative control group (CMC 0.5%) and the treatment group (One Way ANOVA,  $p > 0.05$ ) with homogeneous or the same data variations.

This shows that the ethanol extract of temu tis was not significant in increasing SGOT levels (One Way ANOVA,  $p > 0,05$ ) in white rats. This insignificant result may be because the rats' bodies made repairs to the damaged cells so that the AST levels in the mice's blood did not increase. In addition, rats are also able to repair damaged cells by up to 75% of liver damage within 30 days (Hidayat, 2007). This is supported by the results of research by Maharani and Bachri (2015) in giving ethanol extract of turmeric (*C. longa* Linn.) Doses of 50, 100 and 200 mg / kg BW for 31 days which are not toxic so they do not affect or do not increase the SGOT activity of Wistar male rats where the two enzymes are hepatotoxic parameters. Turmeric rhizome powder is not expected to cause toxic effects on human liver if used for a long time. Research by Laili (2013) shows that giving ginger (*C. xanthorrhiza* Roxb) in capsule form to healthy of men and women aged  $< 30$  years and more than 30 years old has no effect on SGOT levels. This study was conducted to determine whether the consumption of ginger is safe for people healthy.

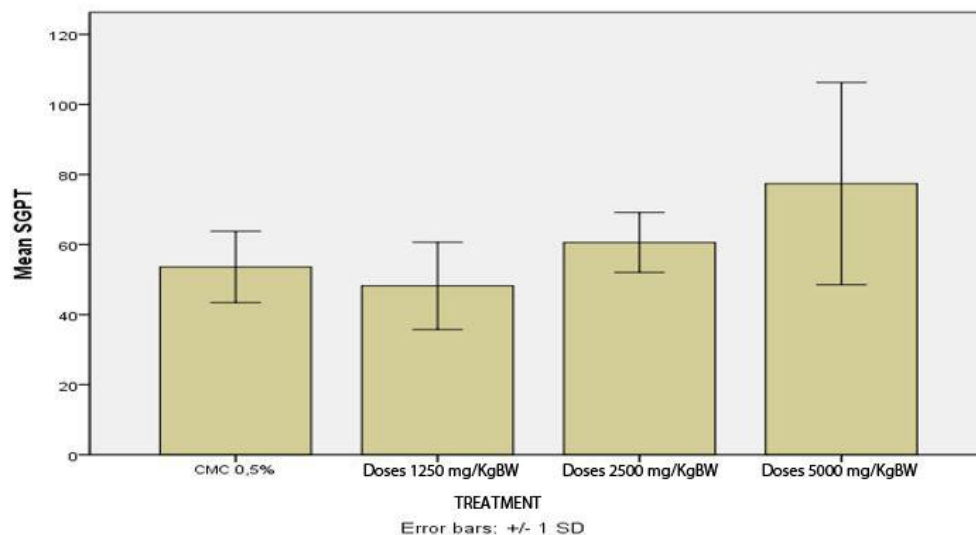
## 2. Levels of SGPT

Of the SGPT level examination of white rats given temu tis rhizome extract with several concentrations results are shown in Table 3.

**Table. 3. Average and standard deviation of SGPT levels**

Treatment	SGPT U/L
	Average $\pm$ SD
(K) CMC 0,5%	56,60 $\pm$ 10,118 U/L
(P1) 1250 mg/KgBW	48,20 $\pm$ 12,478 U/L
(P2) 2500 mg/KgBW	60,60 $\pm$ 8,562 U/L
(P3) 5000 mg/KgBW	77,40 $\pm$ 28,902 U/L

It is known that the highest average SGPT level was in the treatment group of 5000 mg /Kg body weight of  $77.40 \pm 28.902$  U/L (Table 3). While the lowest average SGPT level was in the treatment group with a dose of 1250 mg / Kg body weight of  $48.20 \pm 12.478$  U/L. In the treatment group the dose of 2500 mg / Kg body weight had an average SGPT level of  $60.60 \pm 8,562$  U/L. While the 0.5% CMC treatment group had an average SGPT level of  $56.60 \pm 10,118$  U/L. The distribution of data in each group can be presented in the form of a bar chart (Figure 4).



**Figure 4. Bar diagram of SGPT levels**

The treatment group that was given temu tis extract at doses of 2500 mg/Kg BW and 5000 mg/Kg BW began to increase SGPT levels, but the dose was 12500 mg lower than the control group which was only given 0.5% CMC (Figure 3). Curcumin is one of the most abundant ingredients in the Zingiberaceae family of plants, which has the action of increasing apoptosis in hepatocyte damage (Balaji and Chempakam, 2010). SGPT is an enzyme made in liver cells (hepatocytes), so it is more specifically used as an indication of liver disease compared to other enzymes. This enzyme will increase if there is a liver damage. The increase in SGPT levels is caused by leakage from damaged liver cells or liver necrosis (Alwi et al., 2009). The content of curcumin compounds and their derivatives found in turmeric (*C. longa* L) can cause hepatotoxicity depending on the dose given (Balaji and Chempakam, 2010). The results of the One Way Anova statistical test showed that there was no significant difference in SGPT levels between the control group which was only given 0.5% CMC and the treatment group with doses of 1250, 2500, and 5000 mg/Kg BW ( $p > 0.05$ ). This shows that the ethanol extract of temu tis does not significantly increase SGPT levels in white mice, and does not cause active hepatocellular damage (Goenarwo et al., 2009).

According to Panjaitan et al., (2007) the test animals in the control/untreated group had normal values of SGOT enzyme levels of 330.87 U/L and SGPT of 134.57 U/L. Meanwhile, Petterino and Storino's (2006) stated that the average of SGOT enzyme levels in Sprague Dawley rats in normal conditions (without treatment) had a value of 56.1 U/L - 201.89 U/L, while the average SGPT enzyme level was 34.9 U/L - 218.1 U/L. The average normal values of the SGOT enzyme in humans is 7-40 U/L and the SGPT enzyme is 5-50 U/L (Robin et al., 2012). For this reason, the average value of SGOT and SGPT enzymes based on this study is at the normal level. In addition, it is also supported by the results of research by Winarsih et al., (2012) that the toxic dose of turmeric rhizome extract is  $> 15$  g / kg body weight ( $> 15000$  mg / kg BW). Under normal conditions, the body's cells have the ability to regenerate. If any body cells are damaged, they will be replaced with new cells. This regeneration ability will compensate for cell damage. This is not reflected in the results of the SGOT and SGPT activity tests. It is possible

for an increase in SGOT and SGPT to be above normal, but actually the liver is not in a sick condition, because the dead cells are immediately replaced by new cells (Maharani and Bachri, 2015). The results of Laili's (2013) study show that giving ginger (*Cxanthorrhiza* Roxb) in capsule form to men healthy aged <30 years, women healthy aged < 30 and > 30 years has no effect on ALT levels. Giving *Cxanthorrhiza* or temulawak capsules to healthy men aged > 30 years were not significantly increase of ALT levels (One Way ANOVA,  $p > 0,05$ ) and the ALT levels were still within normal limits. If there is severe cell damage, there will be an increase in SGPT and SGOT levels simultaneously up to twice, even up to 20-100 times the normal level. A very high increase in SGPT enzyme levels accompanied by an increase in the SGOT enzyme is an indicator that indicates liver damage (Purwaningsih et al., 2015). According to Jaffri et al. (2007), an increase in SGPT enzyme levels up to 1.5 times normal in male and female patients indicates acute hepatitis. According to Perlstein et al. (2008) stated that an increase in the level of the SGPT or SGOT enzyme more than doubled indicates liver disease. The increase in SGPT levels that occurred in this study was still within normal limits, and not an indication of liver function damage, so it can be stated that temu tis rhizome extract does not have an effect on liver damage, indicated by the SGPT and SGOT values are still within normal limits.

### **Conclusions and Recommendations**

The administration of ethanol extract of temu tis rhizome (*C.purpuracens*) at a dose of 1250-5000 mg/Kg body weight taken orally does not cause acute toxic symptoms, such as eye damage, changes in skin and hair, behavior (stress), respiratory system, excretion system and comma. Giving the extract also did not cause to death in the test animals, so that it was included in category 5 or not classified according to the Globally Harmonized Classification System (GHS). Sub-chronic examination also did not cause liver toxicity as indicated by the absence of a significant effect on the increase in AST and ALT levels between the control and treatment groups. It is necessary to do an acute toxicity test using a larger dose in order to obtain a dose that can have a significant effect on symptoms of toxicity and death. It is necessary to carry out subchronic toxicity tests with different parameters such as kidney, heart, lung function, and others.

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