SGOT and SGPT level of Wistar rat after the administration of Channa micropeltes extract

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INTRODUCTION

Channa striata (Haruan fish) is one of the local peatland species (Huwoyon and Gustiano, 2013) . Majority of South Kalimantan society believe that Channa striata consumption may accelerate wound healing process due to the albumin content. Albumin is the highest protein to be found in plasma which reaches 60%, and it may accelerate wound healing process by the presence of antioxidant property (Nicodemus *et al.*, 2014) ; (Alamsjah *et al.*, 2014) ; (Agustin *et al.*, 2016) . Channa striata capsule at a 0.7-gram dosage which may accelerate wound healing process has

been widely distributed (Tawali *et al.*, 2012) . However, the high price and the complexity of Channa striata cultivation emerge the necessity for alternative species such as Channa micropeltes (Toman fish) (Audina *et al.*, 2018) .

Channa striata and Channa micropeltes originate from the same family, Channidae. Channa micropeltes is notable for its albumin content which is 5.35%, while Channa striata are reported to contain 3.53% albumin only. Channa micropeltes contains omega-3 fatty acid, omega-6 fatty acid and zinc (Firlianty, 2016); (Fajriani *et al.*, 2018). In a study conducted by (Nicodemus *et al.*, 2014), Channa micropeltes at 16 mL/kg BW dosage may accelerate wound healing process in the absence of chronic disease with 97.21% wound healing rate (Nicodemus *et al.*, 2014).

Channa micropeltes may be produced in the form of a capsule and be used as an alternative herbal drug to accelerate wound healing of the oral mucosa. Yet, a further study of its safety is required to analyze the toxicity prior its consumption. One of the toxicity analysis used is a sub-chronic toxicity test (Hulla *et al.*, 2014).This test should be performed for 28-90 days to identify hepatotoxicity effect by observing the influence of a compound toward the change in *Serum Glutamic Oxaloacetic Transaminase* (SGOT) and *Serum Glutamic Pyruvic Transaminase* (SGPT) level of the liver (Singh *et al.*, 2011); (Wahyuni *et al.*, 2017). The liver is the main organ for drug metabolism. Several drugs may induce the destruction of the liver cell due to hepatotoxic property (Indahsari and Histopatologi, 2017). Hepatotoxicity term refers to liver dysfunction due to over-dosage of drugs or xenobiotic (Singh *et al.*, 2011).

Serum Glutamic Oxaloacetic Transaminase (SGOT) is an enzyme found inside the body which immediately detected in peripheral circulation when necrosis occurred in a tissue. SGOT enzyme is commonly found in cardiac and liver, while *Serum Glutamic Pyruvic Transaminase* (SGPT) is frequently detected in the liver and effectively diagnosed the presence of hepatocellular destruction. This enzyme will be secreted by the liver when there is a destruction of liver cells which is depicted in the increase of SGPT level in blood plasma (Nasution *et al.*, 2015); (Qodriyati *et al.*, 2016). SGOT and SGPT level test should be performed to identify the presence of liver abnormality or destruction due to drug consumption. Average SGOT level is 6-30 IU/L while normal SGPT level is 6-45 IU/L (Nurminha and Gambaran, 2013); (Reza and Rachmawati, 2017). When SGOT and SGPT level is higher than normal, necrosis of hepatocytes in the liver can be detected. The increase in both enzymes level will indicate that the compound should not proceed for further production as an alternative drug (Sari *et al.*, 2015). Based on the background above, it is pivotal to conduct this study to analyze the effect of Channa micropeltes extract capsule at 0.7-gram dosage per oral upon the level of SGOT and SGPT of Wistar rat liver.

Table 1: Formulas of Channa striata Extract Capsule at 0.7 gram dosage

Composition	Unit	Total
Protein	%	85,85
Total lipid	%	4,48
Water level	%	4,61
Ash level	%	4,47
Calsium	Mg/100g	178,91
Phosphor	ppm	7584,68
Magnesium	Mg/100g	137,35
Zinc	ppm	26,06
Iron	ppm	154,39
Vitamin A	Mcg/100g	30,1
Vitamin B2	Mcg/100g	0,69
Vitamin E	Mg/100g	2,52
Vitamin D3	Mcg/100g	14,92
Vitamin B12	Mcg/100g	1,08
Aspartat	ppm	76625,61
Glutamat	ppm	104485,93
Serine	ppm	27022,51
Glisine	ppm	59350,12
Histidine	ppm	36982,73
Arginine	ppm	93522,03
Threonine	ppm	45575,86
Alanine	ppm	39367,7
Proline	ppm	39031,92
Valine	ppm	43852,14
Methyonine	ppm	28142,51
Isoleusine	ppm	39383,8
Leusine	ppm	70774,16
Phenylalanine	ppm	48371,51
Lysine(Lysine HCl)	ppm	66262,24
Tyrosine	ppm	32376,36
Triptophane	ppm	6358,88
Oleic acid/Ω9	%	0,8567
Linoleic acid/Ω6	%	0,1417
Ω3 (Doxosahexaenoic acid)	%	0,0092

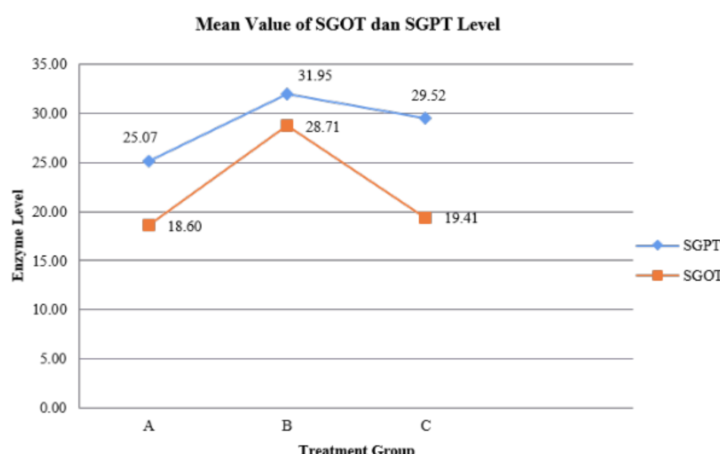

Figure 1: Graphic of SGOT dan SGPT level mean value in Wistar rat (A: Negative control ; B: Positive control of Channa striata extract capsule at 0.7 gram dosage; C: Treatment group of Channa micropeltes extract capsule at 0.7 gram dosage)

Table 2: Formulas of Channa micropeltes extract capsuleat 0,7 gram dosage

Component	Formula	Function
Channa micropeltes dried extract	700 mg	Active compound
Aerosil	30 mg	Adsorbent
Talk	20 mg	Glidan
Mg. Stearat	10 mg	Lubricant
Amilum	260 mg	Filler
Total weight	1000 mg	

MATERIALS AND METHODS

This was an actual experimental study with post-test only and control group design. The ethical clearance was obtained from the Ethics Committee of Health Research, Faculty of Dentistry, Universitas Lambung Mangkurat No 160/KEPKG-FKGULM/EC/I/2019. The study sample comprised of male Wistar rat age 6-8 weeks with 200-300 gram BW.

At the beginning of the study, the experimental animal was adapted at the animal laboratory of Veterinary Centre (BVET) Regional V Banjarbaru for seven days by feeding them with BR2 and aqua dest ad libitum. A total of 12 rats were divided into three treatment groups, with four rats presented in each group. The groups comprised of negative control without given any treatment, positive control with the administration of Channa striata extract at 0.7-gram dosage and treatment group with the administration of Channa micropeltes extract capsule at 0.7-gram dosage. The administration of drugs was performed for 28 days each morning and noon per oral using a nasogastric tube.

Channa micropeltes extraction

Channa micropeltes was obtained from traditional market Martapura, Kalimantan Selatan, and used in this study had a total weight of 11 kg. The part utilized for the study was the flesh of Channa micropeltes. The extract was made at Pharmaceutical Laboratory, Faculty of Mathematics and Science ULM. Fish was cleaned from scale, blood, head and guts

Table 3: The results of Bonferroni test on SGOT and SGPT level of Wistar rat

GROUP		A	B	C
SGOT	A	-	0,011*	1,000
	B	0,011*	-	0,017*
	C	1,000	0,017*	-
SGPT	A	-	0,421	0,968
	B	0,421	-	1,000
	C	0,968	1,000	-

* : Significantly different ($p<0.05$)
A : Negative control
B : Positive control of Channa striata extractcapsule at 0.7 gram dosage
C : Treatment group of Channa micropeltes extractcapsule at 0.7 gram dosage

and the flesh were later weighed at 9.84 kg. The flesh was steamed inside a pan for 30 minutes under 70-80°C temperature. Light yellow liquid was secreted from the flesh to be collected and separated in a total of 750 ml. Channa micropeltes flesh was later covered with flannel fabric and Whatman paper no 1 to be inserted into a hydraulic press for pressing. Channa micropeltes extract was then put into a reaction tube as much as 7.5 ml and centrifuge for 15 minutes on 6000 rpm speed. The supernatant liquid was collected from the centrifuged extract. A total of 700 ml of liquid was obtained to be separated from 50 mL sedimentation. Further, Channa micropeltes extract was evaporated in a rotary evaporator for 8 hours until thickened. The extract was evaporated a second time in a water bath until dried in the form of granules.

Channa striata extract capsule

In this study, the capsulate of 0.7 gram Channa striata extract distributed in the market was employed. Below presents the formulation of 0.7 gram Channa striata extract capsule is shown in Table 1 .

Formulation of Channa micropeltes extract capsule

Dried Channa micropeltes extract was inserted into a mortar and mixed with aerosol, talk, Mg stearate and amylum. All compounds were crushed using stamper until homogenous. Granules were then weighed using an analytical scale and put on a parchment to be inserted inside a gelatinous capsule shell. The capsule was then stored inside dark bottle glass. Formulas of Channa micropeltes extract capsule are presented in Table 2 (Nurani *et al.*, 2017).

Animal treatment

Experimental rats were randomly selected and were administered with standard dosage given orally for 28 days every morning and noon. Calculation of the dosage was obtained from human dose, which is converted by multiplying it with 0.018 (Togubu *et al.*, 2013). The capsule of Channa micropeltes extract was divided into two in which one capsule contained 500 mg granules. Thus, a dosage conversion for rat obtained: 500 mg x 0.018 = 9 mg/g BW.

One capsule of 0.7 gram Channa striata extract in a weight of 750 mg might present a conversion of dosage as 750 mg x 0.018 = 13.5 mg/g BW.

These were the treatment given for each group: Group A (negative control) in which four rats were given BR2 feed for 28 days each morning and noon; Group B (positive control) in which four rats given Channa striata extract capsule at 13.5 mg/g BW dosage dissolved in aqua dest for 28 days each morning and noon using nasogastric tube; Group C (treatment group) in which four rats given Channa micropellets extract capsule at 9 mg/g BW dosage dissolved in aqua dest for 28 days every morning and noon using nasogastric tube.

Collection of blood plasma

On day 29, rats were sacrificed to collect their blood. Each rat was sacrificed by putting it in a container of cotton fumed with 5 ml diethyl ether. The container was covered tightly so that the diethyl ether would not evaporate. After waiting for several minutes until the rat was unconscious, blood was then obtained by an intracardial technique using a syringe. Blood was centrifuged until blood plasma was secreted. Plasma was then separated into a microtube.

Identification of SGOT and SGPT level

SGOT and SGPT analysis was conducted at Toxicology Laboratory of Veterinary Centre (BVET) Regional V Banjarbaru with IFCC methods and interpreted using Genesis 20 spectrophotometry with 365 nm wavelength. Blood plasma mixed with reagent kit under 37°C room temperature. Blood plasma was mixed in a total of 100 μL with a reagent kit in a total of 1000uL. After mixed homogenously, absorbency was observed in minute 1, 2 and 3. Data were presented in the result of absorbance (A). Thus, the result of SGOT and SGPT level activity (IU/L) should be obtained by multiplying the average subtraction from absorbance (A) minute 1, 2 and 3 with 3235 factors. The measurement of activity employed this formula:

$$\left\{\frac{((\triangle \text{A minute 1 and 2})+(\triangle \text{A minute 2 and 3}))}{2}\right\} \text{ x } 3235$$

The result then would be input to computer software SPSS 23.0 for Windows.21

Statistical analysis

Data were analyzed using Saphiro-Wilk test and then proceeded into variance homogeneity test of Levene's. It was revealed that the data were normally distributed and homogenous (p>0.05) thus Oneway ANOVA parametric test with a 95% confidence level (α=0.05) was performed. Data analysis was then followed by Post-Hoc Bonferroni test.

RESULTS AND DISCUSSION

Average SGOT level is 6-30 IU/L, while normal SGOT level is 6-45 IU/L. Graphic of SGOT dan SGPT level mean value in Wistar rat is presented in Figure 1. Based on Figure 1, it can be concluded that the average value for SGOT and SGPT level in group A, B and C are at a normal level. Data were then examined using Saphiro-Wilk and Levene's Test,

which resulted in normal distribution and homogeneity among three groups ($p>0.05$). Data were further analyzed using one-way ANOVA test and a significant value obtained for SGOT level was 0.006 ($p<0.05$) while SGPT level was 0.308 ($p>0.05$) thus presenting a significant difference between each treatment. Data were then continued with Post-hoc Bonferroni analysis which can be observed in Table 3.

Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) enzyme are two enzymes which may detect the destruction of liver cell (Nasution *et al.*, 2015). In this research, there is no significant difference between the SGOT level of negative control and Channa micropeltes extract treatment group. Hence, as between positive control of Channa striata extract group and Channa micropeltes extract treatment group. This reveals that Channa striata extract may increase the SGOT level but still in the normal range; thus, no toxicity effect resulted in the liver. No significant difference was observed on the impact of Channa striata, and Channa micropeltes extract toward SGPT level among all groups, which describes that Channa striata and Channa micropeltes extract do not induce hepatocellular destruction.

Channa micropeltes (*Channa micropeltes)* contains omega-3 fatty acids, omega-6 fatty acid, zinc, vitamin C and albumin (Nicodemus *et al.*, 2014); (Firlianty, 2016); (Irwanda *et al.*, 2015). Albumin content in Channa micropeltes reaches 5.35% (Fajriani *et al.*, 2018).This albumin content will undergo distribution and metabolism (Throop *et al.*, 2004). At a metabolic stage, albumin is synthesized at the liver cell, specifically hepatocytes, and it is converted into preproalbumin (Arroyo *et al.*, 2014). Preproalbumin will then be imported into the endoplasmic reticulum, and fission of N-terminal prepropeptide will present as it is assisted by serine protease to be released into interstitial of the liver, sinusoid and liver vein (Arroyo *et al.*, 2014); (Kebamo and Tesema, 2015). The aerobe route of albumin metabolism in the liver cell will form a by product of oxygen molecules which is classified as *Reactive Oxygen Species* (ROS) (Lee and Wu, 2015); (Li *et al.*, 2015). Albumin possesses the antioxidant property to bind free radical produced by ROS and stimulate antioxidant enzyme such as superoxide dismutase (SOD) through the activation of nuclear factor-erythroid-2 related factor 2 (NRf2) (Widayati *et al.*, 2012); (Ma, 2013); (Cahyani and Rustanti, 2015). NRf2 functions as the first defence against oxidative stress in the cytoplasm. Unless induced by the presence of oxidant and electrophile, NRf2 will be presented in the inactive form (Vriend and Reiter, 2015); (Layal, 2016). It will bind with receptor molecule such as kelch like ECH association protein 1 (Keap-1) and later formulate NRf2-Keap1 complex. In the presence of an oxidant, Nrf2 will be translocated to the nucleus and will form Antioxidants Response Element (ARE) and will be able to stimulate antioxidant enzyme activity such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) which can neutralize ROS component (Widayati *et al.*, 2012); (Vriend and Reiter, 2015).

The increase of superoxide dismutase (SOD) level has a role against free radical inside mitochondria by inducing or changing anion peroxide (O^{2-}) into hydrogen peroxide (H^2O^2), a form of free radical (Widayati *et al.*, 2012); (Fukai and Ushio-Fukai, 2011). Hydrogen peroxide (H2O2) will then be transformed into water (H^2O) and oxygen (O^2) by GPx and CAT (Tsutsui *et al.*, 2011); Werdhasari, 2014; (Qu *et al.*, 2016). The decrease of intracellular or extracellular ROS level will affect the biochemical process, including the protection against microorganisms and the function of the liver cell. When ROS decrease, the occurrence of oxidative stress can be prevented. Thus liver cell remained living and freed from radical (Hardiningtyas *et al.*, 2014). Liver cells which are free from radical will halt cell destruction. Thus, SGOT and SGPT enzyme as identifying marker for cytoplasm and mitochondria destruction in the liver cell will be presented in the normal level (Rachmawati and Ulfa, 2018); (Giannini, 2005).

Channa micropeltes contains several hepatoprotective compounds other than albumins, such as zinc, omega-3 fatty acid and vitamin C. Omega-3 fatty acid is proven to heal liver injury, stabilize and also decrease SGOT and SGPT level (Sukarsa and Studi, 2004); (Chavan *et al.*, 2013). Zinc is shown to reduce SGOT and SGPT level, thus deflate liver cell destruction effect (Unsal *et al.*, 2008). Vitamin C possesses an antioxidant property which depicts a hepatoprotective effect by binding free radical, which decrease oxidative stress in the liver cell (Sabiu *et al.*, 2015).

CONCLUSIONS

It can be concluded from this study that there is no effect of Channa micropeltes extract capsule at 0.7-gram dosage per oral upon SGOT and SGPT level changes in Wistar rat liver. This result should be deployed as the foundation of Channa micropeltes extract capsule development as an alternative herbal drug to accelerate wound healing of the oral mucosa with no destructive effect upon the liver.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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