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We are pleased to inform that your manuscript No. MS/IJBPAS/2021/5599, Titled: "ANTIINFLAMATION AND ACUTE TOXICITY OF *ETLINGERA ELATIOR* LEAVES EXTRACT" received on 23rd September 2020 and under consideration with IJBPAS has been approved by our editorial team for publication in the forthcoming issue Volume 10(9) (Releasing in September 2021) of "International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)". Please submit the following documents within 24 hours (Exclude Holidays) and notify the same via E-mail.

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**ANTIINFLAMMATION AND ACUTE TOXICITY OF *ETLINGERA ELATIOR*
LEAVES EXTRACT****KUSRIANI H^{1,2*}, ALIGITA W², BUDIANA W², FEBRIANTI W² AND NUR ADJI F²****1: Faculty of Pharmacy, Padjadjaran University, Indonesia****2: Faculty of Pharmacy, Bhakti Kencana University, Indonesia*****Corresponding Author: Dr. Kusriani H: E Mail: herni.kusriani@bku.ac.id****Received 27th Sept. 2020; Revised 24th Oct. 2020; Accepted 14th Nov. 2020; Available online 1st Sept. 2021****<https://doi.org/10.31032/IJBPAS/2021/10.9.5599>****ABSTRACT**

Torch ginger leaves (*Etingera elatior*) were proven to have health properties and widely used in community continuously. Phytochemical analysis of torch ginger leaves extract revealed the presence of flavonoid compounds and other polyphenol compounds that are known to have anti-inflammatory and inhibition of alfa glucosidase activity. Anti-inflamamatory activity was tested with the Human Red Blood Cell Stability (HRBC) method. Inhibition of red blood cell lysis due to induction of hypotonic solution was used to measure of anti-inflammatory activity. The toxicity activity of torch ginger leaves was to determine its safety, with LD₅₀ value as a parameter. Torch ginger leaves were extracted using 70% ethanol and concentrated into a thick extract. Anti-inflammatory activity was expressed in IC₅₀ value, which mean the ability of extract to inhibit 50% lysis of red blood cells as a marker of inflammation. IC₅₀ value as antiinflammation activity of leaves extract was 71.24 µg/mL, while the IC₅₀ value of diclofenac sodium as a standard was 48.94 µg /mL. Acute toxicity of leaves extract with oral acute toxicity method was used female mice at aged 8-12 weeks and were grouped randomly. After 14 days of observation, a biochemical examination was carried out and the animals were sacrificed for organ weighing and histopathological examination. The results showed that no deaths or strange behavior were observed during acute toxicity testing. Organ weight and clinical chemical parameters including SGOT, SGPT and creatinine showed no significant difference (p <0.05) when compared to the control group. Torch ginger leaves extract hadpotential antiinflammation properties and showed LD₅₀> 2000mg / kgBW. It is still safe for human consumption.

Keywords: Torch ginger leaves (*Etingera elatior*), anti inflammatory, HRBC (Human Red Blood Cell), oral acute toxicity

INTRODUCTION

Zingiberaceae plants are of considerable as a new and promising source of herbal medicine. They produce a variety of metabolites and can be used for drug development. *Etilingera elatior* or torch ginger is one of a Zingiberacea family, and is classified under the genera of *Etilingera*. This plant is locally known in Indonesia as kecombrang.

Kecombrang has been used for flavoring, vegetable, fish odor remover and cosmetic manufacture and also has medicinal properties (Lachumy, *et al.*, 2010). The flower are edible and some researches are reputed for their antibacteria, antioxidant and cytotoxic activities, and it leaves also have antifungal activity (Abdelwahab, *et al.*, 2010; Chan, *et al.*, 2011), showed a strong antioxidant activity with IC_{50} 14.27 $\mu\text{g} / \text{ml}$ (Kusriani, *et al.*, 2018), inhibit the growth of human colorectal cancer cells (HT29) with IC_{50} 170 $\mu\text{g} / \text{ml}$ (Mai, *et al.*, 2009), and antihyperglycemy activity (Fitrianita, *et al.*, 2018).

The potential of torch ginger plant as traditional medicine is based on the content of these plant compounds, including saponins, flavonoids, polyphenols and essential oils (Chan *et al.*, 2011; Ghasemzadeh, 2015; Kusriani *et al.*, 2018).

From various research results reported, the chemical content of *Etilingera elatiorthat*

has efficacy as anti-inflammatory is flavonoids (Levita *et al.*, 2019). Flavonoids can inhibit cyclooxygenase or lipookigenase and inhibits leukocyte accumulation in the area of inflammation (Garcia *et al.*, 2009; Serafini *et al.*, 2010). Flavonoid and saponin were could stabilize the lysosome membrane, whereas tannins have the ability to bind cations, thus stabilizing erythrocyte membranes and other biological macromolecules (Oyedapo *et al.*, 2010).

Inflammation is a normal protective response to tissue injury involving various physiological processes in the body (Kumar *et al.*, 2012). Inflammation is the body's protection for eliminate harmful stimuli and begin the healing process at network (Robert and Marrow, 2001). However, untreated inflammation can cause diseases such as vasomotor rhinitis, rheumatoid arthritis, atherosclerosis, obesity, diabetes, neurodegenerative diseases and even cancer (Garcia *et al.*, 2009).

Anti-inflammatory drugs which have indications of reducing swelling and pain due to inflammation, but this synthetic chemical drug can cause some risks of unwanted side effects if consumed in the long term. One of the undesirable side effects is gastric ulcer disease and nausea due to the process of inhibiting

was concentrated in vacuo and obtained 9.287% concentrated extract.

Experimental animal

The experimental animals were healthy female white mice and had normal activity with a weight of 20-30 grams and ages of 8-12 weeks.

Antiinflammation Assay

Drug used as Standard: Sodium diclofenac available in the commercial name of Natrium diklofenak^R -50 mg marketed by Kimia Farma, Indonesia was used as a source of sodium diclofenac.

Human Blood: The blood was collected from a healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and collected in heparinized vacutainer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 minutes at 3000 rpm. The packed cells were washed with 0.9% saline and a 40% v/v suspension made using isotonic phosphate buffer which was composed of 154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4 used as Stock erythrocyte or RBC suspension.

Hypotonic solution –induced haemolysis or membrane stabilizing activity

The test sample consisted of stock erythrocyte (RBC) suspension 0.030 ml mixed with 5 ml of hypotonic solution (154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4) containing extract/fractions ranging from

concentration 50-1000 µg/ml. The control sample consisted of 0.030ml RBC suspension mixed with hypotonic buffered solution alone. The standard drug sodium diclofenac was treated similar to test at 10 and 100 µg/ml concentrations. The experiment was carried out in triplicate. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm (Oyedapo et al., 2010).

The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation:

$$\% \text{ Inhibition of haemolysis} = 100 \times [A1 - A2 / A1]$$

A1 = Absorbance of hypotonic buffered solution alone

A2 = Absorbance of test /standard sample in hypotonic solution.

Acute Oral Toxicity Assay

This method based on the OECD 420 (Fixed Dose Method) using female mice as experimental animals (BPOM, 2014, WHO, 2020). Before testing, animals were adapted and must be fasted for 3-4 h. After being fasted, they were weighed and given sample preparation.

The initial dose in the preliminary test was 300 mg / kg BW, given to one mouse as the dose that was expected to cause toxic effects. The first 4 hours after administration of the dose, we observed the sign of toxicity and death. Furthermore,

animals were observed for 24 hours to see if there was any death in mice. If there is no death in mice, the dose can be increased to 2000 mg / kg BW, but if there is death in mice the dose is reduced to 50 mg / kg BW. The main test was carried out with 5 animals for each stage of the test dose. The five animals consist of 1 animal from the preliminary test and 4 additional animals. The doses used were 5, 50, 300 and 2000 mg / kg BW. Observations were made on each animal intensively in the first 4 hours periodically at 30, 60, 120, 180 and 240 minutes after giving the dose extract to see signs of toxicity and death. Furthermore, the animals were observed every 24 hours for 14 days.

On the 15th day of observation, blood through a vein in the tail were used to determine the levels of SGOT, SGPT and creatinine. Then they were sacrificed to evaluate the parameter the organ weight index including the kidneys, heart, liver, lungs and spleen. As well as histopathological examination using the liver and kidneys for each treatment group (BPOM,2014; Hodgson, 2010).

RESULT AND DISCUSSION

Anti-inflammatory activity with Human Red Blood Cell Stability (HRBC) method showed the ability of torch ginger extract to inhibit the lysis of red blood cells as a marker of inflammation.

In a study of torch ginger leaf obtained from Bandung, the results showed that torch ginger leaves extract showed anti-inflammatory activity. The greater the dose given then the greater the activity anti-inflammatory produced (Figure 1). The results of 50 percentage inhibition significantly shown in leaves extract, which is 71.24 $\mu\text{g} / \text{mL}$. Mechanism anti-inflammatory of potential compound such as flavonoid in this extract, allegedly stabilize the red blood cell membrane at the site of inflammation as well inhibits prostaglandin synthesis from arachidonic acid in a way inhibition of cyclooxygenase (COX) work (Serafini *et al*, 2011). The higher doses of extract has caused the higher percentage of inflammatory inhibition. Several types drugs in high doses that cause histamine release directly from cells mast so that the blood vessels become more permeable to the plasma fluid and cause a process inflammation (Gonzales *et al.*, 2007).

The oral acute toxicity assay is a method to detect toxic effects within a short time after oral administration in a single dose or repeated doses given within 24 hours (BPOM, 2014). This acute toxicity test refers to OECD 420 fixed dose procedure which is not necessary a lot of experimental animals (Rao, 2018).

This acute toxicity assay was evaluated with active and healthy female Swiss

webstermice (*Mus musculus*) aged 8-12 weeks with a body weight of 20-30 grams. Prior of testing, mice were acclimatized and placed in sufficient light and air circulating room, far from human activities, quiet and safe from predators (Rejeki, *et al.*, 2018).

Three groups of mice (control group, 300 mg/kg BW group and 2000 mg/kg BW group) were fasted for 3-4 hours first, but still given drinking water. The mice were weighed and were observed intensively since giving the preparation until the 4th hour after administration. Then, observations were still carried out every day for 14 days.

In body weight observations, no significant changes were observed after administering the extract(300mg / kgBW and 2000mg / kgBW) compared to the control group(value represent mean \pm SD (n=3), $p < 0.05$ to control) (Figure 2). The observation of body weight was carried out to determine whether the extract had an effect that could affect body weight after giving the test dose (Mustarichie, *et al.*, 2016).

In observations for 14 days, the tested mice group did not show any signs of clinical symptoms and there were no deaths. The behaviours parameter includes observation of restlessness, seizures, tremors, grooming (licking hair, rubbing feet against the head or mouth), shortness

of breath, diarrhea, defecation, urination, discoloration of hair, hair loss, piloerection (bristling around the nape of the neck), eye discoloration, lacrimation (tears), swelling and bleeding, drooling, stereotypes (repetitive movements such as spinning or somersaults), staub (tail standing), curiosity, active, sleeping, walking backward, walking on the stomach, responding well to food and drink, pounding, limp and dying. The observations showed that both of the control group and tested group did not show any toxic sign, and the behavior of curiosity, activeness and response to eating and drinking was still as usual, but some tested animals showed grooming behavior in the first minute till 2 hours after the giving sample preparation. It because of uncomfortable feeling in their mouth (Burhan, *et al.*, 2016).

The determination Lethal Dose 50 was carried out with observing and counting the death of tested animals during the test. In this study, there is no deaths animal, both in the control group and also in the 300 mg / kgBW and 2000 mg / kgBW dose groups. Because no deaths were found, the LD₅₀ value of torch ginger leaves extract was more than the limit (more than 2000 mg / kgBW). Although, the limit test method is not intended to determine the LD₅₀ value accurately, it can serve as a guide for clarifying that the extracts based on the

dosage level at which the animal is still alive (Jothy, *et al.*, 2011).

After 14 days of observation, the blood mice were taken for biochemical examination (SGOT, SGPT and creatinin levels). SGOT and SGPT are liver transaminase enzymes which is released when the liver is damaged. SGOT is commonly found in the heart, liver, skeletal muscle and kidneys, while SGPT is commonly found in the liver and kidneys, more specific enzyme for liver disease. SGOT and SGPT are the earliest detectors of liver disorders before other clinical signs and symptoms appear (Arneson & Brickell, 2007; Burtis, *et al.*, 2006). Whereas creatinine is synthesized in the kidneys, liver and pancreas as parameter to measure the ability of the glomerulus to filter chemicals from the blood. Increased levels of creatinine from normal levels, indicates that there is interference with kidney function (Dasgupta & Wahed, 2014; Arneson & Brickell, 2007).

The biochemical measurements (Table 1) showed that there was an increase in the levels of SGOT and SGPT in the 300mg / kgBW and 2000mg / kgBW dose groups compared to the normal control group. Meanwhile, there was a decrease creatinin levels in the 300mg / kgBW group and 2000mg / kgBW group compared to the normal control group. However, there were no significant changes ($p > 0.05$) at SGOT,

SGPT and creatinine between the control group and the test dose group. These biochemical parameters still considered normal in this study.

On the 15th day, all the mice that were still alive were sacrificed and dissected to see the weight of the mice organs (liver, kidney, heart, lungs and spleen) and organ histopathology.

Organ weight data were processed at Table 2. There was no significant difference ($p > 0.05$) in the weight of these organs. Torch ginger extract do not affect in the weight and work systems of these organs.

Liver and kidneys were used for histopathological examination. The aim of this examination was to see any damage to the liver and kidneys in the normal control group and the dose group. Liver is the largest internal organ for synthesizing all proteins, and plays a role in a bile production and assists the kidneys to break down toxic compounds. The liver is also able to secrete transaminase enzymes when the cells were damaged. The liver has hepatocyte cells which play a role in producing SGOT and SGPT into the blood (Dasgupta & Wahed, 2014; Arneson & Brickell, 2007).

The kidneys are responsible organ for the formation of urine and the secretion of the end products of body metabolism such as urea, creatinine and uric acid. The kidneys function as an excretory, secrete unwanted

metabolic products, excess inorganic ions, drugs and toxins from the body through the formation of urine (Dasgupta & Wahed, 2014).

Liver and kidney histopathology observations (Table 3) showed some cell damage in the liver and kidneys of mice in both the normal control and dose groups

(Figures 3, 4 and 5, Table 3). Assessment of liver and kidney preparations was carried out using a spoiled Roenigk scoring system by looking at the damage to liver and kidney cells, degeneration parenchymal (DP), hydropic degeneration (DH), and necrosis.

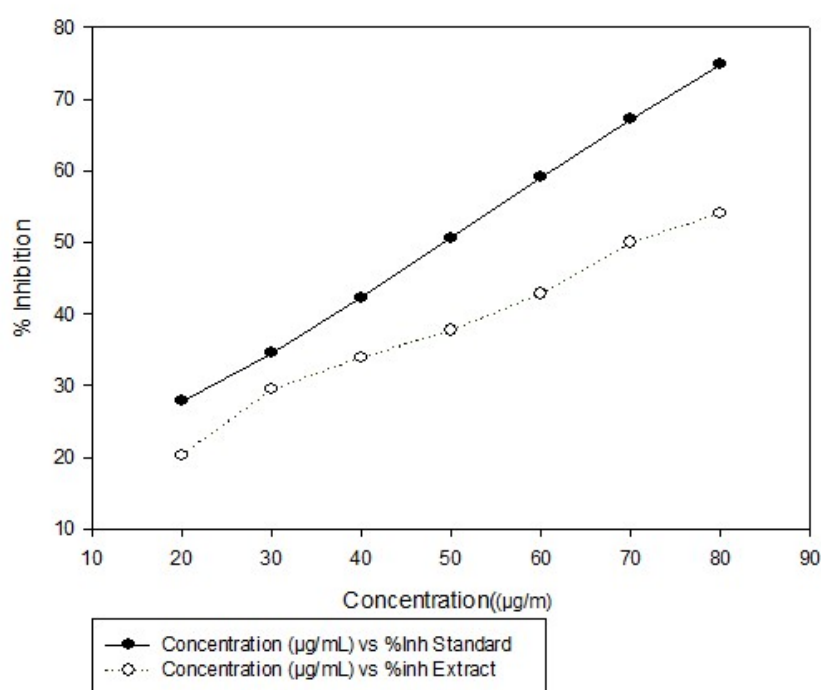


Figure 1: Antiinflammatory activity of diclofenac sodium and *Etlingera elatior* leaves extract

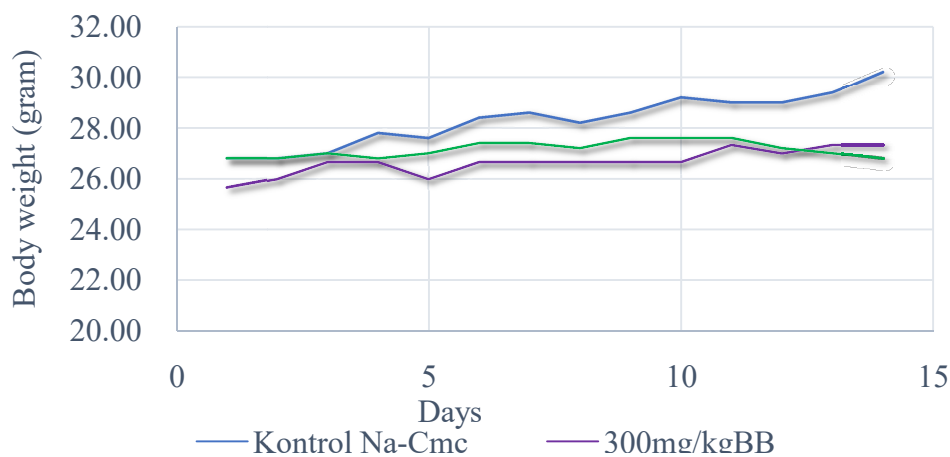


Figure 2: The average of mice weight for 14 days

Table 1: Biochemical parameters

Doses (mg/kg BW)	Biochemical Parameters		
	SGPT	SGOT	Kreatinin
Na-CMC	32.82 ±3.88	84.66 ± 13.86	0.55 ± 0.10
Extract 300	39.93 ± 8.80	94.20 ± 7.80	0.54 ± 0.06
Extract 2000	37.22 ±7.80	74.00 ± 5.01	0.50±0.13

Table 2: Organ weight

Doses (mg/kg BW)	Organ weight (g)				
	Kidney	Hepar	heart	lung	Limpha
Na-CMC	0.44 ±0.08	1.97 ± 0.36	0.18 ±0.06	0.36 ± 0.13	0.56 ±0.19
Extract 300	0.45 ± 0.05	1.77± 0.15	0.19 ±0.06	0.36 ± 0.12	0.52 ± 0.20
Extract 2000	0.45 ±0.07	1.87± 0.29	0.14 ±0.01	0.38 ±0.06	0.41 ±0.11

Table 3: Hispatology parameters

Doses (mg/kg BB)	Hepar score (Hepatosit Cell)	Kidney Score (tubulus)
Na-Cmc	1.32	1.33
Extract 300	1.16	1.03
Extract 2000	1.21	1.1

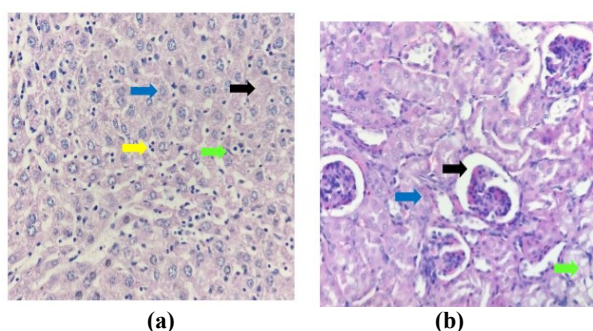


Figure 3: Histopathological observations in the normal control group. (a) liver; (blue arrow) cells tend to be normal; (black arrow) necrotic cells; (green arrow) contains Kupffer cells; and (yellow arrow) there are inflammatory cells in the space between cells.(b) kidneys; (black arrow) The glomerulus is closely spaced on the bowman's capsule; (green arrow) on the tubule, there are several necrotic cells which are indicated by the nucleus condensing and shrinking (picnotic); (blue arrow) normal cells

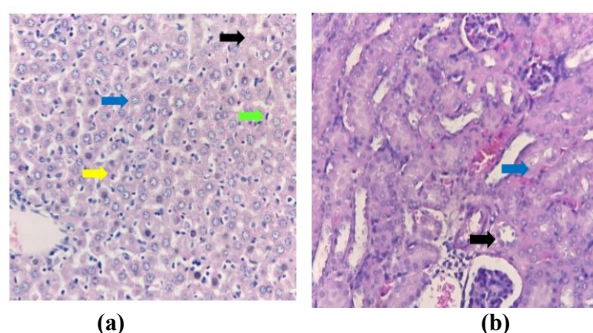


Figure 4: Histopathological observations in the 300mg / kgBW dose group. (a) liver; (blue arrow) cells tend to be normal; (black arrow) necrotic cells; (green arrow) contains Kupffer cells; and (yellow arrow) there are inflammatory cells in the space between cells. (b) kidneys; (black arrow) The glomerulus is closely spaced on the bowman's capsule; (green arrow) on the tubule, there are several necrotic cells which are indicated by the nucleus condensing and shrinking (picnotic); (blue arrow) normal cells

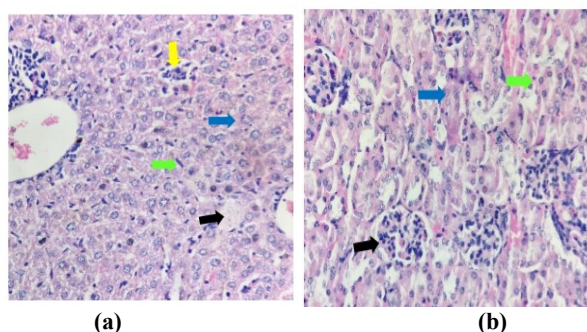


Figure 5: Histopathological observations in the 2000mg / kgBW dose group. (a) liver; (blue arrow) cells tend to be normal; (black arrow) necrotic cells; (green arrow) contains Kupffer cells; and (yellow arrow) there are inflammatory cells in the space between cells.(b) kidneys; (black arrow) The glomerulus is closely spaced on the bowman's capsule; (green arrow) on the tubule, there are several necrotic cells which are indicated by the nucleus condensing and shrinking (picnotic); (blue arrow) normal cells

The administration of torcg ginger leaves extract (*Etingera elatior*) observed for 14 days caused a decrease in liver and kidney histopathological scoring when compared to the normal control group (**Table 3**). These showed that torcg ginger/kecombrang leaves extract (*Etingera elatior*) can repair cell damage, characterized by the number of necrosis cells and scoring were decreased. It fact that this extract had strong antioxidant activity. Based on previous research, antioxidants can prevent liver and kidney cell damage because they can neutralize free radicals in the blood, and prevent cell damage (**Fristiohady, et al., 2020; Ridho, et al., 2020; Kusriani, et al., 2017**).

CONCLUSION

Ktorch ginger/kecombrang leaves extract (*Etingera elatior*) has potential as anti-inflammatory activity which was shown with IC_{50} value of 71.24 $\mu\text{g/mL}$. This extract does not cause toxicity at the limit test dose of 2000 mg / kgBW with $LD_{50} >$

2000mg / kgBW. Kecombrang leaves extract can be well tolerated in short-term therapy.

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