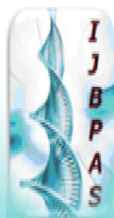


International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

A Bridge Between Laboratory and Reader…

ISSN:2277-4998
IMPACT FACTOR(ISI)2012-13:0-0.663
IMPACT FACTOR(ISI)2012-14:0-0.812
IMPACT FACTOR(ISI)2012-15:1.022
IMPACT FACTOR (ISI) 2017-2018: 1.318
ISI IMPACT FACTOR 2020-2021: 1.709

EDITORIAL TEAM

Our Editorial board is accompanied with a team of well qualified and experienced professionals from academia, industry and research organizations across the world. All have joined hands with a vision to bridge the gap between laboratory and reader.

Dr. PALLAVI JAYAVANATH
MANAGEMENT & SCIENCE UNIVERSITY, SHAH ALAM MALAYSIA

Dr. RAJENDRA GYAWALI
KATHMANDU UNIVERSITY, DEPARTMENT OF PHARMACYNEPAL

Dr. G D TANDON
HOD, DEPT. OF INDUSTRIAL BIOTECHNOLOGY AND MICROBIOLOGY, DR. D. Y. PATIL BIOTECHNOLOGY AND BIOINFORMATICS INSTITUTE, PUNEINDIA

Dr. QURBAN ALI
DEPT. OF PLANT BREEDING & GENETICS UNIVERSITY OF AGRICULTURE, FAISALABAD PAKISTAN

Dr. B G PRAJAPATI
S K PATEL COLLEGE OF PHARMA. EDU. AND RESEARCH, GANPAT UNIVERSITY, MEHSANA, GUJRAT INDIA

Dr. A. LAKSHMANA RAO
VALLABHANENI VENKATADRI INSTITUTE OF PHARMACEUTICAL SCIENCES, KRISHNA (DISTRICT), ANDHRA PRADESH INDIA

Dr. A A HALEEM KHAN
GIRRAJ GOVT. COLLEGE, NIZAMABAD, ANDHRA PRADESH AFFILIATED TO OSMANIA UNIVERSITY HYDRABADINDIA

MR. K MALLIKARJUNA RAO
DEPT. OF PHARMACEUTICS, RATNAM INSTITUTE OF PHARMACY, PIDATHAPOLURE, MUTHUKUR, NELLORE DT, ANDHRA PRADESH

Mr. SHAHZAD AKBAR KHAN
LECTURER DEPARTMENT OF PRE-CLINICAL STUDIES FACULTY OF VETERINARY & ANIMAL SCIENCES UNIVERSITY OF AZAD JAMMU AND KASHMIR RAWALAKOT PAKISTAN

Dr. C SELVAKUMAR
ASST PROFESSOR, DEPT OF BIOCHEMISTRY,MMC&RI, ENATHUR,KANCHEEPURAM,TAMIL NADU INDIA

Dr. SHISHIR KUMAR GANGWAR
COLLEGE OF AGRICULTURE, DEPARTMENT OF ANIMAL RANGELAND AND WILDLIFE SCIENCES, MEKELLE UNIVERSITY, MEKELLE ETHIOPIA

Dr. S SWAMINATHAN
SENIOR CONSULTANT & HEAD, BIOCHEMISTRY DEPARTMENT APOLLO SPECIALITY HOSPITAL, 320,ANNA SALAI CHENNAI INDIA

Dr. A V L N S H HARIHARAN
PROFESSOR & HEAD, DEPT. OF CHEMISTRY GITAM INSTITUTE OF TECHNOLOGY GITAM UNIVERSITY VISAKHAPATNAM INDIA

Dr. HARISH CHANGOTRA
DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY WAKNAGHAT, SOLAN HIMACHAL PRADESH INDIA

Dr. ASHOK PUNJAJI SALAVE
ASSOCIATE PROFESSOR, SHRI DNYANESHWAR MAHAVIDYALAYA, NEWASA, AHMEDNAGAR,UNIVERSITY OF PUNE, GANESHKHIND ROAD, PUNE INDIA

Dr. KARTIK B VYAS
LECTURER SETH L H SCIENCE COLEGE MANSA, GUJRAT INDIA

Dr. RAJESH GUPTA
SRI SAI COLLEGE OF PHARMACY PATHANKOTINDIA

Mr. P GANGASURESH
HOD, PG DEPARTMENT OF MICROBIOLOGY SRI RAM NALLAMANI YADAVA COLLEGE OF ARTS AND SCIENCES KODIKURICHI, TENKASI, TIRUNELVELIN DT, TAMILNADU INDIA

Mr. JAHIR ALAM KHAN
CHIEF EDITOR, BPAS PUBLISHERS LLP, LUCKNOW-226010INDIA

Dr. JYOTI PARKASH
JEAN-PIERRE AUBERT RESEARCH CENTER INSERM U837, 1, PLACE DE VERDUN 59045 LILLE CEDEX FRANCE

Dr. ZAMBARE VASUDEO P.
(M.SC., PH.D., FISBT, FSLSC, FSASC, FIIOAB, FSAB, MSFSN) GENERAL MANAGER (OPERATIONS AND R&D) SEQUENCE BIOTECH PVT. LTD, NASIK 422403 INDIA

Dr. SWAPAN KUMAR GHOSH
DEPARTMENT OF BOTANY RAMAKRISHNA MISSION VIVEKANANDA CENTENARY COLLEGE RAHARA, KOLKATA â€“ 700118 INDIA

Dr. J.K. SRIVASTAVA
Professor and Head Amity Institute of Biotechnology Amity University Uttar Pradesh, Lucknow Campus Malhour (Near Railway Station) Gomti Nagar Extension, Lucknow-227105 (INDIA)INDIA

Dr. P. MUTHURAMAN
DEPT. OF ANIMAL SCIENCE CHONBUK NATIONAL UNIVERSITY JEONJU SOUTH KOREA

Dr. HESHAM EL-BESHBISHY
ASSISSTANT PROFESSOR OF BIOCHEMISTRY, BIOCHEMISTRY DEPARTMENT, FACULTY OF PHARMACY (BOYS), AL-AZHAR UNIVERSITY, NASR CITY 11231, CAIRO, EGYPT

Dr. G.M.NARASIMHA RAO
ASSISTANT PROFESSOR, DEPARTMENT OF BOTANY ANDHRA UNIVERSITY, VISAKHAPATNAM-530 003 INDIA

Dr. RAJESH CHANDRA SHARMA
SENIOR ASSOCIATE PROFESSOR IN PHYSIOLOGY, AIMST, JALAN BEDONG-SEMELING 08100, BEDONG, KEDAH DARUL AMAN MALAYSIA

Dr. AMPAI PANTHONG
ASSOCIATE PROFESSOR DEPARTMENT OF PHARMACOLOGY, FACULTY OF MEDICINE CHIANG MAI UNIVERSITY, CHIANG MAI 50200 THAILAND

Dr. SUMIA FATIMA
HEAD, DEPT.OF BOTANY DR.RAFIQ ZAKARIA COLLEGE FOR WOMEN AURANGABAD â€“ 431 001 INDIA

Dr. HARSHA KHARKWAL
ASST. PROFESSOR (GRADE-II) AND COORDINATOR, AMITY CENTER FOR CARBOHYDRATE RESEARCH, AMITY UNIVERSITY UTTAR PRADESH, NOIDA-201303INDIA

Dr. BHOJ RAJ SINGH
PRINCIPAL SCIENTIST SINCE JUNE 2005, VETERINARY MICROBIOLOGY, IVRI, IZATNAGAR, BAREILLY, UTTAR PRADESH INDIA

Dr. T. RAVI MYCIN
ASSOCIATE PROFESSOR CUM LO, ANNAMALAI UNIVERSITY MADHAVARAM INFO CENTRE, 290 BAZZAAR STREET MADHAVARAM, CHENNAI- 600 060. INDIA

Dr. D.K. DHAWAN
PROFESSOR DEPARTMENT OF BIOPHYSICS & COORDINATOR, CENTER FOR NUCLEAR MEDICINE (UIEAST) PANJAB UNIVERSITY CHANDIGARH INDIA

Dr. SYED FARMAN ALI SHAH
CHEMICAL ENGINEERING DEPARTMENT MEHRAN UNIVERSITY OF ENGINEERING AND TECHNOLOGY, JAMSHORO PAKISTAN

Dr. BHEEMASENA RAO
PROFESSOR, DEPARTMENT OF LIFE SCIENCES, KRISTU JAYANTI COLLEGE, BANGALORE 560 077 INDIA

DR. SUSANA RODRIGUEZ-COUTO
IKERBASQUE RESEARCH PROFESSOR CEIT-IK4 RESEARCH ALLIANCE, UNIT OF ENVIRONMENTAL ENGINEERING, PASEO MANUEL DE LARDIZABAL 15, 20018 DONOSTIA-SAN SEBASTIAN SPAIN

Dr. EMAD M. ABDALLAH
ASSISTANT PROFESSOR OF MICROBIOLOGY, COLLEGE OF SCIENCE AND ATRS AT AL-RASS, QASSIM UNIVERSITY SAUDI ARABIA

Dr. JOSHUA A. OBALEYE
PROFESSOR, UNIVERSITY OF ILORIN, ILORIN. NIGERIA

Dr. KHUSHHALI MENARIA PANDEY
ASSISTANT PROFESSOR, DEPARTMENT OF BIOINFORMATICS, MANIT, BHOPAL INDIA

Dr. ALI FATTAHI BAFGHI
ASSOCIATE PROFESSOR AND SCHOOL MEMBER IN YAZD SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCESIRAN

Dr. AMAL MOHAMED ELIWA
DEPT. OF BIOLOGICAL SCIENCES AND GEOLOGY FACULTY OF EDUCATION AIN SHAMS UNIVERSITY ROXY, CAIRO, P.O. 11341 EGYPT

Dr. SOHAIR ABDEL HAMID SALEH DANDAN
PROFESSOR OF PATHOPHYSIOLOGY IN COLLEGE OF APPLIED MEDICAL SCIENCES, KING SAUD UNIVERSITY, KINGDOM OF (2011-2012) SAUDI ARABIA

Dr. RAJESH KUMAR JHA
PROFESSOR & HEAD, DEPARTMENT OF PHARMACOLOGY JAWAHARLAL NEHRU MEDICAL COLLEGE, D.M.I.M.S. (DEEMED UNIVERSITY)SAWANGI (MEGHE), WARDHA â€“ 442004, MAHARASHTRA STATE INDIA

Dr. DURGA SRINIVASA MURTHY SISTLA
DURGA SRINIVASA MURTHY SISTLA PROFESSOR OF BIOCHEMISTRY, FSPR, FNEA. DEPARTMENT OF BIOCHEMISTRY SRI VENKATESWARA UNIVERSITY TIRUPATI - 517 502INDIA

Dr. RAJESH N
ASST. PROFESSOR & HEAD DEPT. OF BIOCHEMISTRY & NUTRITION CSI HOLDSWORTH MEMORIAL HOSPITAL & COLLEGE MYSORE-570001, KARNATAKA INDIA

Dr. PALLAVI JAYAVANATH
MANAGEMENT & SCIENCE UNIVERSITY, SHAH ALAM MALAYSIA

Dr. KAMAL DEV
Professor, Faculty of Applied Sciences and Biotechnology, Shoolini University, Solan, Himachal Pradesh, India.INDIA

Dr.Fahd Mohammed Abd Al Wahab Abd Al Galil
Assistant professor Department of biology, faculty of sciences University of Bisha, 61922 Bisha. P.O. Box 551, Saudi Arabia

---

DHARY ALEWY HAMMED
ASSOCIATE PROFESSOR, KNOWLEDGE UNIVERSITY, FACULTY OF SCIENCE, DEPARTMENT OF PATHOLOGICAL ANALYSIS, ERBIL, KURDISTAN REGION, IRAQ

Dr. THEYAZN HASSN HADI ALDHYANI
NORTH MAHARASHTRA UNIVERSITY, JALGAON, INDIA

Prof. Dr. Arif Malik
PhD, Professor, Institute of Molecular Biology and Biotechnology University of Lahore, Pakistan

---

Dr. Mohammed Nader Shalaby
LECTURER OF BIOLOGICAL SCIENCES AND SPORTS HEALTH DEPARTMENT, FACULTY OF PHYSICAL EDUCATION, SUEZ CANAL UNIVERSITY,EGYPT

Dr. M. Waqas A. Khan
Associate Professor and CEO at Johar Institute of Professional Studies, 34km Ferozpur Road Nabi Buksh Lahore, Lahore, Pakistan

---

MARC ROMINA ALINA
Lecturer P h D Department of Food Engineering Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine (UASVM) Cluj Napoca, 3 5 Calea M?n??tur street , Cluj Napoca, 400372, www.usamvcluj.roCluj Napoca

---

Professor (Dr.) Mohamed Mabruk, Email: mabruk@squ.edu.om
College of Medicine and Health Sciences, Department of Allied Health Sciences, Sultan Qaboos University,Muscat, Oman

---

Dr. Muhammad Makkey Bhutta
CEO & Founder Institute of Journalism, Media Training and Research E Mail: makkey_k@hotmail.com Multan (Punjab), Pakistan

---

Dr. S.Mohanasundaram
Asst. Professor in Biochemistry Sri Sankara Arts and Science College, Kanchipuram E Mail: sbmohan2007@gmail.com, India

If you also want to be a part of our talented workforce please mail your CV's to the editor at editor@ijbpas.com.

IJBPAS, September, 2021, 10(9): X-X

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTIINFLAMATION AND ACUTE TOXICITY OF *ETLINGERA ELATIOR* LEAVES EXTRACT

KUSRIANI H[1, 2*], ALIGITA W[2], BUDIANA W[2], FEBRIANTI W[2] AND NUR ADJI F[2]

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 (Etlingera 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 LD_{50} value as a parameter. Torch ginger leaves were extracted using 70% ethanol and concentrated into a thick extract. Anti-inflammatory activity was expressed in IC_{50} value, which mean the ability of extract to inhibit 50% lysis of red blood cells as a marker of inflammation. IC_{50} value as antiinflammation activity of leaves extract was 71.24 µg/mL, while the IC_{50} 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 antiinflamation properties and showed LD_{50}> 2000mg / kgBW. It is still safe for human consumption.

Keywords: Torch ginger leaves (Etlingera 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. *Etlingera elatior* or torch ginger is one of a Zingiberacea family, and is classified under the genera of *Etlingera*. 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 µg / ml **(Kusriani, *et al*., 2018)**, inhibit the growth of human colorectal cancer cells (HT29) with IC_{50} 170 µg / ml **(Mai, *et al*., 2009)**, and antihiperglycemy 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 *Etlingera elatior*that has efficacy as anti-inflammatory is flavonoids **(Levita *et al*., 2019)**. Flavonoids can inhibit cyclooxygenase or lipook-igenase 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, neorodegenerative 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

prostalgladin. Besides kidney problems can arise, because of the effects of excessive oxidation of the drug, some cases can also cause heart toxicity **(Robert and Marrow, 2001)**.

Therefore, more plants were chosen as alternative natural remedies to treat various diseases, especially for alternative medicine of drugs that cause unwanted side effects such as antiinflammation properties **(Madhavi *et al*., 2012)**.

However, besides the benefits oftorch ginger leaves for therapeutic purposes, the chemical content cannot be guaranteed to cause therapeutic effects or side effects. To prevent these unwanted toxic effects, it is necessary to carry out the acute toxicity testing to determine the dosage limits which still safe if it consumed.

The results of this acute toxicity assay can be used to provide information about the dangers of the exposure to humans, so the dosage can be determined for human safety **(BPOM, 2014)**.

MATERIALS AND METHODS

The study was conducted in several stages, including preparation of materials, extraction, in vitro anti-inflammatory activity with HRBC assay, and oral acute toxicity test using the OECD 420 fixed dose method. The toxicity parameters were observed body weight of mice, organ weight, levels of SGOT, SGPT, creatinine, mortality of mice (LD50), and histopathological liver and kidneys.

Chemical and Reagent

Sodium citrate, citric acid, sodium chloride, saline 0,9%, Sodium Phosphate Buffer at pH 7.4, TLC (Merck®), diclofenac sodium , human blood, DMSO, Ethanol, trucal u, trulab n, Na-CMC (Carboxy Methyl Celullosa), liquid formaldehyde, SGOT reagent, SGPT reagent, creatinine reagent, standard animal feed and rice husks. All chemicals and reagents were analytical grade.

Plant

Leaves of *Etlingera elatior* were planted at local garden in Bandung City, West Java, Indonesia. Leaves were harvest after 3 months of growth. These plants were identified and confirm by Herbarium Jatinangor, Biology Departement of Padjadjaran University, Indonesia.

Sample Preparation

Leaves of *E. elatior* were washed, cleaned in running tap water, cuts into small pieces and dried to constant weight using oven at 40^0C. The samples were pounded into small size using grinder machine; it was stored in an air tight container till when needed.

Extraction

The dried leaves powder of *E. elatior* (1000g) was extracted sequently by maceration method in ethanol 70% (3x 2000 mL) at room temperature. The filtrate

was concentrated in vacuo and obtained 9.287% concentrated extract.

Experimental animal

The experintal 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 heparinzed 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:

% Inhibition of haemolysis = 100 x [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, theywere 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 hoursafter 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 µg / 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 **(Gonzhales *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 ± 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 parameterincludes observation of restlessness, seizures, tremors, grooming (licking hair, rubbing feet against the head or mouth), shortness of breath, diarrhea, defication, 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 LD_{50} 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 LD_{50} 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 transminase 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, indicatesthat 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 parameterars 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, degenerationparenchymal (DP), hydropic degenation (DH), and necrosis.

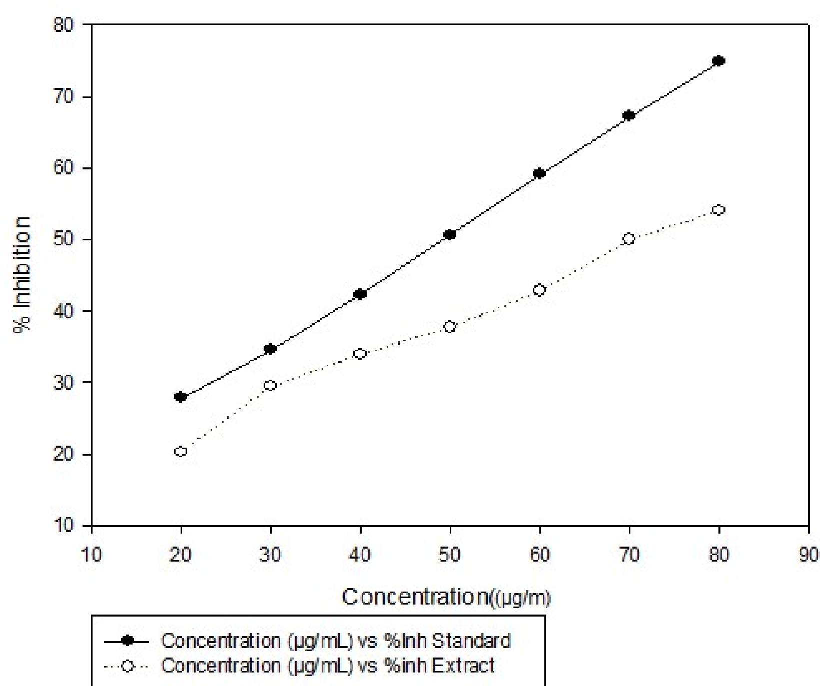


Figure 1: Antiinflammatory activity of diclofenac sodium and *Etlingera elatior*leaves extract

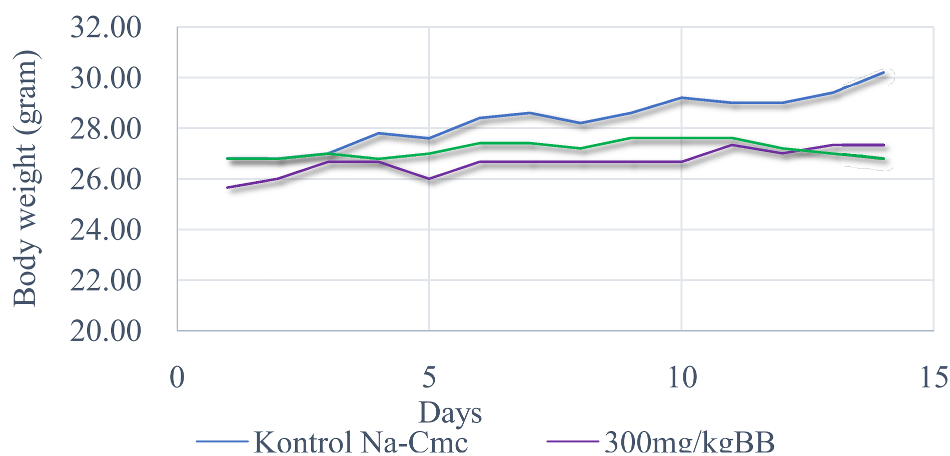


Figure 2: The average of mice weight for 14 days

Table 1: Biochemical parameters

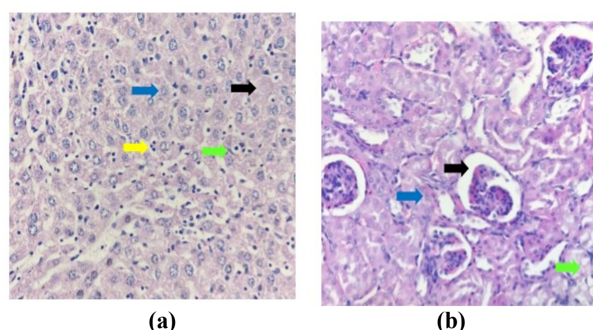
Doses (mg/kg BW)	Biochemical Parameters		
	SGPT	SGOT	Kreatinin
Na-CMC	32.82 ±3.88	84.66 ± 13.86	0.55 ± 0.10
Extract 300	39.93 ± 8.80	94.20 ± 7.80	0.54 ± 0.06
Extract 2000	37.22 ±7.80	74.00 ± 5.01	0.50±0.13

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

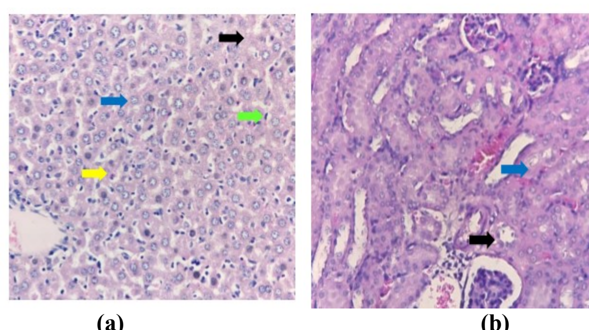
Table 3: Hispatology parameters

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



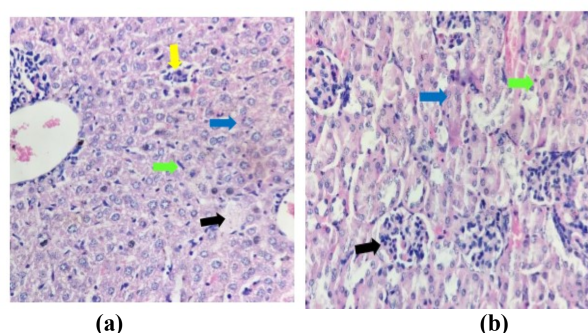
(a) (b)

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 gromerulus 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



(a) (b)

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 gromerulus 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



(a) (b)

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 gromerulus 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 (*Etlingera 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 (*Etlingera 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 (*Etlingera elatior*) has potential as anti-inflammatory activity which was shown with IC_{50} value of 71.24 µg/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.

REFERENCES

[1] Lachumy SJT, Sasidharan S, Sumathy V, Zuraini Z. Pharmacological activity, phytochemical analysis and toxicity of methanol extract of *Etlingera elatior* (torch ginger) flowers. Asian Pacific Journal of Tropical Medicine 2010; 3(10): 769–774.

[2] Abdelwahab SI, Zaman FQ, Mariod AA, Yaacob M, Abdelmageed AHA, Khamis S. Chemical composition, antioxidant and antibacterial properties of the essential oils of *Etlingera elatior* and *Cinnamomum pubescens Kochummen*. J Sci Food Agric 90(15), 2010; 2682.

[3] Chan, E., Voon, P., Tan, V. & Low, Y., Antioxidant and Antibacterial Properties of Alpinia galanga,

Curcuma longa, and Etlingera elatior (Zingiberaceae). *Pharmacognosy Journal,* 3(22), 2011; 54-61.

[4] Kusriani, H. *et al*., Activity-guided Isolation of Antioxidant Phenolic Compound from Etlingera elatior Leaves. 2018; 43-46.

[5] Levita J. *et al*, Perspektif Molekular Aktivitas Antiinflamasi Tanaman Kecombrang (Etlingera elatior Jack RM Smith), ed. 1, Deepublish, Sleman, 2019

[6] Mai, C. et al., Antiproliferative and Apoptotic Studies of the Standardised Extracts of Etlingera elatior on Human Colorectal Carcinoma Cells. *Malaysian Journal of Chemistry,* 11(1), 2009; 136-142.

[7] Fitrianita, A., Yardi & Musir, A., Uji Efek Antihiperglikemia Ekstrak Etanol 70% Daun Kecombrang (Etlingera Elatior) pada Tikus Sprague Dawley dengan Penginduksi Aloksan. *Jurnal Ilmiah Farmasi,* 14(1), 2018; 9-16

[8] Ghasemzadeh A., Secondary Metabolites Constituents and Antioxidant, Anticancer and Antibacterial Activities of *Etlingera elatior* (Jack) R. M. Sm Grown in Different Locations of Malaysia, *International Society for Complementary Medicine Research (ISCMR).* 2015; 15, 335

[9] Garcia-Lafuente A., E. Guillamo´n, A.V. Mauricio, A. R. Jose, and A. Martı´nez., Flavonoids as Antiinflammatory Agents : Implications in Cancer and Cardiovascular Disease. *Inflam. Res.* 2009; 58

[10] Oyedapo OO, BA Akinpelu, KF Akinwunmi, MO Adeyinka and FO Sipeolu. Red Blood Cell Membrane Stabilizing Potentials of Extracts og Lantana camara and its fractions. *International Journal of Plant Physiology and Biochemistry.* 2010; 2(4), 46-51

[11] Serafini M., I. Peluso, and A. Raguzzini. Flavonoids as Anti-inflammatory Agents. *Proc. Nutr. Soc.69,* 2010; 273-278

[12] Kumar V, Zulfiqar A.B, Dinesh K, N.A Khan, I.A Chashoo. Evaluation of anti-inflamatory potential of leaf extracts of *Skimmia anquetilia*, *Asian Pasific Journal of Tropical Biomedicine*. 2012; 627-630.

[13] Roberts LJ II, Marrow JD. Analgesic-antipyretic and Anti-inflammatory Agents and Drugs Employed in the Treatment of Gout. In : Hardman JG, Limbird LE,editors. The Pharmacological

Basis of Therapeutics, 10 th. edition. Mc Graw Hill. New York, 2001; 687–731

[14] Madhavi P, Maruthi R, Kamala V, Habibur Rahman, M. Chinna E., Evaluation of Antiinflamatory Activity of Citrullus lanatus Seed Oil by Invivo and In-vitro Models. *International Research Journal of Pharmaceutical and Applied Sciences*. 2(4), 2012; 104-108

[15] Peraturan Kepala Badan Pengawas Obat dan Makanan (BPOM) Republik Indonesia No. 7 Tahun 2014, *Pedoman Uji Toksisitas Non Klinik Secara In Vivo.* 2014.

[16] WHO.World Health Organization, Teratogenicity Study. Geneva; http://ecb.jrg.it/Document/testing-method/annexv B31, 1998

[17] Hodgson, E., *A Textbook of Modern Toxicity.* A John Wiley & Sons, Inc. Publication. North Carolina. 2010.

[18] González-Gallego, Sánchez-4, Campos S., and Tunon M., Anti-inflammatory properties of Dietary Flavonoids. *Nutrición Hospitalaria*. 22. 2007; 287-293

[19] Rao, K. S., Acute Oral Toxicity (A Review). *BEM Reports,* 4(2), 2018; 39 - 41.

[20] Rejeki, P. S., Putri, E. A. C. & Prasetya, R. E., *Ovariektomi Pada Tikus dan Mencit.* Airlangga University Press.Surabaya: 2018.

[21] Mustarichie, R., Warya, S., Saptarini, N. M. & Musfiroh, I., Acute and Subcroninc Toxicities of Indonesian Mistletoes Dendrophthoe pentandra L. (Miq.) Ethanol Extract. *Journal of Applied Pharmaceutical Science,* 6(09), 2016; 109-114.

[22] Burhan, A., Rahim, A. & Regina, Standarisasi Parameter Spesifik dan Non Spesifik Ekstrak Etanol Daun Kecombrang (Etlingera elatior (Jack) RM. Smith). *Journal of Pharmaceutical and Medicinal Sciences,* I(2), 2016; 21-24.

[23] Jothy, *et al*., Acute Oral Toxicity of Methanolic Extract of Cassia Fistula in Mice. *Molecules,* Issue 16, 2011; 5268-5282.

[24] Arneson, W. & Brickell, J., *Clinical Chemistry: A Laboratory Perspective.* United States of America: F.A Davis Company.2007.

[25] Burtis, C. A., Ashwood, E. R. & Bruns, D. E., *Tietz textbook of Clinical Chemistry and Molecular Diagnostics.* 4th ed. Elsevier Saunders. Canada.2006.

[26] Dasgupta, A. & Wahed, A., *Clinical Chemistry, Immunology and Laboratory Quality Control.*

(A Comprehensive Review for Board Preparation, Certification and Clinical Practice). 1st ed. Houston, : Elsevier. Texas. 2014.

[27] Fristiohady, A. *et al*. Nephroprotective Effect of extract Etlingera elatior (Jack) R.M. Smith on CCL4-induced Nephrotoxicity in Rats. *Current Research on Biosciences and Biotechnology,* I(2), 2020; 62-65.

[28] Ridho, M. R., Prasetyo, A. & Hairrudin, Hepatoprotector Effect of Coconut Water (Cocos nucifera L.) and Folic Acid to the Liver Histopathology of Pregnant Wistar Female Rats (Rattus norvegicus) Induced by Carbamate. *Journal of Agromedicine and Medical Sciences,* 6(1), 2020; 53-61.

[29] Kusriani, H. et al., Antioxidant and Cytotoxic Activities, Total Phenolic Content of Leave, Flower, and Rhizome Extracs of Torch Ginger (*Etlingera elatior*). *Pharmacy,* 14(01), 2017; 51-63.

Herni IJBPAS_2021_5599

by R Herni Kusriani

Submission date: 01-Sep-2021 09:03PM (UTC-0500)
Submission ID: 1639806937
File name: Galley_Proof_MS_IJBPAS_2021_5599.pdf (1.42M)
Word count: 4413
Character count: 23792

IJBPAS, September, 2021, 10(9): X-X

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTIINFLAMATION AND ACUTE TOXICITY OF *ETLINGERA ELATIOR* LEAVES EXTRACT

KUSRIANI H[1, 2*], ALIGITA W[2], BUDIANA W[2], FEBRIANTI W[2] AND NUR ADJI F[2]

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 (Etlingera 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 LD_{50} value as a parameter. Torch ginger leaves were extracted using 70% ethanol and concentrated into a thick extract. Anti-inflammatory activity was expressed in IC_{50} value, which mean the ability of extract to inhibit 50% lysis of red blood cells as a marker of inflammation. IC_{50} value as antiinflammation activity of leaves extract was 71.24 µg/mL, while the IC_{50} 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 antiinflamation properties and showed LD_{50}> 2000mg / kgBW. It is still safe for human consumption.

Keywords: Torch ginger leaves (Etlingera 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. *Etlingera elatior* or torch ginger is one of a Zingiberacea family, and is classified under the genera of *Etlingera*. 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 µg / ml **(Kusriani, *et al.*, 2018)**, inhibit the growth of human colorectal cancer cells (HT29) with IC_{50} 170 µg / ml **(Mai, *et al.*, 2009)**, and antihiperglycemy 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 *Etlingera elatior*that has efficacy as anti-inflammatory is flavonoids **(Levita *et al.*, 2019)**. Flavonoids can inhibit cyclooxygenase or lipook-igenase 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, neorodegenerative 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

prostalgladin. Besides kidney problems can arise, because of the effects of excessive oxidation of the drug, some cases can also cause heart toxicity **(Robert and Marrow, 2001)**.

Therefore, more plants were chosen as alternative natural remedies to treat various diseases, especially for alternative medicine of drugs that cause unwanted side effects such as antiinflammation properties **(Madhavi *et al*., 2012)**.

However, besides the benefits oftorch ginger leaves for therapeutic purposes, the chemical content cannot be guaranteed to cause therapeutic effects or side effects. To prevent these unwanted toxic effects, it is necessary to carry out the acute toxicity testing to determine the dosage limits which still safe if it consumed.

The results of this acute toxicity assay can be used to provide information about the dangers of the exposure to humans, so the dosage can be determined for human safety **(BPOM, 2014)**.

MATERIALS AND METHODS

The study was conducted in several stages, including preparation of materials, extraction, in vitro anti-inflammatory activity with HRBC assay, and oral acute toxicity test using the OECD 420 fixed dose method. The toxicity parameters were observed body weight of mice, organ weight, levels of SGOT, SGPT, creatinine, mortality of mice (LD50), and histopathological liver and kidneys.

Chemical and Reagent

Sodium citrate, citric acid, sodium chloride, saline 0,9%, Sodium Phosphate Buffer at pH 7.4, TLC (Merck®), diclofenac sodium , human blood, DMSO, Ethanol, trucal u, trulab n, Na-CMC (Carboxy Methyl Celullosa), liquid formaldehyde, SGOT reagent, SGPT reagent, creatinine reagent, standard animal feed and rice husks. All chemicals and reagents were analytical grade.

Plant

Leaves of *Etlingera elatior* were planted at local garden in Bandung City, West Java, Indonesia. Leaves were harvest after 3 months of growth. These plants were identified and confirm by Herbarium Jatinangor, Biology Departement of Padjadjaran University, Indonesia.

Sample Preparation

Leaves of *E. elatior* were washed, cleaned in running tap water, cuts into small pieces and dried to constant weight using oven at 40^0C. The samples were pounded into small size using grinder machine; it was stored in an air tight container till when needed.

Extraction

The dried leaves powder of *E. elatior* (1000g) was extracted sequently by maceration method in ethanol 70% (3x 2000 mL) at room temperature. The filtrate

was concentrated in vacuo and obtained 9.287% concentrated extract.

Experimental animal

The experintal 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 heparinzed 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:

% Inhibition of haemolysis = 100 x [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, theywere 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 hoursafter 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 µg / 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 **(Gonzhales *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 ± 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 parameterincludes observation of restlessness, seizures, tremors, grooming (licking hair, rubbing feet against the head or mouth), shortness of breath, diarrhea, defication, 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 LD_{50} 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 LD_{50} 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 transminase 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, indicatesthat 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 parameterars 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, degenerationparenchymal (DP), hydropic degenation (DH), and necrosis.

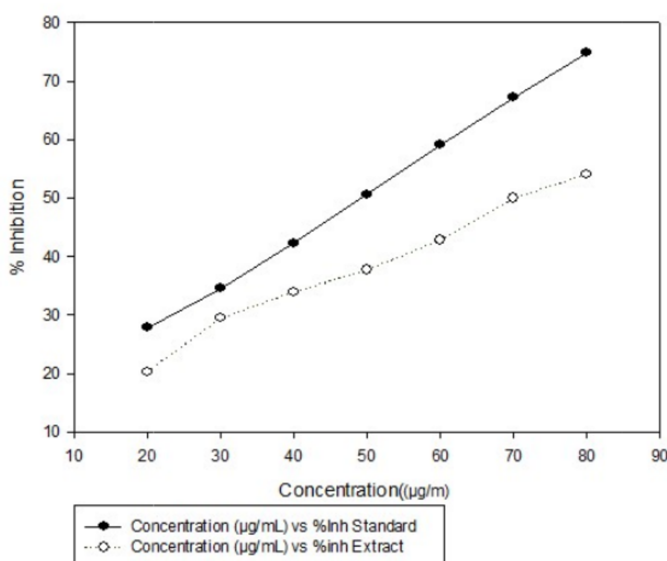


Figure 1: Antiinflammatory activity of diclofenac sodium and *Etlingera elatior*leaves extract

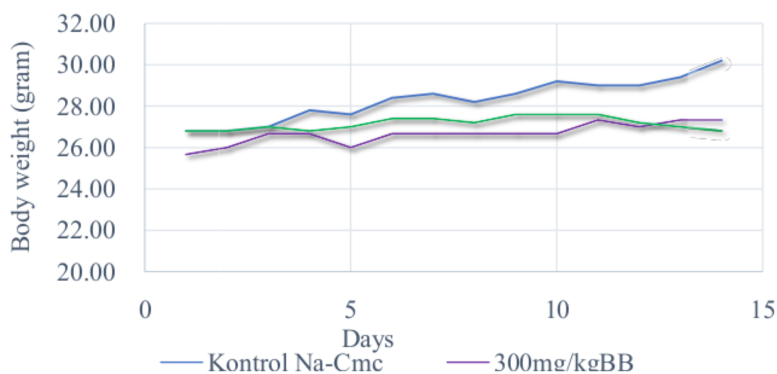


Figure 2: The average of mice weight for 14 days

Table 1: Biochemical parameters

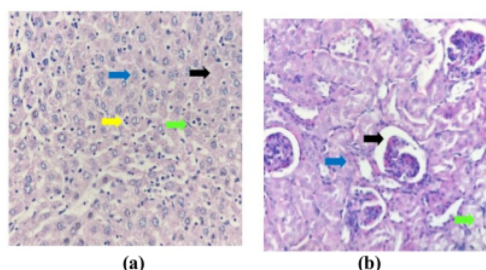
Doses (mg/kg BW)	Biochemical Parameters		
	SGPT	SGOT	Kreatinin
Na-CMC	32.82 ±3.88	84.66 ± 13.86	0.55 ± 0.10
Extract 300	39.93 ± 8.80	94.20 ± 7.80	0.54 ± 0.06
Extract 2000	37.22 ±7.80	74.00 ± 5.01	0.50±0.13

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

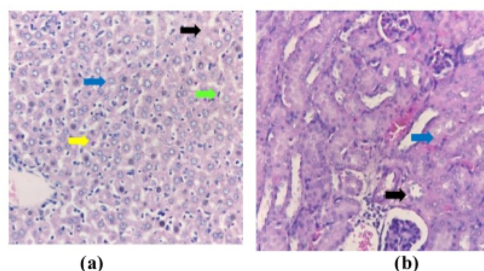
Table 3: Hispatology parameters

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



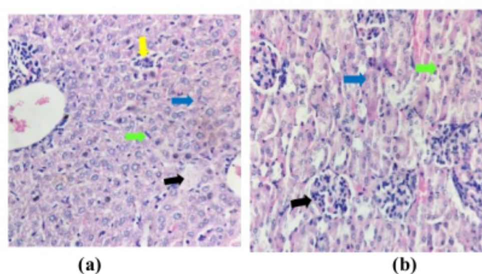
(a) (b)

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 gromerulus 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



(a) (b)

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 gromerulus 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



(a) (b)

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 gromerulus 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 (*Etlingera 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 (*Etlingera 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 (*Etlingera elatior*) has potential as anti-inflammatory activity which was shown with IC_{50} value of 71.24 μg/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.

REFERENCES

[1] Lachumy SJT, Sasidharan S, Sumathy V, Zuraini Z. Pharmacological activity, phytochemical analysis and toxicity of methanol extract of *Etlingera elatior* (torch ginger) flowers. Asian Pacific Journal of Tropical Medicine 2010; 3(10): 769–774.

[2] Abdelwahab SI, Zaman FQ, Mariod AA, Yaacob M, Abdelmageed AHA, Khamis S. Chemical composition, antioxidant and antibacterial properties of the essential oils of *Etlingera elatior* and *Cinnamomum pubescens Kochummen*. J Sci Food Agric 90(15), 2010; 2682.

[3] Chan, E., Voon, P., Tan, V. & Low, Y., Antioxidant and Antibacterial Properties of Alpinia galanga,

Curcuma longa, and Etlingera elatior (Zingiberaceae). *Pharmacognosy Journal,* 3(22), 2011; 54-61.

[4] Kusriani, H. *et al*., Activity-guided Isolation of Antioxidant Phenolic Compound from Etlingera elatior Leaves. 2018; 43-46.

[5] Levita J. *et al*, Perspektif Molekular Aktivitas Antiinflamasi Tanaman Kecombrang (Etlingera elatior Jack RM Smith), ed. 1, Deepublish, Sleman, 2019

[6] Mai, C. et al., Antiproliferative and Apoptotic Studies of the Standardised Extracts of Etlingera elatior on Human Colorectal Carcinoma Cells. *Malaysian Journal of Chemistry,* 11(1), 2009; 136-142.

[7] Fitrianita, A., Yardi & Musir, A., Uji Efek Antihiperglikemia Ekstrak Etanol 70% Daun Kecombrang (Etlingera Elatior) pada Tikus Sprague Dawley dengan Penginduksi Aloksan. *Jurnal Ilmiah Farmasi,* 14(1), 2018; 9-16

[8] Ghasemzadeh A., Secondary Metabolites Constituents and Antioxidant, Anticancer and Antibacterial Activities of *Etlingera elatior* (Jack) R. M. Sm Grown in Different Locations of Malaysia, *International Society for Complementary Medicine Research (ISCMR).* 2015; 15, 335

[9] Garcia-Lafuente A., E. Guillamo´n, A.V. Mauricio, A. R. Jose, and A. Martı´nez., Flavonoids as Antiinflammatory Agents : Implications in Cancer and Cardiovascular Disease. *Inflam. Res.* 2009; 58

[10] Oyedapo OO, BA Akinpelu, KF Akinwunmi, MO Adeyinka and FO Sipeolu. Red Blood Cell Membrane Stabilizing Potentials of Extracts og Lantana camara and its fractions. *International Journal of Plant Physiology and Biochemistry.* 2010; 2(4), 46-51

[11] Serafini M., I. Peluso, and A. Raguzzini. Flavonoids as Anti-inflammatory Agents. *Proc. Nutr. Soc.*69*,* 2010; 273-278

[12] Kumar V, Zulfiqar A.B, Dinesh K, N.A Khan, I.A Chashoo. Evaluation of anti-inflamatory potential of leaf extracts of *Skimmia anquetilia*, *Asian Pasific Journal of Tropical Biomedicine*. 2012; 627-630.

[13] Roberts LJ II, Marrow JD. Analgesic-antipyretic and Anti-inflammatory Agents and Drugs Employed in the Treatment of Gout. In : Hardman JG, Limbird LE,editors. The Pharmacological

Basis of Therapeutics, 10 th. edition. Mc Graw Hill. New York, 2001; 687–731

[14] Madhavi P, Maruthi R, Kamala V, Habibur Rahman, M. Chinna E., Evaluation of Antiinflamatory Activity of Citrullus lanatus Seed Oil by Invivo and In-vitro Models. *International Research Journal of Pharmaceutical and Applied Sciences*. 2(4), 2012; 104-108

[15] Peraturan Kepala Badan Pengawas Obat dan Makanan (BPOM) Republik Indonesia No. 7 Tahun 2014, *Pedoman Uji Toksisitas Non Klinik Secara In Vivo*. 2014.

[16] WHO.World Health Organization, Teratogenicity Study. Geneva; http://ecb.jrg.it/Document/testing-method/annexv B31, 1998

[17] Hodgson, E., *A Textbook of Modern Toxicity*. A John Wiley & Sons, Inc. Publication. North Carolina. 2010.

[18] González-Gallego, Sánchez-4, Campos S., and Tunon M., Anti-inflammatory properties of Dietary Flavonoids. *Nutrición Hospitalaria*. 22. 2007; 287-293

[19] Rao, K. S., Acute Oral Toxicity (A Review). *BEM Reports,* 4(2), 2018; 39 - 41.

[20] Rejeki, P. S., Putri, E. A. C. & Prasetya, R. E., *Ovariektomi Pada Tikus dan Mencit.* Airlangga University Press.Surabaya: 2018.

[21] Mustarichie, R., Warya, S., Saptarini, N. M. & Musfiroh, I., Acute and Subcroninc Toxicities of Indonesian Mistletoes Dendrophthoe pentandra L. (Miq.) Ethanol Extract. *Journal of Applied Pharmaceutical Science,* 6(09), 2016; 109-114.

[22] Burhan, A., Rahim, A. & Regina, Standarisasi Parameter Spesifik dan Non Spesifik Ekstrak Etanol Daun Kecombrang (Etlingera elatior (Jack) RM. Smith). *Journal of Pharmaceutical and Medicinal Sciences,* I(2), 2016; 21-24.

[23] Jothy, *et al*., Acute Oral Toxicity of Methanolic Extract of Cassia Fistula in Mice. *Molecules,* Issue 16, 2011; 5268-5282.

[24] Arneson, W. & Brickell, J., *Clinical Chemistry: A Laboratory Perspective.* United States of America: F.A Davis Company.2007.

[25] Burtis, C. A., Ashwood, E. R. & Bruns, D. E., *Tietz textbook of Clinical Chemistry and Molecular Diagnostics.* 4th ed. Elsevier Saunders. Canada.2006.

[26] Dasgupta, A. & Wahed, A., *Clinical Chemistry, Immunology and Laboratory Quality Control.*

(A Comprehensive Review for Board Preparation, Certification and Clinical Practice). 1st ed. Houston, : Elsevier. Texas. 2014.

[27] Fristiohady, A. *et al*. Nephroprotective Effect of extract Etlingera elatior (Jack) R.M. Smith on CCL4-induced Nephrotoxicity in Rats. *Current Research on Biosciences and Biotechnology,* I(2), 2020; 62-65.

[28] Ridho, M. R., Prasetyo, A. & Hairrudin, Hepatoprotector Effect of Coconut Water (Cocos nucifera L.) and Folic Acid to the Liver Histopathology of Pregnant Wistar Female Rats (Rattus norvegicus) Induced by Carbamate. *Journal of Agromedicine and Medical Sciences,* 6(1), 2020; 53-61.

[29] Kusriani, H. et al., Antioxidant and Cytotoxic Activities, Total Phenolic Content of Leave, Flower, and Rhizome Extracs of Torch Ginger (*Etlingera elatior*). *Pharmacy,* 14(01), 2017; 51-63.