

Effect of Dayak onion Tuber (*Eleutherine Americana* Merr.) Extract to prevent Increased Necrosis of Kidney Tubular Epithelial Cells in Mice (*Mus Musculus*) To oral Lead Acetate Exposure

by Gadis Meinar Sari

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HOME / ARCHIVES / Vol 11, Special Issue 5 (Sep), 2019

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ORIGINAL ARTICLE(S)

ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT BAWANG DAYAK (ELEUTHERINE BULBOSA (MILL.) URB) IN CREAM AGAINST PROPIONIBACTERIUM ACNES

SYAHRIDA DIAN ARDHANY, SUSI NOVARYATIIN

Pages 1-4

[View PDF](#) [Abstract](#) [Download PDF](#)

ASSESSMENT OF THE QUALITY OF OUTPATIENT PRESCRIPTIONS FROM VARIOUS CLINICAL SETTING IN A TERTIARY HOSPITAL, SAUDI ARABIA

SULTAN M ALSHAHRANI

Pages 5-8

[View PDF](#) [Abstract](#) [Download PDF](#)

AMELIORATIVE EFFECT OF PHOENIX DACTYLIFERA ON ADVERSE EFFECTS OF LINEZOLID IN MALE ALBINO RATS

MAHMOUD AHMED ABDO SAID, SAYED A AZIZ, SAMEH M ELNABTITY

Pages 9-13

[View PDF](#) [Abstract](#) [Download PDF](#)

ANTIBACTERIAL ACTIVITY OF CELERY LEAVES (APIUM GRAVEOLENS L.) FORMULATED IN TOOTHPASTE AGAINST STREPTOCOCCUS MUTANS

ERZA GENATRIKA, FITA SATRIANI, INDRI HAPSARI

Pages 14-16

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

IN VITRO CYTOTOXIC AND APOPTOTIC ACTIVITIES OF SULFATED POLYSACCHARIDE FROM CODIUM EDULE P.C. SILVA AGAINST BREAST CANCER ADENOCARCINOMA

²⁶ARIANE MARIE G BAYRO, MARY JHO-ANNE T CORPUZ, ROSS D VASQUEZ

Pages 17-21

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

THE ANTIBACTERIAL ACTIVITY OF BAWANG DAYAK (ELEUTHERINE BULBOSA (MILL.) URB.) FROM CENTRAL KALIMANTAN AGAINST ACNE-CAUSING BACTERIA

SUSI NOVARYATIIN, SYAHRIDA DIAN ARDHANY

Pages 22-25

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

IN VITRO ANTICOAGULANT AND ANTIOXIDANT ACTIVITIES OF PRASAPLAI RECIPE AND ZINGIBER CASSUMUNAR ROXB. EXTRACTS

SURIYAN SUKATI, KHEMJIRA JARMKOM, SURACHAI TECHAOEI, NAKUNTWALAI WISIDSRI, WARACHATE KHOBJAI

Pages 26-30

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

EFFECT OF LATEX FROM MUSA PARADISIACA VAR. SAPIENTUM AND CARICA PAPAYA L. ON PROLIFERATION AND MIGRATION FIBROBLAST CELL NIH3T3

ELZA SUNDHANI, ENDAH NUR IROHIM, RUMIYANA HARTININGSIH, ERZA GENATRIKA, NUNUK ARIES NURULITA

Pages 31-35

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

CYP2D6 PHENOTYPES AMONG JAVANESE AND SUNDANESE SUBJECTS IN INDONESIA

PRI ISWATI UTAMI, SUGIYANTO SUGIYANTO, SUDIBYO MARTONO, LUKMAN HAKIM

Pages 36-39

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

ANTIBACTERIAL, IN VITRO CYTOTOXIC, AND ANTIOXIDANT ACTIVITIES OF ELECTROLYZED OXIDIZING/REDUCING WATER

ASMIYENTI DJALIASRIN DJALIL, ELZA SUNDHANI, RETNO WAHYUNINGRUM, DWI HARTANTI, NONI LESTIOWATI, PRAYOGO PANGESTU, RIDWAN SETIAJI, SUWANDRI SUWANDRI

Pages 40-43

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

FACTOR PROMOTING WOUND HEALING: RADICAL SCAVENGING AND ANTI-INFLAMMATORY ACTIVITY AND GROWTH FACTOR PROMOTION OF HELIOTROPIUM INDICUM

NAKUNTWALAI WISIDSRI, SURADWADEE THUNG MUNG MEE, WARACHATE KHOBJAI

Pages 44-48

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

DETERMINATION OF LUPEOL FROM MAKHAEN AT THAILAND AND TAIWAN BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

SURACHAI TECHAOEI, KHEMJIRA JARMKOM, NAKUNTWALAI WISIDSRI, SURADWADEE THUNG MUNG MEE, WARACHATE KHOBJAI

Pages 49-51

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

THE CHARACTERISTICS AND ANTIOXIDANT ACTIVITIES OF CHABA MAPLE (HIBISCUS ACETOSELLA) HOMEMADE JAM

SURADWADEE THUNG MUNG MEE, NAKUNTWALAI WISIDSRI, WARACHATE KHOBJAI, THISAKORN DUMRONGPHUTTIDECHA, KHEMJIRA JARMKOM, SURACHAI TECHAOEI

Pages 52-55

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

CORRELATION OF RADIOGRAPHIC DAMAGE AND METABOLIC SYNDROME IN SPONDYLOARTHRITIS: A CROSS-SECTIONAL STUDY

HENDRA GUNAWAN, ²⁵SONY WIBISONO MUDJANARKO, AWALIA, LITA DIAH RAHMAWATI, JOEWONO
SOEROSO, AGUNG PRANOTO
Pages 56-60

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

¹**FORMULATION AND CHARACTERIZATIONS OF SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM OF EXTRACT PETIVERIA ALLIACEA (SINGAWALANG) LEAVES**

ARIFA MUSTIKA, NURMAWATI FATIMAH, GADIS MEINAR SARI
Pages 61-65

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

METABOLITES PROFILE OF COLORECTAL CANCER CELLS AT DIFFERENT STAGES

¹⁰HAZWANI MOHD YUSOF, SHARANIZA AB-RAHIM, WAN ZURINAH WAN NGAH, SHEILA NATHAN,
RAHMAN A JAMAL A, MUSALMAH MAZLAN
Pages 66-70

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

EXPRESSION OF THE MICROFOLD CELLS IN THREE-DIMENSIONAL COCULTURE SYSTEM FOR IN VITRO CULTIVATION OF HUMAN NOROVIRUS

MIZANURFAKHRI GHAZALI, SHARANIZA AB-RAHIM, MUDIANA MUHAMAD
Pages 71-74

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

ABERRANT N-GLYCOSYLATION REGULATES INVASION OF MG-63 CELLS THROUGH EXTRACELLULAR MATRIX REMODELING

¹⁰SARMILA HANIM MUSTAFA, MUDIANA MUHAMAD, SHARANIZA AB-RAHIM
Pages 75-79

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

TOCOTRIENOL-RICH FRACTION MODULATE THE PHOSPHOINOSITIDE 3-KINASES/AKT SIGNALING PATHWAY GENES AND PREVENT OXIDATIVE STRESS IN NICOTINE-INDUCED PRE-IMPLANTATION EMBRYOS

NURUL HAMIRAH KAMSANI, SHARANIZA AB-RAHIM, YUHANIZA SHAFINIE KAMSANI, NOR ASHIKIN
MOHAMED NOOR KHAN, MOHD HAMIM RAJIKIN
Pages 80-85

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

ANTIOXIDANT AND ¹⁴FREE RADICAL SCAVENGING ACTIVITY OF HIBISCUS ACETOSELLA LEAVES EXTRACTS

THISAKORN DUMRONGPHUTTIDECHA, SURADWADEE THUNG MUNG MEE, WARACHATE KHOBJAI,
NAKUNTWALAI WISIDSRI, SURACHAI TECHAEI
Pages 86-89

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

CYTOTOXIC ACTIVITY EVALUATION OF ERIOCAULON CINEREUM R.BR. ON HELA AND VERO CELL LINES

PINUS JUMARYATNO, ARDE TOGA NUGRAHA, ADILIA TRI HIDAYATI, BAIQ RISKY WAHYU LISNASARI,
WIDYANUR MAYA DIAHANDARI, NANANG FAKHRUDIN
Pages 90-93

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

CYTOTOXIC ACTIVITY OF ERIOCAULON CINEREUM R.BR TO MCF-7 AND VERO CELL LINE

ARDE TOGA NUGRAHA, ASGAR PURNAMA, SITI NURUL KOMARIAH , HADY ANSHORI T, HADY
ANSHORI T
Pages 94-96

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

DISSOLUTION ENHANCEMENT OF TETRAHYDROCURCUMIN USING OPTIMIZED SELF-NANOEMULSIFYING DRUG DELIVERY SYSTEM

IKA YUNI ASTUTI, TRI SULIATIN, RETNO WAHYUNINGRUM
Pages 97-102

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

EVALUATION OF ACETYLCHOLINESTERASE ACTIVITY AND CYTOTOXICITY OF DIFFERENT PARTS OF NELUMBO NUCIFERA GAERTN ON HUMAN NEUROBLASTOMA CELL LINE (SH-SY5Y)

KHEMJIRA JARMKOM, SURACHAI TECHAEI, NAKUNTWALAI WISIDSRI, WARACHATE KHOBJAI

Pages 103-105

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

THE ANTI-INFLAMMATORY ACTIVITY OF ESSENTIAL OIL OF CLOVE (SYZYGIVM AROMATICUM) IN ABSORPTION BASE OINTMENT WITH ADDITION OF OLEIC ACID AND PROPYLENE GLYCOL AS ENHANCER

NINING SUGIHARTINI, RANI PRABANDARI, TEDJO YUWONO, DESTY RESTIA RAHMAWATI

Pages 106-109

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

BIOLOGICAL ACTIVITY OF SS-GLUCANS FROM EDIBLE MUSHROOM, SCHIZOPHYLLUM COMMUNE IN THAILAND

SAMRAJ RATTANADILOK NA PHUKET, TITIMA SANGKAEW, PIYATIDA CHANAPAN, SURACHAI TECHAOEI

Pages 110-112

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

IN VITRO ANTIMICROBIAL ACTIVITY OF SOME ESSENTIAL OILS AGAINST BACTERIAL PATHOGENS CAUSING SKIN DISEASES IN VAPOR PHASE

PURIT PATTANAPANIT, SUNISA MITHONGLANG, SUNITA MITHONGLANG, SURACHAI TECHAOEI

Pages 113-115

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

THE EFFECTS OF CURCUMIN AND VITAMIN D COMBINATION AS INHIBITOR TOWARD SALMONELLA TYPHI BACTERIA GROWTH IN VIVO

AMI FEBRIZA, VIVIEN NOVARINA A KASIM, HASTA HANDAYANI IDRUS, MOCHAMMAD HATTA

Pages 116-120


 [View PDF](#)  [Abstract](#)  [Download PDF](#)

ANTIBACTERIAL ACTIVITIES OF SAPODILLA FRUIT EXTRACT INHIBITING SALMONELLA TYPHI ON MICE BALB/C

HASTA HANDAYANI IDRUS, MOCHMAMMAD HATTA, AMI FEBRIZA, VIVIEN NOVARINA A KASIM

Pages 121-126

 **8** [View PDF](#)

 [Abstract](#)

 [Download PDF](#)

THE EFFECT OF PSYCHOLOGICAL STRESS ON MPF INTRAFOLLICULAR

REVI GH NOVIKA, BUDI SANTOSO, WIDJIATI WIDJIATI

Pages 127-130

 [View PDF](#)

 [Abstract](#)

 [Download PDF](#)

EFFECT OF SIMVASTATIN ON HISTOPATHOLOGY OF THE HEART AFTER 5/6 SUBTOTAL NEPHRECTOMY

PUTU NITA CAHYAWATI

Pages 131-133

 [View PDF](#)

 [Abstract](#)

 [Download PDF](#)

THE EFFECT OF HOMALANTHUS POPULNEUS (GIESEL.) PAX. EXTRACT IN EXPRESSION OF T-CELL RECEPTOR: INHIBITION STUDY OF HIV INFECTION

SINTYA E, WIJAYANTI N, NORAENI A

Pages 134-137

 [View PDF](#)

 [Abstract](#)

 [Download PDF](#)

IN VITRO ENTRAPMENT AND RELEASE STUDIES OF LEVOFLOXACIN USING EPICHLOROHYDRIN-CROSSLINKED HYDROGEL

ANGELI ANN S RESCOBER

Pages 138-144

 [View PDF](#)

 [Abstract](#)

 [Download PDF](#)

ATHEROPROTECTIVE EFFECT OF SOLANUM BETACEUM ON RAT EXPOSED TO CIGARETTE SMOKE

SITI KHAERUNNISA, HANIK BADRIYAH HIDAYATI, JONI SUSANTO, YUANI SETIAWATI, SUHARTATI

SUHARTATI

Pages 145-149

 [View PDF](#)

 [Abstract](#)

 [Download PDF](#)

ANTI-TYROSINASE ACTIVITY FROM VARIOUS SOLVENTS OF PEANUT SHELL (ARACHIS HYPOGAEA L.) EXTRACTS IN VITRO

RISHA FILLAH FITHRIA, MELLA DWI KRISDIANA, ETIKA MUSLIMAH, SARIF MUSYafa, NINING SUGIHARTINI

Pages 150-152

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

THE IMPACT OF CASSYTHA FILIFORMIS BUTANOL FRACTION TO THE PREGNANCY AND FETAL DEVELOPMENT ON MICE

ARMENIA NAZAR, FIRLICIA AYUNING, ALMAHDY AHMADIN

Pages 153-156

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

COMPARISON TENSILE STRENGTH OF NATURAL AND SYNTHETIC ABSORBABLE SUTURES

TJOKORDA GDE TIRTA NINDHIA, PUTU ASTAWA, TJOKORDA SARI NINDHIA, WAYAN SURATA I

Pages 157-159

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

SYNTHESIS AND ANTIOXIDANT EVALUATION OF 3-BROMO-FLAVONE

AYU MASYITA, RISFAH YULIANTY, YUSNITA RIFAI

Pages 160-162

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

STUDY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ENZYME AND ISOPROSTANE ON PREECLAMPSIA WITH NIFEDIPINE, METHYLDOPA, AND MAGNESIUM SULFATE THERAPY

LINA LUKITASARI, ADITIAWARMAN ADITIAWARMAN, MASYHUDI MASYHUDI, SITI KHAERUNNISA, SUHARTATI SUHARTATI

Pages 163-166

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

SOLANUM BETACEUM IMPROVES COGNITIVE FUNCTION BY DECREASING N-METHYL-D-ASPARTATE ON ALZHEIMER RATS MODEL

INDRI SAFITRI, HANIK BADRIYAH HIDAYATI, AGUS TURCHAN, SUHARTATI, SITI KHAERUNNISA

Pages 167-170

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

EFFECT OF DAYAK ONION TUBER (*ELEUTHERINE AMERICANA* MERR.) EXTRACT TO PREVENT INCREASED NECROSIS OF KIDNEY TUBULAR EPITHELIAL CELLS IN MICE (*MUS MUSCULUS*) TO ORAL LEAD ACETATE EXPOSURE

GADIS MEINAR SARI, DAENG AGUS VIEYA PUTRI, TJITRA WARDANI, LILIK HERAWATI

Pages 171-173

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

THE DEGREE OF SECRETORY IMMUNOGLOBULIN A IN BURN PATIENTS WITH PROBIOTIC THERAPY

LYNDA HARIANI, IRAWAN WAHYUDI, ISWINARNO DOSOSAPUTRO, SJAIFUDDIN NOER M

Pages 174-176

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

DIGITAL HEARING AID SIGNAL PROCESSING SYSTEM USING ANDROID PHONE

YEH-HUANN GOH, YOON-KET LEE, MUM-WAI YIP, KOK-SENG EU, YANN LING GOH, KIN-YUN LUM

Pages 177-181

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

AN APPROACH FOR QUANTITATIVE EVALUATION OF TRANSFEMORAL PROSTHESIS SOCKET BY FINITE ELEMENT ANALYSIS

LE VAN TUAN, AKIHIKO HANAFUSA, SHINICHIROU YAMAMOTO

Pages 182-185

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

APPLICATION OF LASER-INDUCED BREAKDOWN CAVITATION BUBBLES FOR CELL LYSIS IN VITRO

DARINA JASIKOVA, MIROSLAVA RYSOVA, MICHAL KOTEK

Pages 186-190

 [View PDF](#)  [Abstract](#)  [Download PDF](#)

EVALUATION OF CEFOPERAZONE/SULBACTAM AND VITAMIN K USE IN PATIENTS WITH BACTERIAL INFECTIONS

THEERAPONG SEESIN, PITIPONG PENGSAUPIN, SARAWUT WEESAPHEN, PEERAYA SRIPHONG,
UAEPONG LIMPAPANASIT, SIRIN BHONGCHIRAWATTANA

Pages 191-193

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EFFECT OF DAYAK ONION TUBER (*ELEUTHERINE AMERICANA* MERR.) EXTRACT TO PREVENT INCREASED NECROSIS OF KIDNEY TUBULAR EPITHELIAL CELLS IN MICE (*MUS MUSCULUS*) TO ORAL LEAD ACETATE EXPOSURE

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ABSTRACT

Objective: This study aims to determine the effect of *Dayak* onion tuber extract to prevent an increase in necrosis of kidney proximal tubular epithelial cells exposed to lead acetate.

Methods: Fresh *Dayak* onion tuber was extracted with 96% ethanol and then given orally to mice with doses of 65 mg/kg, 130 mg/kg, and 260 mg/kg for 21 days, accompanied by 14 days of lead acetate on the 8th-21st days. Histological preparations of the mice's kidney were made by hematoxylin and eosin staining, and then, the necrosis of tubular cells was examined using a $\times 400$ magnification light microscope that observed 5 views randomly marked by the presence of pyknosis, karyorrhexis, and karyolysis.

Results: This study showed the higher the dose of *Dayak* onion tuber extract and the lower the number of tubular epithelial cell necrosis ($p < 0.05$).

Conclusion: Extract of *Dayak* onion tuber could prevent an increase in necrosis of kidney proximal tubular cells of mice that exposed to lead acetate.

Keywords: *Dayak* onion extract, Lead acetate, Necrosis, Tubular epithelial cells.

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INTRODUCTION

The use of traditional medicines in Indonesia is essentially part of the Indonesian culture. The biodiversity of the Indonesian reaches to 30,000 species of plant and 940 of them are traditional medicinal plants. The advantage of using traditional medicine is it easily available in our surrounding, and empirically traditional medicine is able to cure various diseases, but its efficacy and capabilities have not been clinically proven yet [1].

Antioxidant compounds have an important role in the health of the human body. Various scientific evidence shows that antioxidant compounds can reduce various diseases because the main character of this compound is its ability to capture free radicals [2]. Free radicals are molecules that have too much or too few electrons to cause instability. Free radicals try to steal or give their electrons to other molecules so they can change their chemical structure [3]. Oxidative stress is a condition imbalance of the number of oxidants and pro-oxidants in the body which causes the activity of free radical molecules, or reactive oxygen species (ROS) attacks lipids, sugar, protein, and DNA so that it can cause cellular and genetic damage [4].

Lead is a toxic heavy metal used in the industrial world. Lead is found in everyday life, for example, in canned food, cosmetic ingredients, motor vehicle fuel, wall paint, plant fertilizers, and much more. The lead that enters the body through the digestive tract will accumulate in the blood and work to inhibit the δ -aminolevulinic acid dehydratase (ALAD) enzyme, where this enzyme functions in hemoglobin synthesis [5]. In a series of chemosynthesis, because of this inhibition, there is an increase in the levels of ALA substrate which causes the formation of reactive bonds and produces ROS compounds [6]. The mechanism of ROS inflicts in cellular dysfunction and cell membrane lipid peroxidation which results in loss of calcium homeostasis from the mitochondria and endoplasmic reticulum. Then, because of the calcium levels are not well controlled, it

can lead to cause tissues damage. Necrosis is irreversible damage that by the presence of degeneration, karyorrhexis, pyknosis, and karyolysis.

In this case, the kidney is the largest organ that receiving blood about 20-25% of cardiac output. Kidney damage can occur due to various diseases and commonly damaged the mass of the kidney. The proximal tubule is the most part that often damaged due to lead exposure. The predisposing factor that results in a perishable proximal tubule is the role that reabsorbs 60-80% of glomerular filtration. Other factors are the surface area of tubular re-absorption, the active transport system for ions and organic acids, and the ability of the tubules to concentrate the active transport system substances for ions, organic acids, low molecular proteins, peptides, and heavy metals that mostly occurring in the proximal tubules which cause its to be damaged. Loose proximal tubular epithelium makes various components into tubular cells. This is also the cause of affecting tubular damage [7].

Indonesia's biodiversity is more than 30,000 species of plants and 940 of them are traditional medicinal plants. One of the traditional medicinal plants derived from onion *Dayak* (*Eleutherine americana* Merr.). There are many methods available in preventing costs due to exposure to free radicals, especially in this case lead which antioxidant content found in the *Dayak* onion tuber (*E. americana* Merr.). According to phytochemical analysis, it has a higher level of phenol and flavonoid. Previous research by Yuswa [8] proved that this plant has a total phenol content and high levels of flavonoids. The presence of antioxidants in the body is expected to compensate for free radical reactions that produced from various cells [9]. Phenol is a natural chemical compound that works as an antioxidant through a reducing mechanism, free radical capture, metal chelating to reduce the formation of reactive bonds, and electron donors. Flavonoids as antioxidants can protect cells from oxidative stress and reduce the risk of various degenerative diseases that associated with oxidative damage, and almost all flavonoids have strong antioxidant effects

because they contain chemical elements responsible for antioxidant activity [10] quoted from Sandhar *et al.* [11].

Based on the description above, this study examined the effect of *Dayak* onion tuber extract (*E. americana* Merr.) to prevent an increase necrotic kidney tubular proximal epithelial cells of mice (*Mus musculus*) that exposed to peroral lead acetate, with the content framework as follows:

METHODS

Laboratory experimental research using the post-test, only control group design was applied. There were five groups consisting of the negative control and given a placebo orally for 21 days daily and positive control also given a placebo orally for 21 days and lead acetate by 50 mg/kg dose on 8th-21st days.

Group K1 was given *Dayak* onion tuber extract at a dose of 65 mg/kg for 21 days and lead acetate 50 mg/kg on 8th-21st days. Then, Group K2 was given *Dayak* onion tuber extract with a dose of 130 mg/kg for 21 days and lead acetate 50 mg/kg on 8th-21st days, and Group K3 was given *Dayak* onion tuber extract with a dose of 260 mg/kg for 21 days and lead acetate 50 mg/kg on 8th-21st days.

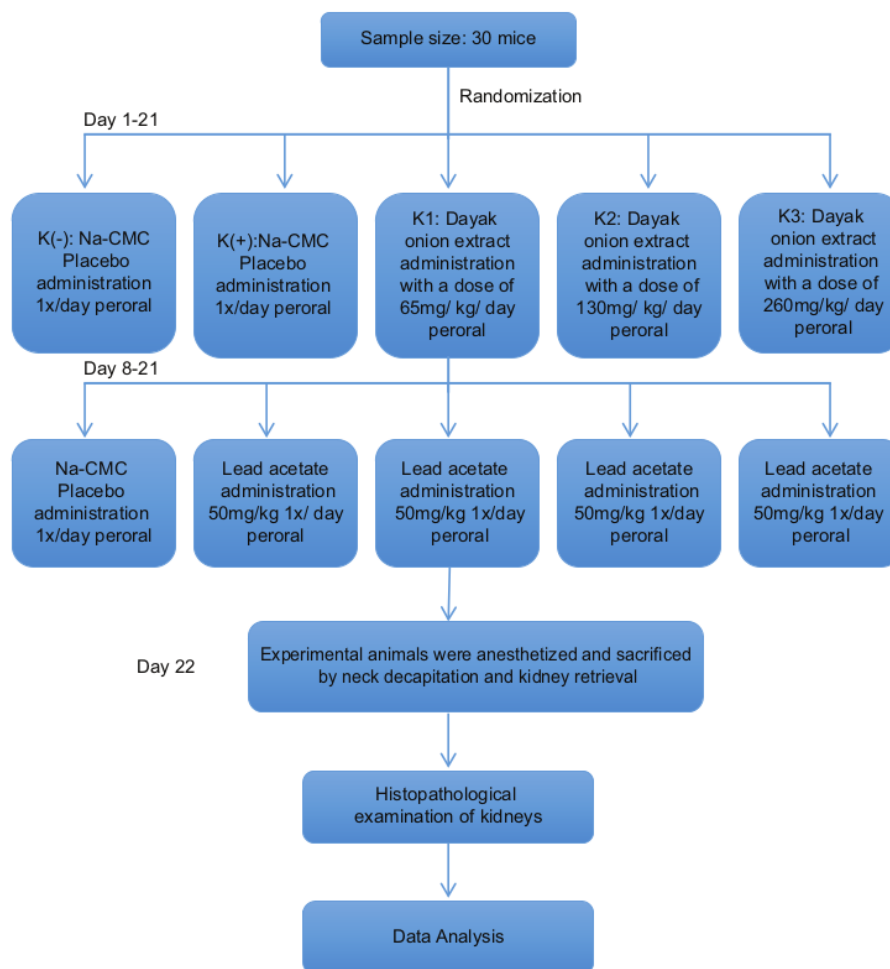
In addition, on the 22nd day, all the mice were sacrificed to be dissected to taken their kidneys. Preparation in making kidney histology was using hematoxylin and eosin staining. Then, the necrosis of tubular cells was examined using a ×400 magnification light microscope that observed 5 views randomly marked by the presence of pyknosis cells (the cell nucleus looks smaller, dense, and black), karyorrhexis (cell nucleus breaks into several clots), and karyolysis (nucleus cells pale, spread, or dissolve).

RESULTS AND DISCUSSION

Provision of *Dayak* onion tuber extract has been proven to prevent an increased kidney tubular epithelial cells in mice that exposed to oral lead acetate. Lead acetate is used as a trigger for the formation of free radicals so that ROS was formed, resulting in increased necrosis in proximal tubular epithelial cells.

Normal proximal tubular epithelial cell is seen in the negative control group (a) and necrotic proximal tubular epithelial cells (b), as shown in Fig. 1.

Based on the statistical test results, the data on the amount of necrotic epithelial cells in proximal tubules tested for normality with the Shapiro-Wilk test were obtained; all the data were normally distributed ($p > 0.05$).



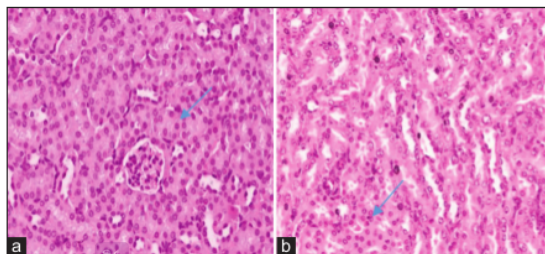


Fig. 1: (a) Normal proximal tubular epithelial cells, (b) necrotic proximal tubular epithelial cells, with hematoxylin and eosin staining ($\times 400$)

Table 1: Average of necrotic proximal tubular epithelial cells

Group	Necrosis (%) (Mean \pm SD)	Brown-Forsythe sig.
K (-)	(2.967 \pm 1.856)	0.001*
K (+)	(14.833 \pm 3.829)	
K (1)	(17.633 \pm 7.938)	
K (2)	(6.167 \pm 1.717)	
K (3)	(4.433 \pm 1.799)	

*Significant value ($p < 0.05$). SD: Standard deviation

Based on the normality test above, then variance homogeneity of the data using Levene test has a result of $p < 0.05$ means that the data were not homogeneous or have different variants ($p < 0.002$). Hence, the reading of the Brown-Forsythe test resulted in $p < 0.05$ which means statistically significant. This shows that the extract of *Dayak* onion tuber can prevent an increasing percentage of necrotic epithelial cells in proximal tubules in the treatment group significantly, as seen in Table 1.

This study used the extract of *Dayak* onion tuber (*E. americana* Merr.) by giving in multilevel doses to each treatment groups (K1, K2, and K3). Group K1 was given a dose of 65 mg/kg, the Group K2 was 130 mg/kg, and K3 was 230 mg/kg. The percentage of necrotic epithelial cells in proximal tubules decreases by increasing the doses given of *Dayak* onion tuber extract. The content of phenol and flavonoids contained in the union work as an antidote to free radicals due to oxidative stress. The antioxidant activity in *Dayak* onion tuber extract was due to the content of total phenol and high flavonoid levels in 96% ethanol. The higher the total phenol value, the higher the antioxidant activity produced. Antioxidant activity in flavonoid and phenol compounds with -OH groups which were bound to carbon aromatic rings [12].

The effect of lead hematotoxicity is to inhibit the conversion of the δ -ALAD enzyme to porphobilinogen and the corporation from iron (Fe) into the protoporphyrin IX to form hemoglobin. The inhibition of the δ -ALAD enzyme resulted in the accumulation of δ -ALA and protoporphyrin resulting in an inhibition of hemoglobin synthesis. In addition, lead inhibits the ferrochelatase enzyme, which causes iron (Fe) ions unable to bind to the protoporphyrin ring, and therefore, competition occurs between lead and Fe [13].

The physiological system of the body has the ability to reduce the damage of cells by peroxidation. However, if the body immune is weak or too much exposure to ROS, additional body protection mechanisms were needed. One form of additional protection was through the consumption of antioxidants which contained in many natural ingredients. All antioxidants can prevent lipid peroxidation [14].

Antioxidants are compounds that able to neutralize or stabilize free radicals by completing the lack of electrons in these free radicals. Antioxidants can prevent the occurrence of chain reactions from the formation of free radicals that can cause oxidative stress [15].

The mechanical action of antioxidants in free radicals has 3 ways, namely primary antioxidants, secondary antioxidants, and tertiary antioxidants. Here, the antioxidant content in *Dayak* onion tuber extract works on the mechanism of secondary antioxidants, namely Vitamin C, Vitamin E, Vitamin B, beta-carotene, and phytochemical compounds (flavonoids and carotenoids) that work by chelating metals as proxies, for example, lead, capturing radicals, and preventing fission reaction. Secondary antioxidants called as a chelating agent [16].

This study was a basic research on the effects of herbal extracts in improving organ function which was still at the treatment stage of experimental animals. Further research is still needed on the effectiveness of *Dayak* onion extracts and clinical trials in humans gradually. Considering that *Dayak* onion is easily available and affordable, thus it is expected to be able to be used as a natural alternative medicine to prevent increased necrosis in renal tubular epithelial cells due to lead acetate exposure.

CONCLUSION

Based on this study, it was proven that administration of *Dayak* onion tuber extract by doses of 65 mg/kg, 130 mg/kg, and 260 mg/kg could prevent an increase in necrosis proximal tubular cells of mice that exposed to lead acetate. The percentage of necrotic epithelial cells in proximal tubules decreases by increasing the doses given of *Dayak* onion tuber extract.

CONFLICTS OF INTEREST

All authors have none to declare.

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PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12

PAGE 13

PAGE 14

PAGE 15

PAGE 16

PAGE 17
