ORIGINAL ARTICLE

Toxicity Effects of Clitoria Ternatea L. Extract in Liver and Kidney Histopathological Examination in Mus Musculus

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ABSTRACT

INTRODUCTION: *Clitoria ternatea* L. or butterfly pea is more increasingly being consumed as traditional medicine though it contains secondary metabolites, which can be non-beneficial to its consumer. LD_{50} and histopathological examination are used frequently in toxicity studies. Thus we aimed to determine the toxicity level of *Clitoria ternatea* L. flower extract and its effects on the liver and kidney in mice. **MATERIALS AND METHOD:** We carried out this analytical experimental study with from August 2021 to June 2022. Mice were categorised into five dose groups. They were treated with aquades, paracetamol-induced, and flower extract at 500, 1000, and 2000 mg/kg BW orally for 14 consecutive days. **RESULTS**: *C.ternatea* flower was classified as having very low toxicity (LD_{50}>2000 mg/kg BW). We detected toxicity signs in mice such as lethargy and tremor in the group treated with more than 1000 mg/kg BW. Histopathological examinations showed leucocytes, vacuolation, and necrosis in the liver and kidneys of mice. There were significant differences in liver and kidney histopathological scores between the five study groups ($p<0.05$). **CONCLUSION:** *C.ternatea* flowers are safe to consume at a dosage of 500 and 1000 mg/kg BW since no mice died after being treated for 14 days. Liver and kidney damage appeared at 2000 mg/kg BW dosage in histopathological examination. Hence, its consumption at this dosage should be limited.

INTRODUCTION

Plant-derived products are mostly considered safe because of their "natural" trait and nutrients. Regardless of this, secondary metabolites in plants are utilised as a defense mechanism for human health that can actually be toxic for humans. Some of these metabolites cannot be distinguished from active ones.[1,2] Thus, the safety of herbal products is still questionable.[3] These herbal products may cause side effects such as herb-induced liver injury (HILI) and nephrotoxicity since both these organs are mainly involved in metabolism of food and excretion.[4,5] Herbal plants are used widely by humans to treat various diseases. Normally, phytochemical studies and their effects on traditional drugs are conducted to investigate their safety and effectiveness. Some components in herbal plants may be toxic, teratogenic, and carcinogenic.[6] *Clitoria ternatea* L. has been increasingly popular in use as an herbal medicine. Anthocyanins in this plant give a blue color to its petals. Thus, the flower is often used as a dye in food, drink, and manufacturing industries, especially in Asia.[7] *C.ternatea* has potency as an antidiabetic, anti-inflammatory, antimicrobial, anticancer, analgesic, antipyretic, antiplatelet, and antioxidant agent.[8] *C.ternatea* is usually consumed as brewed drinks.[9] Studies investigating leaves of *C.ternatea* found declined levels of AST, ALT, and ALP(in full) with a dosage of 1000 mg/kg BW. The plant also has nephroprotective effects marked by declining levels of urea and creatinine in mice.[10] Despite its benefits, some studies showed that *C.ternatea* leaf extract was hepatotoxic and nephrotoxic at 2000 mg/ kg BW.[11] *C.ternatea* roots were toxic at doses above 2500 mg/ kg BW. Another toxicity study was carried out using *C.ternatea* root extract and obtained LD_{50} of 15000 mg/ kg BW with damaged liver in the histopathology examination.[12] Toxicity study in plants is crucial to assess their effects on organs in humans

by measuring various parameters. The most frequent parameters used are LD_{50}, liver, and kidney function. LD_{50} is a parameter to evaluate possible dose that causes death in 50% of the study population. Liver and kidney function in animals is examined from histopathological studies. Mammals, including mice, rats, rabbits, and sheep, are commonly used for such genetic and molecular studies.[13] Acute toxicity study using mice is generally conducted in approximately 14 days. This type of study helps predict appropriate and safe dosage for consumption. It also determines which dosage is toxic and causing damage to certain organs.[14] Thus far, there has been no report about the toxicity effect of *C.ternatea* flowers on the liver and kidney, although it has been widely used by the public. Thus, this study was performed to investigate this aspect.

MATERIALS AND METHOD

STUDY DESIGN

This was an experimental and post-test-only control group design approach using white mice (*Mus musculus*) as the animal model and was carried out at the Anatomical Pathology Laboratory, Medical Faculty, Andalas University; Biomedicine Laboratory, Medical Faculty, Jambi University; Pharmacology Laboratory, Faculty of Pharmacy, Andalas University; Chemical Laboratory, Faculty of Mathematics and Natural Sciences, Andalas University; and Genbinesia Biology Laboratory, East Java, Indonesia. This study was conducted from August 2021 until June 2022. We obtained ethical approval from The Research Ethics Committee of Medical Faculty Andalas University (No. UN.16.2/KEP-FK/2022).

The plant had been identified by a certified botanist (Heri Santoso, S.Si) and the data specimen had been stored at the Biology Laboratory of Genbinesia, East Java (voucher specimen number 08.175/Genbinesia/III/2022). Mice were categorised into five groups with each group containing six mice: a) the negative control group was given 10 mL/kg BW of 2% aqueous; b) induced group was given paracetamol with a toxic dosage to the liver and kidney; c) treatment group used 500 mg/ kg BW of *C.ternatea* flower extract; c) treatment group used 1000 mg/ kg BW of *Clitoria ternatea* L. flower extract; and d) The treatment group used 2000 mg/ kg BW of *C.ternatea* flower extract . The total samples in each group were calculated using Federer's formula. We considered a total number of 30 mice as necessary and expected a 10% dropout throughout the study. White mice aged 6-8 weeks old, weighing 20-30 grams, and had no anatomical abnormalities were included. We excluded mice that showed weakness, inactivity, inability to eat, or had previously been used in other research. Samples were taken by purposive quota sampling technique for the samples to be considered as overall population representation based on the inclusion and exclusion criteria. White mice were obtained from the Pharmacology laboratory in the Faculty of Pharmacy, Andalas University, and divided randomly into five groups. Each group contained six mice. Mice were assigned to different racks and weighed upon arrival. Each mouse was given random numbers. Thirty male white mice (*Mus musculus)* were used.

Before treatment, we used mice aged 6-8 weeks old and weighed 26.3±1.1 g. The cages were assigned randomly into five categories. Three different investigators were involved in this study. The first investigator was responsible for animal preparation, treatment administration, and organ harvesting. The second investigator was aware of the treatment group allocation. The third investigator performed the histopathological examination and data analysis. This study assessed the lethal dose (LD) of *C.ternatea* flower extract, toxicity signs, histopathological features, and organ (liver and kidney) damage. According to the global classification system by the United Nations, there are five groups of chemicals based on their lethal dose (LD). Organ damage was examined on a 100x and 400x magnification microscope for focal necrosis in hepatic cells, central vein area, and degeneration in the middle zone of the liver. In acutely damaged liver tissue, bleeding, polymorphonuclear cells, hepatic cell vacuolisation, and mononuclear cell infiltration were found. Vacuolation, tubular necrosis, bleeding, and infiltration of mononuclear cells were assessed in the damaged kidney after treatment.

STATISTICAL METHODS

The data in the study was abnormally distributed with the Shapiro-Wilk test. We used purposive quota sampling as a

sampling technique for this study. Kruskal Wallis non-parametric test determined the differences in liver and kidney histopathological scores among the negative control group (K-), the positive control group (K+), and the treatment group (P) followed by the Post Hoc test of Mann Whitney analysis. A p-value<0.05 is considered statistically significant. Analysis was done using SPSS version 26.

PLANT EXTRACTION

We obtained fresh *C.ternatea* flowers from a hydroponic plantation in Padang, West Sumatera, Indonesia. The total weight of fresh flowers needed for 14 days is 5880 mg. We considered the amount of lost water component during the drying process as much as 90% and the involvement of the maceration process using ethanol as much as 20% yield. Hence, 294 grams of fresh flowers were needed. They were separated from dirt and other foreign substances such as pests, dust, and other impurities. Then, they were cleaned under running water. They went through the drying process by keeping them protected from sun rays for three days.

This method ensured fewer compounds that might damage their chemical compound. After that, they were blended into powder form. They were macerated in 96% ethanol concentration for 3x24 hours inside a room far from sunlight. The maceration product was collected, evaporated by distillation vacuum, and thickened with a rotary evaporator at a temperature of 40° C; thus, the extract was produced. The white mice were acclimatized for at least five days. They were under treatment for 14 days in a row.

We gave the extract to mice orally (as in humans). We observed the pharmacology and toxicological effects of the mice after treatment. The observation was made at least every 6 hours, such as neurological and behavior changes, dysfunction, and other signs of nervous system toxicity. A series of inspections were made during the maintenance period. Mice organ harvesting was conducted after mice were found dead to minimise possible autolysis and affect the histopathological results. Mouse was euthanized and sanitized using 70% ethanol. The peritoneal cavity was opened by cutting vertically from the lower end of the abdomen to the ribcage, then horizontally through the middle section of the abdomen to both sides. Intestines were placed sideward to reveal the portal vein and the vena cava until the liver could be accessed. We perfused the liver through the vena cava until the vasculature became engorged. At this point, we cut the portal vein, and remove the gallbladder and liver ligaments. The liver was collected. Kidney harvests were performed after that. When the bowel was retraced sideways, we observed one side of the kidney, abdominal aorta, and inferior vena cava. These vasculatures were completely divided. At this moment, the whole kidney was dissected free. We fixed the liver and kidneys in 4% formaldehyde treated them with paraffin wax, and then sliced them into 8 µm of thickness and stained them with haematoxylin-eosin. Liver and kidney damage was measured using parameters from Alves et al and Niizuma et al respectively.[15,16]

RESULTS

Description

C. ternatea is a type of liana plant that can grow up to 3 meters annually. It has caulking rods that twist to its host. It consists of three compound leaves 3, alternately arranged, rounded, blunt end, flat edge, rounded base, pinnate leaves based on bone structure, smooth upper surface, dark green colored at the upper part, and pale green underside the leaf. Its flower grows about 4 cm long and 3 cm wide and shapes resembling a butterfly. It possesses five pieces of stamens, a piece of pistil, and five blue strands of the crown with whitish to yellowish gradations. It has striated, black to brown colored pods which measure 5–7 cm long with six to ten flat and round shaped seeds, diameter 2−3 cm.

The Effect of *Clitoria Ternatea* L. on Liver Histology

We prepared liver tissue using haematoxylin-eosin staining to carry out a histopathological examination of the organ. The treatment groups showed differences in histopathology examination, as shown in Figure 1. Liver tissue in the negative control group showed organised hepatocytes and sinusoids with few leukocytes (arrow) in the sinusoids. Liver damage, vascular dilation, severe inflammation with leukocyte distribution around

the sinusoid, degenerating hepatocytes, and necrosis (arrowhead) were seen in the paracetamol-induced group. The treatment group using *Clitoria ternatea* L. flower extract of 500 mg/kg BW and 1000 mg/kg BW did not show significant histological differences compared to negative controls. Meanwhile, the treatment group of 2000 mg/kg BW extract, showed increasing leukocyte distribution in the sinusoids. We also recognised degeneration of hepatocytes and necrosis in this group.

Figure 1: *Clitoria ternatea* L. plant (a) Parts of *Clitoria ternatea* L.; flower (b), root (c), pod (d), and leaf (e).

The Effect of *Clitoria Ternatea* L. on Kidney Histology

We prepared kidney tissue using haematoxylin-eosin staining to carry out a histopathological examination of the organ. The treatment groups showed differences in histological images, as shown in Figure 2. Kidney tissue in the negative control group revealed organised tubules and glomeruli with few leukocytes in the interstitial tissues. Treatment with paracetamol manifested kidney damage, edema, vascular dilation, and bleeding, as well as tubules with degenerating epithelium and necrosis.

Treatment of *C.ternatea* flower extract at 500 mg and 1000 mg/kg BW did not exhibit significant histological differences from negative controls. Treatment of *C.ternatea* flower extract at 2000 mg/kg BW caused dilation of blood vessels in focal area, multiple tubules with degenerated epithelium, and necrosis. There was no sign of toxicity manifested in the negative control and treatment group using *C.ternatea* flower extract at a dosage of 500 mg/kg BW. Two mice in group 2 (dose 1000 mg/ kg BW) exhibited lethargies (33.3%). A mouse in group 3 (dose 2000 mg/kg BW) displayed lethargy and tremors (50.0%). Two mice died in the positive control group (paracetamol induced), and all mice presented lethargy (100%).

Relationship of Dosage to Liver Histopathological Examination

We presented the distribution of liver histopathology scores based on Alves et al liver damage parameters according to dose group. We examined six histopathological slides for each group of dosage with a total of 30 slides. Microscopic examination was done with 40x and 100x visual field magnification. Scoring system for liver damage based on histopathological examination:

0: no steatosis area and inflammation
1: <30% of steatosis area, watering degeneration, and some small necrosis
2: 30-50% of steatosis area, ballooning degeneration, more small necrosis, presence of Mallory body, and infiltration of local PMN
3: >50% of steatosis area, severe degeneration, necrosis, and bridging necrosis.

The higher the dose, the greater the average scores of liver histopathology findings. There were significant differences in liver histopathological scores among the treatment group ($p<0.05$).

Table 1: The distribution of liver histopathology scores according to the group

Variable	Mean	SD	95% CI	P-value
Negative Control (Aquades)	0.10	0.0	0.10-0.19	
Group 1 (Dosage of 500 mg/kg	0.40	0.1	0.21-0.59	
Group 2 (Dosage of 1000 mg/kg	1.2	0.3	0.85-1.55	0.005
Group 3 (Dosage of 2000 mg/kg	2.40	0.3	2.05-2.75	
Positive Control (Paracetamol	2.73	0.1	2.62-2.84	

*Kruskal Wallis test is significant if $p<0.05$

There was a difference in liver histopathological scores among the negative control with the treated group using 2000 mg/kg BW of extract ($p=0.002$), the negative control group with the paracetamol-induced group ($p=0.0005$), and the treated group using 500 mg/ kg BW of the extract with a paracetamol-induced group ($p=0.007$).

Table 2: The significance of differences among treatment groups in liver histopathological scores with Post hoc Dunn's test.

Group	Negative Control	Group 1	Group 2	Group 3	Paracetamol Induced
Negative Control	-	1.000	0.189	0.002*	0.0005*
Group 1	1.000	-	1.000	0.120	0.007*
Group 2	1.000	1.000	-	1.000	0.246
Group 3	0.002*	0.120	1.000	-	1.000
Paracetamol Induced	0.0005*	0.007*	0.246	1.000	-

*Post hoc Dunn's test is significant if p<0.05

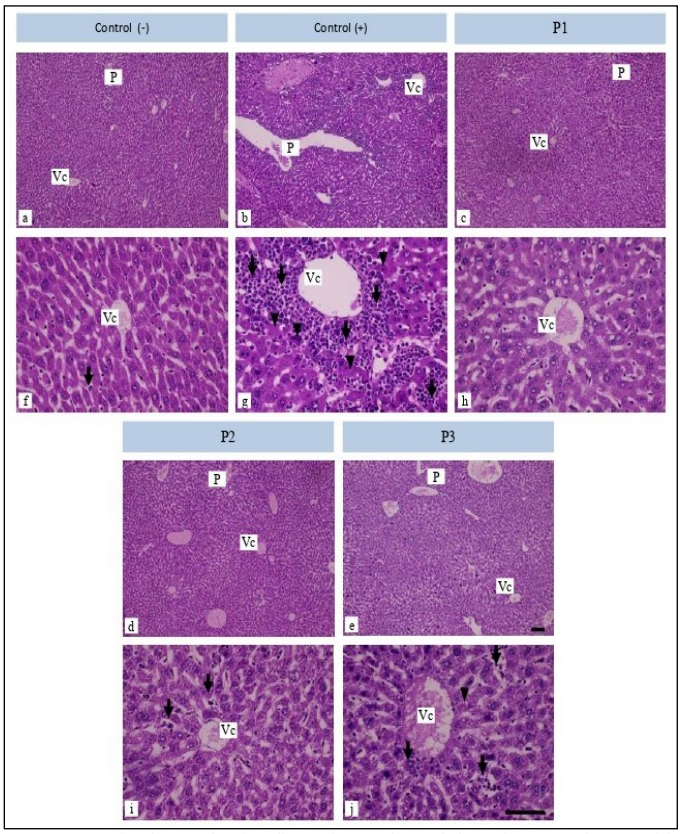

Figure 2: Histology of mice liver tissue showed hepatic parenchyma with hepatocytes arranged in lobules, central veins (Vc), and portal areas with (P). Negative control group (a.f), positive control with paracetamol (b, g), treatment of *Clitoria ternatea* L. flower extract at a dosage of 500mg/kg BW (c, h), 1000 mg/ kg BW (d, i), and 2000 mg/ kg BW (e, j). Few leucocytes in sinusoid (arrows). Liver damage, vascular dilation, severe inflammation with distributed leucocytes around vasculature, degenerated hepatocytes and necrosis (arrowhead). Hematoxylin-eosin stained. The top panel displayed a magnification of 100x, and the bottom panel 400x. The e, j scale;100µm.

Relationship of Dosage to Kidney Histopathological Picture

The higher the dose, the greater the histological score of the kidney. Scoring system for kidney damage based on histopathological examination was measured by the degree of glomerular sclerosis, interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, and arteriosclerosis of medium-sized arteries. Specific percentages are as follows:

0: Normal; 1: 1-24%; 2: 25-50% and 3: >50%

We concluded that there was a significant difference in renal histopathological scores based on Niizuma *et al* renal damage parameter according to the dose group (p<0.05).

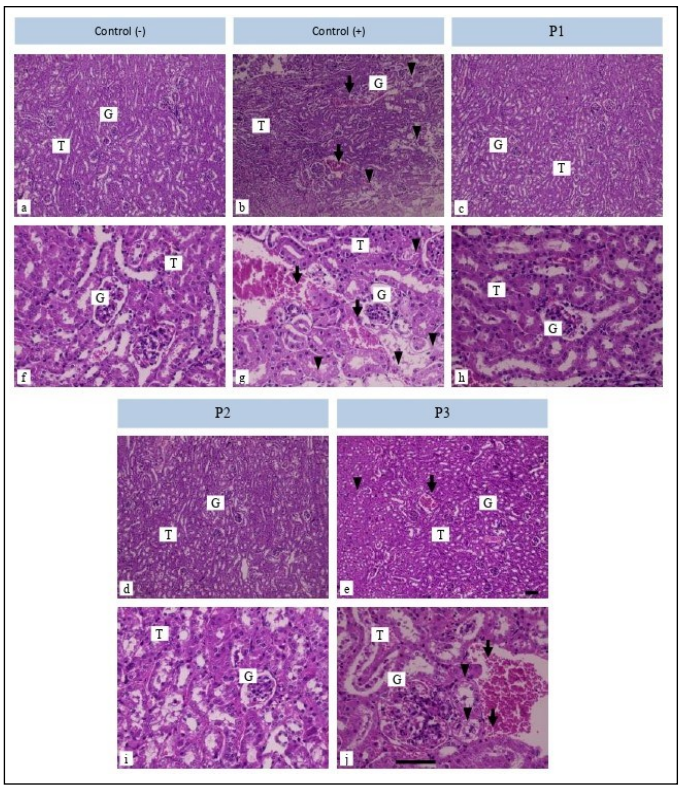

Figure 3: Kidney tissue histology of the mice showed the renal cortex with tubules (T) and glomeruli (G). Negative control group (a.f), positive control with paracetamol (b, g), treatment of *Clitoria ternatea* L. flower extract at a dosage of 500mg/kg BW (c, h), 1000 mg/ kg BW (d, i), and 2000 mg/kg BW (e, j). Kidney damage, edema, vascular dilation and bleeding (arrows). Degenerated epithelium and necrosis in tubules (arrowhead). Hematoxylin-eosin stained. The top panel displayed a magnification of 100x, and the bottom panel 400x. The e, j scale; 100µm.

Table 3: The distribution of the average score of renal histopathology according to dose.

Variable	Mean	SD	95% CI	p-value
Negative Control (Aquades)	0.11	0.00	0.11-0.15	0.005
Group 1 (Dosage of 500 mg/kg BW)	1.07	0.21	0.85-1.28	
Group 2 (Dosage of 1000 mg/kg BW)	2.30	0.41	1.86-2.73	
Group 3 (Dosage of 2000 mg/kg BW)	2.37	0.23	2.12-2.61	
Positive Control (Paracetamol Induced)	2.63	0.20	2.43-2.84	

*Kruskal Wallis test is significant if p<0.05

Based on the histopathological findings, we found that *C.ternatea* flower extract caused kidney damage in mice and a significant increase in histopathological scores at a dosage of 2000 mg/kg BW given orally. There was a difference in kidney histopathological scores among the negative control with the treated group using 2000 mg/kg BW of extract (p=0.001), the negative control group with

the paracetamol-induced group (p=0.0005), and the treated group using 500 mg/ kg BW of the extract with a paracetamol-induced group (p=0.014).

Table 4: The significance of differences in renal histopathological scores between doses with Post Hoc Dunn's test.

Group	Negative Control	Group 1	Group 2	Group 3	Paracetamol Induced
Negative Control	-	1.000	0.014*	0.010*	0.0005*
Group 1	1.000	-	0.452	0.355	0.023*
Group 2	0.014*	0.452	-	1.000	1.000
Group 3	0.010*	0.355	1.000	-	1.000
Paracetamol Induced	0.0005*	0.023*	1.000	1.000	-

* Post Hoc Dunn's test is significant if p<0.05

DISCUSSION

LD_{50} Dose of *Clitoria Ternatea* L. Flower Extract

Several studies have shown numerous pharmacological effects on *C.ternatea* flowers. However, awareness of its potential toxicity is still lacking. This study was performed to examine the acute toxicity of *C.ternatea* flower extract in mice by following OECD guidelines. Mice were used in this study since they are closer to representing the toxicity effect of substances on humans.[17] We categorised treatment groups as follows:

1. The lowest dose can induce the lowest toxic response in mice but above the potential dose (500 mg/ kg BW).
2. The intermediate dose is high enough to cause a moderate toxic effect on mice (1000 mg/kg BW).
3. The highest dose which can induce a higher toxic response in mice (2000mg/ kg BW)

According to the global classification system, toxicity levels can be grouped into five categories based on lethal dose (LD). *Clitoria ternatea* L. flower extract was included in the group with LD_{50} of more than 2000 mg/kg (group 5), which fell into the low toxicity category.[18]

Sign of Toxicity in Mice After Being Given Clitoria Ternatea L. Flower Extract

In this study, five mice presented toxicity manifestations which were lethargy and tremors, after being given *C.ternatea* extract ranging from a dose of 500-2000 mg/kg BW. There were no previous reports of death using the same extract and dose. However, this study was consistent with the acute toxicity study using Saccharum munja (*S.munja*) extracts, in that no mice were found dead. We also found changes in mice behaviors, such as increased respiration, weakness, and tremors in the treatment group within the first 24 hours. Signs of toxicity after herbal plant consumption were reported in several studies, such as diarrhea, vomiting, seizures, tremors, and death.[19] Lethargy in mice was presented as sluggish behavior, fainting, coma, hypoactivity, or bowed posture. These were clinical signs to mark any disorder or disease in mice.[20,21] Due to exposure to toxic materials, lethargy was influenced by inflammatory mediators with the inhibition of orexin. Tremors in mice exposed to toxic components are usually more frequent, severe, and unintentional and can be accompanied by seizures.[22]

Histological Score of Mice Liver Damage After Being Given *Clitoria Ternatea* L. Flower Extract

The higher the dose, the greater the histological score of the liver sample. We found differences in liver histopathological scores according to the dose group (p<0.05). Liver damage on the histopathological examination was marked with local lymphocyte aggregation, enlargement, degeneration, vacuolation, and cell necrosis. The wider the damage, the greater the histopathological score given. We found vacuolisation in the liver and kidney of treated mice at 2000 mg/ kg BW of extract. This result aligned with other toxicity studies that also observed the presence of cytoplasmic vacuolation and changes in hepatocyte fat with sinusoidal dilatation and congestion.

The liver in this group showed histological damage characterised by expanded infiltration of inflammatory cells in the pericentral region, necrotic cells, and pyknotic nuclei.[23] In acute and subacute liver injuries, swelling is usually seen with nonlipid cytoplasmic vacuolation in evenly distributed hepatocytes. This condition is related to fluctuation in the intracellular concentration of the cytochrome P450 enzyme due to exposure to toxic components such as plants.[24] Degeneration (vacuolation) is caused by glycogen accumulation.[25] Irreversible vacuolation is a cytopathological condition that causes cell death caused by the cytotoxic stimulus. It affects non-

acidic organelles, the endoplasmic reticulum (ER), and the Golgi body. Its inducers include natural and synthetic compounds in medical drugs, herbal plants, industrial pollutants bacterial-infected cells' protein toxins, and viral sheaths. In addition, irreversible vacuolation is also seen in bacterial-infected cells' protein toxins and viral sheaths.[26] Under certain conditions, irreversible vacuolisation leads to cell death.[27] The inducers of paraptosis, swelling, and vacuolation of cells recorded to date are celastrol (triterpenoid group), hesperidin (bioflavonoid group), and gypenoside (triterpenoid saponin group).[28–30] Several studies found that *Clitoria ternatea* L. flowers have triterpenoid components, flavonoids, and saponins.[31,32]

Celastrol modulates extracellular signal-regulated kinase (ERK) and Jun N-terminal kinase (JNK) routes which impede proteasomes and impair protein folds in the endoplasmic reticulum (ER). Accumulation of Ca^{2+} causes the production of reactive oxygen species (ROS) and dysfunction. This inhibits proteasomes and degrades inositol triphosphate (IP3R) and mitochondrial calcium uniporter (MCU). Ca^{2+} amplifies the celastrol effect and disrupts endoplasmic reticulum-associated degradation (ERAD) function thus creating vacuolization. This event leads to stress and cell death.[28] Gypenoside increases ROS and releases Ca^{2+} followed by the forms of cytoplasmic vacuolation that happens and leads to cell death.[30,33] It is known that curcumin shows antioxidant and ROS-producing activity.[34] When intracellular concentrations are low, curcumin acts to cleanse free radicals and protect from deoxyribonucleic acid (DNA) deterioration.[35]

Conversely, curcumin can disrupt the cell's antioxidant capacity and provide cytotoxic effects when a high concentration is reached. Activation of JNK and ERK2 causes proteasome inhibition, ER stress, and vacuolization.[36] Several studies found that *C.ternatea* flowers have flavonoid components. Flavonoids have benefits as an anti-inflammatory, antiallergic, and antioxidant, as well as preventing cardiac disease and cancer.[37] The mechanism by which flavonoids in *C.ternatea* flowers cause cell vacuolisation is not yet known. *C.ternatea* flowers have a saponin component where saponins are associated with the cell apoptosis-induced mechanism and ER vacuolisation through increased ROS and activation of caspase-3 and caspase-9.[38] Saponins could activate apoptosis in hepatoma G2 (HepG2) cells, whose bioactivity is mainly by binding to receptors on the cell surface to increase the number of ROS. Increased ROS can damage the mitochondrial membranes and cause 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to open, releasing Ca^{2+} and cytochrome complex (Cyt-C). An increase in cytoplasmic Ca^{2+} can further encourage the opening of MPTP so that matrix metalloproteinase (MMP) levels decrease and induce apoptotic factors.[39]

Histological Score of Kidney Damage Degree of Mice

There was a difference in renal histopathological scores according to the dose group ($p<0.05$). The kidneys are responsible for toxins and large amounts of free radicals secretion that can form oxidative stress to cause kidney damage. In toxicity studies, the kidneys are one of the organs used as the toxicity indicator. Disorders in the kidneys can be caused by metabolite-induced cellular damage from toxic and discretionary materials the kidneys. The degree of kidney damage depends on the number of erythrocytes present in the glomerulus and the diameter of the tubules on histopathological examination.[16,40]

Plants with high saponin concentrations can cause changes in the kidney structure. Administration of saponins at a dosage of 200 mg/kg BW can increase the damage to the renal tubules. The renal portion of mice fed with lipopolysaccharide (LPS) and cannabis displayed pronounced degeneration of the tubular, dilatation, glomerular atrophy, degeneration or hypercellularity, and capillary congestion. In the tubule structure, clear vacuolisation and dilation were observed.[40,41]

CONCLUSIONS

C.ternatea flowers are safe to consume at a dosage of 500 and 1000 mg/kg BW since no mice died after being treated for 14 days. There were no histopathological changes and insignificant differences among these two doses. However, liver and kidney damage began appearing at a 2000 mg/kg BW dosage in histopathological examination. There were significant differences in liver and kidney histopathological scores among treated groups. Hence, its consumption at this dosage should be limited.

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