Journal of Tropical Biodiversity and Biotechnology
Volume 07, Issue 01 (2022): jtbb69389
DOI: 10.22146/jtbb.69389

Research Article

Single-dose Acute Oral Toxicity Study of Chloroform Extract of Snake Plant (*Sansevieria trifasciata* Prain.) Leaf in Wistar Rats (*Rattus norvegicus* Berkenhout, 1769)

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Keywords:
acute oral toxicity
chloroform extract
medicinal plants
OECD 420
Sansevieria trifasciata
Submitted:
26 September 2021
Accepted:
09 January 2022
Published:
23 March 2022
Editor:
Ardaning Nuriliani

ABSTRACT

Sansevieria trifasciata is one of popular ornamental plants which also believed possessing therapeutic effects due to their phytochemical constituents. Secondary metabolites of plants can be toxic to other organisms; therefore, toxicity studies must be carried out to investigate adverse effects prior to further exploration as potent candidates of medicinal plants. This research aimed to evaluate toxicity and safety of consuming chloroform extract of *S. trifasciata* leaf (CESTL) in acute phase using female Wistar rats as model animal. Procedure referred to OECD Guidelines for the Testing of Chemicals, Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure with single-dose administration of 2000 mg/kg bw. Results demonstrated that during 14 days of the experiment, neither mortality and sublethal effects as signs of toxicity were detected. There were no significant differences during the experiment between treatment groups and control in body weight, core temperature, individual and social behavior, food and water intake, as well as hematological profile, clinical biochemistry parameters, and relative organ weight (visceral organs indices). Almost all values were maintained within normal range (baseline) with fluctuation as normal physiological dynamics appeared relatively similar in all groups. Therefore, it can be concluded that no-observed-adverse-effect-level (NOAEL) for single-dose oral administration of CESTL with the dose 2000 mg/kg bw and can be classified in the hazard of Category 5 based on Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Based on this finding, we will continue to conduct further study to assess the repeated-dose acute oral toxicity.

INTRODUCTION

Secondary metabolites (SMs) are by-products of plants which are not needed in their life processes. These compounds rather play a role in plant defense mechanisms against other organisms and dealing with stress from their surrounding environment. Many SMs can be used for medicinal purposes and health supplements for human and animals. The bioactive ingredients were isolated by extraction using various solvents, then the potential therapeutic properties are assessed through pharmacological research (Pagare et al. 2015).

Sansevieria trifasciata is one of popular ornamental plants as well as bio-remediation agent. A little is known about its efficacy as medicine and health nourishment. So far, the practice of using *S. trifasciata* for medication is only empirical, based on folk medicine and ethnobotany studies. The use of *S. trifasciata* as traditional medicine is due to its phytochemical constituents which are believed able to cure various diseases as well as to maintain body health (Dewatisari et al. 2021). Aqueous extract of *S. trifasciata* leaves has anti-diabetic effect (Qomariyah et al. 2012), ethanolic extract of *S. trifasciata* leaves possess antiallergic and antianaphylactic properties (Andhare et al. 2012). Chloroform extract of *S. trifasciata* leaves (CESTL) is potential to be developed as pharmaceutical agent due to the high content of triterpenoids, steroids, phenols, flavonoids, and alkaloids (Dewatisari 2020). However, there is still no scientific evidence provided.

As SMs are potential to be toxic to other organisms, while inappropriate dose of medicinal substances may lead to various adverse effects; therefore, series of toxicity and safety studies need to be conducted. *In vivo* experiment or preclinical trial is one of three methods commonly used for testing compounds that is essential in drug development process. The result will provide information for further test, the clinical phase trial (Derelanko & Hollinger 2002; Parasuraman 2011).

One common parameter on toxicity test is the lethal dose (LD50). So far, we cannot find any publication on the toxicity studies of CESTL. Therefore, we did not get any information regarding its LD50. However, we collected some data of LD50 of acute oral administration of *S. trifasciata* but with different solvents: LD50 of the ethanolic extract of *S. trifasciata* in Wistar rats at the dose 18000 mg/kg bw (Ighodaro et al. 2017) and LD50 of the methanolic extract of *S. trifasciata* in Wistar rats at the dose 500 mg/kg bw (Dey et al. 2014). Anbu et al. (2009) conducted acute oral toxicity test of *S. trifasciata* in Swiss mice which LD50 of the ethanolic extract at the dose 1513.5 ± 21.5 mg/kg bw, whereas the aqueous extract at the dose 1426 ± 43.6 mg/kg bw.

Toxicity test should not be limited to determining the LD50 value, as is the case with conventional procedure. As concern for animal welfare and ethics, the classical LD50 test protocol has been revised and evaluated to use fewer animals, to reduce the level of suffering, and to adopt internationally accepted methods (United Nations 2011). One of recommended methodology on toxicity tests is developed by The Organization for Economic Co-operation and Development (OECD), of which Indonesia is one of the key partners. Toxicity studies consist of acute, subchronic, and chronic periods. The acute oral toxicity tests consisted of two parts: First, the single-dose, which the substance is administered only once and the observation takes 14 days since the administration (OECD 2002). Second, the repeated-dose, which the substance is administered daily during 28 days. The dose used in the repeated-dose test is considered from the result from single-dose test (OECD 2008).

Based on the dose, Globally Harmonized System of Classification and Labelling of Chemicals (GHS) defines the level of acute oral toxicity into five categories, from Category 1 (the highest hazard) to Category 5 (the lowest hazard) as follows: 5, 50, 300, 2000, and 5000 mg/kg bw. To protect animal welfare, testing at a dose of 5000 mg/kg bw is discouraged and should not be carried out unless there is a strong reason that has a direct relevance to health and can be justified (United Nations 2011). This research aimed to study single-dose acute oral toxicity and safety levels of CESTL which follows OECD Test Guideline No 420: Acute Oral Toxicity - Fixed Dose Procedure with the dose 2000 mg/kg bw using female Wistar rats as model animal.

MATERIALS AND METHODS

Ethical Clearance

All procedures in this study which related to the care and use of animal for experimental model in preclinical trial have complied with animal welfare principles and did not violate the animal ethics. This is supported by the approval and issuance of Ethical Clearance by the Research Ethics Commission of Faculty of Veterinary Medicine, Universitas Gadjah Mada with the Number: 00034/EC-FKH/Eks./2021 dated on April 12th, 2021.

Plant Material and Extraction Method

Species identification has been carried out and the result refers to *Sansevieria trifasciata* Prain. which has been approved with the issuance of Certificate of Identification by the Head of Laboratory of Plant Systematics, Faculty of Biology UGM No. 014526/S.Tb./II/2019 dated on February 25, 2019. We also grew the plant for collection (Figure 1).

Figure 1. Morphology of *Sansevieria trifasciata* Prain. we used in this study.

S. trifasciata leaves were collected by Mrs. Whika Febria Dewatisari, S.Si., M.Sc., a doctoral student of Faculty of Biology, UGM. The preparation of CESTL based on graded maceration method as follows: leaves were rinsed, finely chopped, and dried in the oven (50 ℃). The dried material *(simplicia)* then were ground into powder and soaked in chloroform with a ratio of powder : chloroform = 1 : 3 (w/v) for three days, shaking regularly to optimize the extraction. The solution was filtered and evaporated using an electric fan until completely dried. Stock of CESTL was stored in air-tight glass containers or wrapped with aluminum foil in the refrigerator (4 ℃), and taken as needed to be processed as a working solution (Dewatisari et al. 2021).

Experimental Animals

Sample animals were nine female nulliparous Wistar rats *(Rattus norvegicus* Berkenhout, 1769) aged eight weeks old with body weight range at 135-173 (157.25±11.11) grams. Rodents, especially rats, has been used extensively in descriptive toxicity studies and drug safety tests as their systems represent human physiology (Greaves 2012).

Experiment took place at Animal House, the animal facility of Faculty of Biology UGM, the same place where they were originated. The procedure of animal care and maintaining followed standard procedures for rearing laboratory rats (NRC 2011). Rats were housed in communal cage designed for laboratory rats, made of transparent polypropylene with the size 38 x 25 x 23.5 cm^3, equipped with metal wire mesh for the lid, wood shaving for bedding, feeder, and rodent drinking bottle (Figure 2).

Figure 2. Housing for female Wistar rats as experimental animals in single-dose acute oral toxicity study of CESTL

Environmental parameters are as follows: room temperature 27-29 °C, relative humidity 68-81%, standard photoperiod with artificial lighting 12 hours light : 12 hours dark. Cages were cleaned up twice a week with detergent and disinfectant.

Rats were fed with standard chow diet (Ratbio®, P.T. Citra Ina Feedmill, Jakarta) and mineral water (P.T. Berkah Tirta Jaya, Yogyakarta). Initial feed weight and water volume were determined for calculating daily food intake and water consumption.

Study Design

Rats were assigned into three groups: the first group received CESTL, the second group received 5% Tween80 (v/v) as chloroform extract emulsifier (TWEEN), and the third group received distilled water as control-placebo (CTRL). The procedure of experiment referred to OECD Test Guideline No. 420 (OECD 2002) with a dose of 2000 mg/kg bw (Sighting study). CESTL, Tween80, and distilled water were administered orally 1 mL/individual once at the beginning of the experiment (Day 0).

Parameters

Quantitative parameters consisted of body weight, rectal temperature, food intake, water consumption, hematological profile based on complete blood count (CBC), liver function tests based on alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) activities, renal function tests based on creatinine (CRE) and blood urea nitrogen (BUN) levels, as well as levels of fasting blood glucose, total cholesterol, and triglycerides. All parameters were observed on Day 0, 7, and 14.

Qualitative parameters as signs of toxicity consisted of mortality, sublethal effects, and clinical manifestations that lead to illness, including: morphological or physical examination, individual and social behaviors and activities, as well as stool conditions. These parameters were monitored soon after administration, intensively for four hours post-administration, and continued every day until Day 14.

Anesthesia and Euthanasia

Before performing blood collection, rats were anesthetized by intramuscular injection of Ketamine (Kepro®, Holland) and Xylazine (Interchemie®, Holland) cocktail 0.1 mL/100 g bw (K=50 mg/kg bw, X=5 mg/kg bw). On the last day of the experiment (Day 14), rats were sacrificed by similar anesthesia procedure followed with phlebotomy for exsanguination (sera were preserved for further analysis) and perfusion using physiological saline (0.9 % NaCl w/v, Otsuka®, Indonesia).

Collection of Visceral Organs and Calculation of Relative Organ Weights

Soon after rats being euthanized, necropsy was performed, visceral organs consisted of liver, kidney, spleen, lungs, heart, brain, and internal genital organs (ovaries and uterus) were removed, rinsed in saline, and weighed. Relative organ weight was calculated by dividing the absolute organ weight by

final body weight, then multiplied by 100 (Cattley & Cullen 2013). The formula is as follows:

$$Relative\ organ\ weight = \frac{Absolute\ organ\ weight}{Final\ body\ weight} \times 100$$

Organs were preserved in 10% neutral buffered formalin (NBF) fixative. Organs were processed for histopathological observation when the results of their functions tests indicated significant functional impairment.

Blood Analysis

Blood samples for hematological and clinical biochemical profiles were withdrawn from orbital sinus. As much as 1 mL blood were collected in 1.5 mL ethylene-diamine-tetra-acetic acid (EDTA)-coated microtube as anticoagulant. Hematological profiles were analyzed using Sysmex®XP-100. Glucose and total cholesterol levels were measured directly using EasyTouch® rapid test strips.

Plasma for measurement of ALT, AST, creatinine, BUN, and triglycerides were separated from whole blood using centrifuge (Eppendorf®5418R) and then analyzed using Microlab®300.

Data Analysis

Qualitative data were shown as table to compare each group. Quantitative data was tabulated in Microsoft®Excel® v.2019 and statistical analysis were performed using IBM®SPSS® v.23. based on Repeated-measures ANOVA Test to compare means over time from related group ($a = 0.05$). If significant difference is detected ($p < 0.05$), the test is continued with Bonferroni Post Hoc Test to discover which specific means differed. One-way ANOVA Test was employed to compare means between groups ($a = 0.05$). If significant difference is detected ($p < 0.05$), the test is continued with Tukey's Honestly Significant Difference (HSD) Post Hoc Test to discover which specific means differed. Reference interval for normal values were constructed based on the lowest and highest values of each variable from the animal population in this study at Day 0 or "The Baseline" (Poitout-Belissent & McCartney 2010). Results are displayed in comparison table as descriptive statistic values (mean ± standard deviation).

RESULTS AND DISCUSSION

There is a new approach in the methodology for assessing acute toxicity study, in which it should not only focus on determining LD_{50} or LC_{50} since not all chemicals cause death of animals. Animals may be still alive or survive during the experiment but suffering due to physiological disturbances (sublethal effect). Therefore, signs of toxicity in animals must be clearly observed (OECD 2002).

Signs of Toxicity

We used transparent rats cage thus we can see their natural behavior to monitor signs of toxicity. The old-style rat cages with opaque walls did not allow researcher to observe animals from the sides. Watching from the top of the cage will only interfere their activities and is stressful to the animals (ARRP 2008). In addition, the wall of the cage should be high enough to enable rats standing upright (rearing and stretching) as shown in Figure 2, because this is one of their normal behaviors and a sign of healthy rat (ARRP 2008; NC3RS 2017).

During 14-day experiment, no death or ill rats were found. Observations on general morphological and physical condition of animals, as well as individual and social behavior and their activities showed similar results as control (Table 1). It means that neither CESTL nor Tween80 which used as emulsifier of chloroform extract in aqueous media generated toxic effects that harmed the health.

The health status and psychological conditions (distress) of laboratory rats can be observed based on their morphology, behavior, and physiology. Unhealthy or stressed rats are passive, reduced activity, decreased appetite and drinking, licked their body frequently, stiffed body and guarded limbs, self-mutilate, aggressive, a lot of vocalizations as response to handling, reluctant to interact with their conspecific. Rats with health problem have messy appearance as they do not do grooming properly, fur are coarse and stiff or piloerection, hunched posture, red eyes discharge like bloody tears due to porphyrin secretion, partially closed eyelids, dilated pupils, nasal discharge or

Table 1. Signs of toxicity in female Wistar rats as experimental animals in single-dose acute oral toxicity study of CESTL.

PARAMETER	CESTL (n=3)	TWEEN (n=3)	CTRL (n=3)
Mortality	0	0	0
Body weight (g)	Increased by 34.67±5.37[a] R^2= 0.999	Increased by 25.00±4.01[a] R^2= 0.990	Increased by 18.33±7.13[a] R^2= 0.985
Rectal temperature (°C)	Fluctuated, mean= 33.98±0.82[a]	Fluctuated, mean= 34.24±0.75[a]	Fluctuated, mean= 33.73±0.51[a]
General morphological/ physical condition	Normal/healthy, face, body, and tail are clean	Normal/healthy, face, body, and tail are clean	Normal/healthy, face, body, and tail are clean
Individual behavior and activities	Active, normal	Active, normal	Active, normal
Social behavior and activities	Active, normal, positive interaction	Active, normal, positive interaction	Active, normal, positive interaction
Food intake (g/ individual/day)	Fluctuated, mean= 16.68±3.59[a]	Fluctuated, mean= 15.68±3.89[a]	Fluctuated, mean= 14.05±3.41[a]
Water consumption (mL/ individual/day)	Fluctuated, mean= 25.03±3.69[a]	Fluctuated, mean= 24.43±3.19[a]	Fluctuated, mean= 22.33±3.85[a]
Stool condition	Structure, color, and smell are normal, no diarrhea detected	Structure, color, and smell are normal, no diarrhea detected	Structure, color, and smell are normal, no diarrhea detected

Abbreviations: CESTL= chloroform extract of *Sansevieria trifasciata* leaf, TWEEN= 50 % Tween80, CTRL= control/ placebo/distilled water.

Values are expressed as the mean±SD. Values ended with same superscript letters in a row indicate no significant difference between groups based on one-way ANOVA test ($p > 0.05$).

runny nose, and abnormal resting position. Physiologically, ill rats have sleep disruption, they sleep most of the time or easily awakening, hypothermia, rapid and shallow breathing, abnormal heartbeat, expiration with grunting sound (Carstens & Moberg 2000; Wang et al. 2019). We did not find these signs in all animals we used in this experiment (Table 1).

Ekeanyanwu and Njoku (2014) used dimethyl sulfoxide (DMSO) as emulsifier as well as vehicle for oral administration of chloroform extract in rats. Unfortunately, we failed to dissolve CESTL in DMSO; therefore, we used Tween80 as alternative agent to emulsify chloroform extract which is nonpolar or hydrophobic, so that it can be dissolved in aqueous medium thoroughly (Parker et al. 2021). Tween80 is polysorbates, one of the food additives commonly used as emulsifier. Single-dose acute oral toxicity test resulted in very low toxicity level: dose 22 g/kg bw did not exhibit toxicity symptoms in rats (NOAEL). Acceptable daily intake of Tween80 is 0-25 mg/kg bw (FSCJ 2007).

Santos-Lopez et al. (2010) used 1% Tween80 to dissolve chloroform extract of aerial parts of *Phytolacca icosandra*, Christian et al. (2014) used 3% Tween80 to dissolve *Persea americana* leaf extract, Zakaria et al. (2015) used 8% Tween80 to dissolve chloroform extracts of *Muntingia calabura* and *Melastoma malabathricum* leaves. We used 5% Tween80 because with that concentration CESTL was completely dissolved in distilled water.

Oral administration of 5-10% Tween80 can cause diarrhea in female rats (FSCJ 2007). However, according to The Joint FAO/WHO Expert Committee on Food Additives (JECFA), oral administration of 5% Tween80 (equivalent to 2500 mg/kg bw) is considered safe (NOAEL). In this experiment, rats administered with Tween80 or CESTL did not experience diarrhea as indicated by normal stool condition (Table 1). Diarrhea is physiological response against toxic substances which enter the body via digestive tract, therefore it is one of basic parameters in acute oral toxicity tests (OECD 2002; Wang et al. 2019).

In addition to cause gastrointestinal disorders, toxic compounds can reduce or lead to loss of appetite, which can be observed by calculating daily food intake and water consumption. A decrease in food consumption followed by reduction of water consumption result in weight loss and suppress the immunity. This is a secondary effect of toxic substance on physiological condition (Morita et al. 2017). Results pointed out that oral administration of CESTL did not cause diarrhea and digestive problems, in fact, there was an increase in food intake, water consumption, and body weight compared to control (Table 1). Further toxicity studies are needed to investigate the effects of CESTL on the digestive system over a longer period of time, as well as its potential to stimulate appetite, improve digestion, and promote weight gain for underweight individuals.

Besides having impacts on the digestive physiology and growth, toxic substances may also suppress the immune system which is characterized by alteration in leukocyte count and susceptibility to disease, increased risk of

anemia, liver and kidney dysfunctions, impaired normal energy metabolism, and even mental disorders, such as stress and depression (Morita et al. 2017; Wang et al. 2019). Hematological analysis, measurement of relative organ weights, evaluation of liver and renal functions, as well as examination of metabolic profiles consisted of blood glucose, total cholesterol, and triglycerides levels can provide a comprehensive data on the toxicity and safety studies of potential therapeutic agents on physiological conditions of experimental animals in preclinical research, which will later be translated to humans dose in clinical trial phase (Etame et al. 2017; Sutrisni et al. 2019; Sukandar & Sheba 2019).

Effect of CESTL on Hematological Profile

Evaluation of or erythrocyte profile (Table 2) demonstrated that values of red blood cell count (RBC), hematocrit (HCT), and hemoglobin (HGB) in all groups fell below the baseline on Day 7 but gradually increased on Day 14 (significant in group received Tween80). Based on this, it is vital to investigate further to see the indication of anemia or the values will recover to normal (baseline range). This condition, however, is not due to CESTL toxicity since it occurred in all groups.

The decrease of RBC, HCT, and HGB resulted in elevation of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Although this is a natural regulation of the body to anticipate anemia, but it must be considered because the alteration of erythrocyte indices may change the size of the cell. It is interesting because this alteration is not significant in group received CESTL, which means the extract does not disrupt the structure and function of the erythrocyte.

We used 5% Tween80 as emulsifier to dissolve in distilled water. According to Mantskava et al. (2018), Tween80 can alter erythrocyte profile but only temporarily; therefore the use of Tween80 as emulsifier is relatively safe. The decline of MCH and MCHC correlate with hypochromia which means the anemia is caused by less hemoglobin concentration. In opposite, the elevation of MCH and MCHC do not refer to hyperchromia. The increase can be caused by erythrocyte morphology or spherocytic (Vilchez 2020).

The value of MCV in groups received Tween80 and control exceeded the baseline but it was not significant. Significant increment indicates macrocytic anemia, liver disease, and vitamin B12 deficiency (Maner & Moosavi 2021). Alteration of erythrocyte profile in control may occur with age and natural processes. Observing that erythrocyte profile in group received CESTL is more stable than the other groups, we hypothesize that this substance can be used as supplement to improve the health. Based on this finding, we are preparing to continue exploring the therapeutic effect of CESTL with lowering the concentration of Tween80 to anticipate its adverse effect on erythrocyte profile.

Table 2. Erythrocyte profile in female Wistar rats as experimental animals in single-dose acute oral toxicity study of CESTL.

VARIABLE	DAY	GROUPS			BASELINE
		CESTL	TWEEN	CTRL	
RBC (´10⁶/mL)	0	7.88±0.38[a]	7.75±0.16[a]	8.49±0.35[a]	7.38 – 8.95
	7	7.27±0.16[a]	6.64±0.11[b]	7.41±0.18[a]	
	14	7.21±0.09[a]	6.71±0.25[ab]	7.14±0.29[a]	
HCT (%)	0	42.77±2.75[a]	42.37±0.87[a]	46.67±2.30[a]	39.20 – 49.90
	7	41.10±0.60[a]	37.10±0.40[b]	42.00±0.86[a]	
	14	40.07±1.10[a]	37.57±1.27[ab]	40.87±1.42[a]	
HGB (g/dL)	0	14.80±0.43[a]	14.23±0.34[a]	15.50±0.93[a]	13.90 – 16.80
	7	14.55±0.45[a]	13.45±0.15[a]	14.37±0.05[a]	
	14	14.13±0.25[a]	13.80±0.28[a]	14.23±0.39[a]	
MCV (fL)	0	54.20±0.90[a]	54.67±0.42[a]	55.00±0.75[a]	53.10 – 55.80
	7	55.23±1.96[a]	54.80±1.57[a]	56.70±0.51[a]	
	14	55.57±0.97[a]	55.97±0.41[a]	57.30±1.10[a]	
MCH (pg)	0	18.83±1.26[a]	18.40±0.08[a]	18.70±0.56[a]	17.50 – 20.60
	7	20.00±0.20[a]	20.25±0.12[b]	19.40±0.43[b]	
	14	19.60±0.45[a]	20.30±0.51[ab]	19.93±0.29[ab]	
MCHC (g/dL)	0	34.80±2.83[a]	33.57±0.19[a]	33.23±0.59[a]	32.40 – 38.80
	7	35.40±0.60[a]	36.25±0.05[b]	34.23±0.61[a]	
	14	35.27±0.53[a]	36.23±0.66[ab]	34.83±0.52[a]	
RDW-SD (fL)	0	28.63±0.90[a]	27.33±0.39[a]	27.23±0.61[a]	26.80 – 29.80
	7	29.57±1.19[a]	28.13±0.50[a]	28.63±0.82[a]	
	14	28.60±0.70[a]	28.80±0.29[a]	28.83±0.82[a]	

Abbreviations: CESTL= chloroform extract of *Sansevieria trifasciata* leaf, TWEEN= 50 % Tween80, CTRL= control/ placebo/distilled water, RBC= red blood cell count, HCT= hematocrit, HGB= hemoglobin, MCV= mean corpuscular volume, MCH= mean corpuscular hemoglobin, MCHC= mean corpuscular hemoglobin concentration, RDW-SD= red blood cell distribution width- standard deviation.

Values are expressed as the mean±SD. Values ended with same superscript letters in a column for each variable indicate no significant difference between days based on repeated-measures ANOVA test ($p>0.05$).

Values of leukocyte profile were fluctuating in all groups, however most of them were within baseline. Monocytes, eosinophils, and basophils were not shown as they were not detected by our machine, possibly because their numbers were very low (Table 3).

The total number of leukocytes (WBC) in group received Tween80 exceeded baseline due to the significant increase of neutrophil count. According to Thamir et al. (2013), Tween80 can increase and activate neutrophils, in the other hand, it decreases lymphocyte count. The number of neutrophils in group received CESTL fell down the baseline on Day 7 but returned to baseline on Day 14 (not significant). This result is similar to the work by Ayalogu et al. (2011) using aqueous extract of *S. senegambica*.

Elevation of neutrophil count resulted in significant increase of neutrophil lymphocyte ratio (N/L); however, the value was still within baseline. N/L is a biomarker that describes two aspects of immune system, acute and chronic inflammation (neutrophil count) and adaptive immunity (lymphocyte count). It is also one of predictive factors to identify the presence of critical illness (Liu et al. 2020; Song et al. 2021). As N/L value in group received CESTL was relatively stable during the experiment; therefore, we conclude that the extract is safe to consume as it did not trigger immune response.

Table 3. Leukocyte profile in female Wistar rats as experimental animals in single-dose acute oral toxicity study of CESTL.

VARIABLE	DAY	GROUPS			BASELINE
		CESTL	TWEEN	CTRL	
WBC (´10³/mL)	0	10.20±2.78[a]	11.20±1.00[a]	9.73±0.41[a]	6.90 – 13.70
	7	10.70±1.39[a]	14.75±0.12[a]	10.13±0.45[a]	
	14	11.10±1.84[a]	15.37±3.10[a]	12.07±0.99[a]	
NEU (´10³/mL)	0	1.70±0.08[a]	2.40±0.36[a]	2.97±0.05[a]	1.60 – 3.00
	7	1.40±0.75[a]	2.37±1.36[a]	2.73±0.26[a]	
	14	2.03±0.62[a]	4.60±0.80[b]	2.90±1.02[a]	
LYM (´10³/mL)	0	8.50±2.82[a]	8.80 ±1.14[a]	6.77±0.37[a]	5.10 – 12.00
	7	8.80±1.14[a]	11.45±0.45[a]	7.40±0.28[a]	
	14	9.07±1.23[a]	10.77±2.36[a]	9.17±0.59[a]	
NEU (%)	0	17.93±5.61[a]	21.93±4.16[a]	30.17±0.85[a]	12.10 – 31.00
	7	18.60±1.28[a]	27.33±7.34[a]	27.00±1.85[a]	
	14	17.73±2.79[a]	30.10±1.84[a]	23.57±6.74[a]	
LYM (%)	0	82.07±5.61[a]	78.07±4.16[a]	69.83±0.85[a]	69.00 – 87.90
	7	81.40±1.28[a]	72.67±7.34[a]	73.00±1.85[a]	
	14	82.27±2.79[a]	69.90±1.84[a]	76.43±6.74[a]	
N/L	0	0.15±0.01[a]	0.21±0.01[a]	0.45±0.00[a]	0.14 – 0.45
	7	0.15±0.00[a]	0.23±0.02[ab]	0.43±0.02[a]	
	14	0.15±0.00[a]	0.24±0.00[b]	0.41±0.04[a]	

Abbreviations: CESTL= chloroform extract of *Sansevieria trifasciata* leaf, TWEEN= 50 % Tween80, CTRL= control/ placebo/distilled water, WBC= white blood cell count, NEU= neutrophil, LYM= lymphocyte, N/L= neutrophil lymphocyte ratio.

Values are expressed as the mean±SD. Values ended with same superscript letters in a column for each variable indicate no significant difference between days based on repeated-measures ANOVA test ($p>0.05$).

Platelet count (PLT) is strongly influenced by technical factors particularly in collecting blood and waiting time at which blood samples are handled. Problems during blood collection resulted in platelet aggregation and blood clotting, so that platelet count decreased and, consequently, this affected on other variables (Tien 1995). Results showed that most of the values of thrombocyte profile were within the baseline with fluctuations as normal physiological dynamics (Table 4). Some values exceed the baseline but are not significant. Mean platelet volume (MPV) and platelet distribution width (PDW) values at group received CESTL declined significantly but returned to the baseline. This result indicated that CESTL did no harm on the normal hemostatic process.

Effect of CESTL on Clinical Biochemistry Profile

Evaluation of Liver and Renal Functions

ALT and AST are main parameters to evaluate liver functions. Hepatocyte injury causes the leak of those enzymes from cells into blood circulation, so that the elevation of both enzymes are detected in plasma or serum (Debelo et al. 2016). Results showed that ALT and AST activities fluctuated in all groups, some values were maintained in the baseline, whereas some others exceed the baseline but not significant (Table 5).

This result brought to conclusion that CESTL is relatively safe, did not induce hepatotoxicity. Extract of *S. senegambica* also did not show hepatotoxi-

Table 4. Thrombocyte profile in female Wistar rats as experimental animals in single-dose acute oral toxicity study of CESTL.

VARIABLE	DAY	GROUPS			BASELINE
		CESTL	TWEEN	CTRL	
PLT (´10³/mL)	0	1052.67±134.70[a]	870.67±447.88[a]	1092.67±66.55[a]	239 – 1227
	7	869.67±470.71[a]	1247.00±17.96[a]	917.67±573.76[a]	
	14	1234.67±95.83[a]	819.00±497.39[a]	1213.00±86.46[a]	
PCT (%)	0	0.70±0.05[a]	0.56±0.28[a]	0.71±0.05[a]	0.16 – 0.79
	7	0.79±0.05[a]	0.79±0.02[a]	0.79±0.08[a]	
	14	0.81±0.05[a]	0.75±0.04[a]	0.77±0.07[a]	
MPV (fL)	0	6.70±0.37[a]	6.53±0.19[a]	6.50±0.14[a]	6.30 – 7.20
	7	6.43±0.34[b]	6.23±0.09[a]	6.37±0.05[a]	
	14	6.53±0.26[ab]	6.40±0.14[a]	6.37±0.12[a]	
PDW (fL)	0	7.60±0.57[a]	7.47±0.31[a]	7.17±0.17[a]	7.00 – 8.40
	7	7.17±0.59[b]	6.63±0.39[a]	6.93±0.25[a]	
	14	7.47±0.39[ab]	7.03±0.05[a]	6.90±0.22[a]	
P-LCR (%)	0	4.87±1.59[a]	4.53±0.90[a]	4.20±1.02[a]	3.20 – 7.00
	7	4.97±1.39[a]	4.20±0.73[a]	3.90±0.37[a]	
	14	4.43±1.18[a]	4.07±0.95[a]	4.00±0.42[a]	

Abbreviations: CESTL= chloroform extract of *Sansevieria trifasciata* leaf, TWEEN= 50 % Tween80, CTRL= control/ placebo/distilled water, PLT= platelet count, PCT= plateletcrit, MPV= mean platelet volume, PDW= platelet distribution width, P-LCR= platelet large cell ratio.

Values are expressed as the mean±SD. Values ended with same superscript letters in a column for each variable indicate no significant difference between days based on repeated-measures ANOVA test ($p>0.05$).

city (Ayalogu et al. 2011). *S. liberica* even has hepatoprotective activity (Ikewuchi 2012a). Hepatoprotective activity of chloroform extract is due to the flavonoids as powerful antioxidant (Khan et al. 2012). Phytochemical analysis exhibited that CESTL is rich of flavonoids (Dewatisari 2020); therefore, it is potential to be developed as new candidate of hepatoprotective agent.

The high values of ALT and AST in group received Tween80 possibly may because this substance can impair liver function through hemolysis and cholestasis (Ellis et al. 1996). However, this effect of Tween80 did not appear in group that administered with CESTL. We assume that CESTL may nourish liver structure and functions, thereby eliminate the adverse effect of Tween80. We will follow up this finding by reducing the concentration of Tween80 in the next toxicity study to anticipate the bias caused by this substance.

Creatinine (CRE) and BUN are the main parameters for evaluating renal function. These metabolic wastes are consistently excreted through the kidneys, dissolved in urine. Therefore, the increased levels of them in plasma or serum can be used as indicator of impaired renal functions (Fitria et al. 2019). Results showed that CRE and BUN levels in all groups fluctuated. Some values were outside of the baseline and significant (Table 6).

Increasing values of both compounds did not necessarily denote impaired renal function if the levels were within reference interval (baseline) or the alterations were not significant (Mulyati et al. 2019). This indicated that CESTL did not induce nephrotoxicity and safe for kidney health. Extract of

Table 5. Evaluation of liver functions in female Wistar rats as experimental animals in single-dose acute oral toxicity study of CESTL.

VARIABLE	DAY	GROUPS			BASELINE
		CESTL	TWEEN	CTRL	
ALT (mg/dL)	0	52.93±10.49^{ab}	54.90±13.98^{a}	39.27±3.23^{a}	35.40 – 74.50
	7	92.80±0.00^{a}	83.05±9.68^{a}	78.43±6.78^{b}	
	14	47.87±2.39^{b}	90.23±43.86^{a}	55.90±4.38^{ab}	
AST (mg/dL)	0	57.83±32.96^{a}	90.73±12.80^{a}	75.37±11.90^{a}	12.60 – 108.40
	7	89.00±8.16^{a}	50.50±14.29^{a}	77.33±9.39^{a}	
	14	91.03±11.80^{a}	149.27±36.67^{a}	141.63±45.25^{a}	

Abbreviations: CESTL= chloroform extract of *Sansevieria trifasciata* leaf, TWEEN= 50 % Tween80, CTRL= control/ placebo/distilled water, ALT= alanine aminotransferase, AST= aspartate aminotransferase.
Values are expressed as the mean±SD. Values ended with same superscript letters in a column for each variable indicate no significant difference between days based on repeated-measures ANOVA test (p>0.05).

Table 6. Evaluation of renal functions in female Wistar rats as experimental animals in single-dose acute oral toxicity study of CESTL.

VARIABLE	DAY	GROUPS			BASELINE
		CESTL	TWEEN	CTRL	
CRE (mg/dL)	0	0.35±0.05^{a}	0.40±0.02^{a}	0.37±0.04^{a}	0.30 – 0.40
	7	0.27±0.06^{ab}	0.28±0.05^{b}	0.30±0.05^{a}	
	14	0.39±0.05^{b}	0.33±0.01^{c}	0.45±0.00^{b}	
BUN (mg/dL)	0	19.08±2.42^{a}	17.09±1.53^{a}	14.68±2.54^{a}	12.0 – 22.50
	7	16.22±3.47^{a}	19.52±1.60^{b}	14.91±1.89^{a}	
	14	18.14±3.40^{a}	18.60±3.19^{ab}	18.73±1.43^{a}	

Abbreviations: CESTL= chloroform extract of *Sansevieria trifasciata* leaf, TWEEN= 50 % Tween80, CTRL= control/ placebo/distilled water, CRE= creatinine, BUN= blood urea nitrogen.
Values are expressed as the mean±SD. Values ended with same superscript letters in a column for each variable indicate no significant difference between days based on repeated-measures ANOVA test (p>0.05).

S. senegambica also showed the same result (Ayalogu et al. 2011). Nephroprotective effect is even found in *S. roxburghiana* (Aclan et al. 2020). The nephroprotective activity of chloroform extract is due to the content of phenolics, flavonoids, and amino acids as antioxidants (Jain & Singhai 2010). Work of Dewatisari (2020) revealed that CESTL has a high content of phenols and flavonoids, which means it is potential as nephroprotective agent.

Effect of CESTL on Glucose Level and Lipid Profile

CESTL contains triterpenoids, steroids, phenols, flavonoids, and alkaloids (Dewatisari 2020). Those phytochemical compounds have antidiabetic activity (Ota & Ulrih 2017). Alkaloids, phenolics, and flavonoids also possess antihypercholesterolemic and antilipidemic activities (Asghar et al. 2018). Flavonoids, alkaloids, and terpenoids attenuate atherosclerosis (Liu et al. 2019). Phytosterols have antidiabetic, antihypercholesterolemic, and antiatherosclerotic activities (Salehi et al. 2021). Phytochemical screening (Dey et al. 2014) and *in vitro* assay (Yumna et al. 2018) served that *S. trifasciata* has antidiabetic activity. This property is also found in *S. liberica* (Ifebi et al. 2021). The potential of CESTL as blood lipid lowering agent has not been studied. Research

by Sanad (2020) directed that the chloroform extract of *Vangueria infausta* leaf has ability to regulate blood cholesterol level. Research on *S. liberica* (Johnkennedy et al. 2014) and *S. senegambica* (Ikewuchi 2012b) demonstrated that both species possess hypocholesterolemic effect. *S. senegambica* is also able to control triglyceride level (Ikewuchi et al. 2011).

Prior to explore the potential of CESTL efficacy as herbal product to overcome health problems, toxicity studies must be carried out to determine its toxicity and safety on glucose, cholesterol, and triglyceride metabolism on normoglycemic and normolipidemic models, as we did in this study. Results showed that glucose level was maintained within baseline in all groups. Total cholesterol level increased above baseline, as well as the other groups, but then recovered. Triglyceride level exhibited similar dynamics with cholesterol level, but according to the statistical analysis the result is different. The fluctuation of cholesterol level in group received CESTL is not significant, whereas the fluctuation of triglyceride level is significant (Table 7). However, by looking the physiological dynamics (the initial value, the fluctuation, and the final result), CESTL is promising to lower cholesterol and triglyceride levels.

Based on this finding, we hypothesize that neither CESTL nor Tween80 as emulsifier disrupt the normal metabolism of glucose, cholesterol, and triglycerides, confirmed by reasonable weight gain due to normal growth of the animal. However, these results are provisional since CESTL was administered only single-dose. Therefore, we are preparing to conduct a further study with repeated-dose administration to study the effect of CESTL when consumed routinely during the acute period.

Table 7. Glucose level, lipid profile, and growth in female Wistar rats as experimental animals in single-dose acute oral toxicity study of CESTL.

VARIABLE	DAY	GROUPS			BASELINE
		CESTL	TWEEN	CTRL	
Fasting glucose (mg/dL)	0	94.33±14.27^{a}	145.67±13.52^{a}	177.67±41.25^{a}	75 – 233
	7	129.00±10.80^{a}	180.33±47.30^{a}	200.33±25.32^{a}	
	14	120.33±16.05^{a}	165.33±49.78^{a}	106.00±14.45^{a}	
Total cholesterol (mg/dL)	0	138.33±23.46^{a}	138.67±10.87^{a}	106.00±5.35^{a}	100 – 171
	7	276.00±93.34^{a}	247.33±68.05^{a}	240.33±16.36^{ab}	
	14	122.00±18.71^{a}	165.00±84.92^{a}	231.00±21.26^{b}	
Triglycerides (mg/dL)	0	47.77±8.35^{a}	48.93±2.65^{a}	56.90±6.19^{a}	38 – 66
	7	64.90±14.86^{b}	59.05±7.80^{a}	67.43±14.99^{a}	
	14	34.083±5.39^{ab}	41.63±5.20^{a}	42.40±5.74^{a}	
Body weight (g)	Initial	145.33±7.84^{a}	162.00±4.97^{a}	157.33±7.71^{a}	135 – 173
	0	156.33±1.88^{a}	169.67±5.91^{b}	161.33±6.54^{a}	
	7	167.33±3.85^{a}	176.33±3.77^{ab}	168.67±7.32^{a}	
	14	180.00±7.87^{a}	187.00±1.41^{ab}	175.67±6.94^{a}	

Abbreviations: CESTL= chloroform extract of *Sansevieria trifasciata* leaf, TWEEN= 50 % Tween80, CTRL= control/ placebo/distilled water.

Values are expressed as the mean±SD. Values ended with same superscript letters in a column for each variable indicate no significant difference between days based on repeated-measures ANOVA test ($p>0.05$).

Effect of CESTL on Relative Organ Weight

Organ weight, or to be precise is relative organ weight, is endpoint parameter in toxicity study. In adults, ratio of organ weight to body weight is constant; therefore, changes in its value indicates body response to a treatment (Cattley & Cullen 2013), one of which is exposure to toxic compounds in both acute and chronic phases (Greaves 2012; Cattley & Cullen 2013). Results showed that values of almost all organs in treatment groups did not experience significant changes, except the liver (Table 8).

Liver is critical target organ in toxicity studies because it functions as the center for regulation of nutrient metabolism, producing various functional proteins, providing energy for homeostasis, as well as drug metabolism and detoxification. Liver weight in rats occupies 2-3 % of body weight (Rogers & Dintzis 2018). Decreasing of liver weight is common with age. Conversely, increasing of liver weight can occur due to the accumulation of lipids, glycogen, and other compounds due to cell damage, congestion, hypertrophy, and hyperplasia of liver cells (Greaves 2012).

Toxic effect generally elevates liver weight. In this study, treatment groups had lower liver weight than the control. Liver weight reduction is uncommon, it can be caused by hepatocyte atrophy or death due to injury or apoptosis (Cattley & Cullen 2013). Liver weight is normally influenced by physiological factors, especially blood circulation, so it is difficult to evaluate based on histopathological examination. Hepatic enzymes can increase liver weight, but this is not always the case (Greaves 2012). ALT and AST values **(**Table 5**)** indicate that the activities of both liver enzymes fluctuated during the experiment, some exhibited significant increase or decrease, however, they are not salient to cause alteration in liver weight. In this study, CESTL was administered only once (single-dose), hence, we will continue with repeated-dose administration to get more information regarding effect of CESTL on liver weight before conducting histopathological assessment.

Table 8. Relative organ weight in female Wistar rats as experimental animals in single-dose acute oral toxicity study of CESTL.

ORGAN	RELATIVE WEIGHT (g)		
	CESTL	TWEEN	CTRL
Brain	1.037±0.009[a]	1.029±0.031[a]	0.989±0.003[a]
Gastrointestinal tract	9.135±1.706[a]	9.357±0.588[a]	9.585±0.694[a]
Heart	0.356±0.034[a]	0.369±0.034[a]	0.356±0.023[a]
Internal genital organ	0.428±0.020[a]	0.767±0.373[a]	0.407±0.106[a]
Kidney, left	0.380±0.035[a]	0.435±0.028[a]	0.423±0.075[a]
Kidney, right	0.469±0.029[a]	0.460±0.039[a]	0.387±0.019[a]
Liver	3.861±0.106[a]	3.998±0.170[a]	4.567±0.160[b]
Lungs	0.867±0.043[a]	0.750±0.025[a]	0.743±0.056[a]
Spleen	0.309±0.033[a]	0.357±0.066[a]	0.283±0.030[a]

Abbreviations: CESTL= chloroform extract of *Sansevieria trifasciata* leaf, TWEEN= 50 % Tween80, CTRL= control/ placebo/distilled water.

Values are expressed as the mean±SD. Values ended with same superscript letters in a row for each variable indicate no significant difference between groups based on one-way ANOVA test ($p>0.05$).

CONCLUSION

No mortality and any sublethal effects as signs of toxicity were detected on female Wistar rats used as model animal during the study. Based on all parameter values and statistical analysis results, it can be concluded that acute oral administration of chloroform extract of *Sansevieria trifasciata* leaf (CESTL) at the dose 2000 mg/kg bw (single-dose) generated no-observed-adverse-effect-level (NOAEL). Therefore, CESTL can be classified in the hazard of Category 5 based on Globally Harmonized System of Classification and Labelling of Chemicals (GHS). Build upon this finding, we are preparing to conduct further study to assess the repeated-dose acute oral toxicity of CESTL to provide more information prior to the exploration of its potential therapeutic effects.

AUTHORS CONTRIBUTION

LF designed the research and supervised all the process; ICPG and WBTS conducted the experiment and responsible for data collection; ICPG, WBTS, and MIM analysed the data and constructed interpretations of the results for discussions. LF wrote the manuscript as compilation of concepts from all authors.

ACKNOWLEDGMENTS

This work is supported financially by the "Hibah Kolaborasi Dosen dan Mahasiswa" (KDM) of the Faculty of Biology Gadjah Mada University of the year 2021 (Grant Number: 1009/UN1/FBI/KSA/PT.01.03/2021, dated on March 25, 2021). We are very grateful to Mrs. Whika Febria Dewatisari, S.Si., M.Sc. for the idea, and for providing the valuable CESTL, as well as the time for sharing her knowledge with us.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare, and there is no financial interest to report.

REFERENCES

Aclan, J.B.P. et al., 2020. Determination of nephroprotective activity of *Sansevieria roxburghiana* Schult. & Schult.f. (Agavaceae) methanolic crude extract in gentamicin-induced nephrotoxicity in male Wistar albino rats. *Asia Pacific Journal of Education, Arts and Sciences,* 7(4), pp.107–114.

Anbu, J.S.J. et al., 2009. Analgesic and antipyretic effects of *Sansevieria trifasciata* leaves. *African Journal of Traditional, Complementary and Alternative Medicines,* 6(4), pp. 529–533.

Andhare, R.N. et al., 2012. Evaluation of antiallergic and anti-anaphylactic activity of ethanolic extract of *Sansevieria trifasciata* leaves (EEST) in rodents. *Journal of Ethnopharmacology,* 142, pp.627–663. doi: 10.1016/j.jep.2012.05.007

ARRP, 2008. *Guideline 20: Guidelines for the Housing of Rats in Scientific Institutions,* Animal Research Review Panel, Animal Welfare Branch, NSW Department of Primary Industries, pp.1–74.

Asghar, N. et al., 2018. Phytochemical composition, antilipidemic and antihypercholestrolemic perspectives of Bael leaf extracts. *Lipids in Health and Disease,* 17(1), 68. doi: 10.1186/s12944-018-0713-9

Ayalogu, E.O. et al., 2011. Effects of an aqueous leaf extract of *Sansevieria senegambica* Baker on plasma biochemistry and haematological indices of salt-loaded rats. *South African Journal of Science,* 107(11-12), 481. doi: 10.4102/sajs.v107i11/12.481

Carstens, E. & Moberg, G.P., 2000. Recognizing pain and distress in laboratory animals. *ILAR Journal,* 41(2), pp.62–71. doi: 10.1093/ilar.41.2.62

Cattley, R.C. & Cullen, J.M., 2013. Liver and gall bladder. In *Haschek and Rousseaux's Handbook of Toxicologic Pathology*. London, UK: Academic Press/Elsevier Inc., pp 1509–1566.

Christian, E.O. et al., 2014. Acute toxicity investigation and anti-diarrhoeal effect of the chloroform-methanol extract of the leaves of *Persea americana. Iranian Journal of Pharmaceutical Research,* 13(2), pp.651–658.

Debelo, N. et al., 2016. Assessment of hematological, biochemical and histopathological effects of acute and sub-chronic administration of the aqueous leaves extract of *Thymus schimperi* in rats. *Journal of Clinical Toxicology,* 6(2), pp.1–9. doi: 10.4172/2161-0495.1000286

Derelanko, M.J. & Hollinger, M.A., 2002. *Handbook of Toxicology*. In Boca Raton: Taylor & Francis, CRC Press LLC.

Dewatisari, W.F., 2020. Perbandingan pelarut kloroform dan etanol terhadap rendemen ekstrak daun lidah mertua (*Sansevieria trifasciata* Prain.) menggunakan metode maserasi. *Prosiding Seminar Nasional Biologi di Era Pandemi COVID-19,* 6(1), pp.127–132. doi: 10.24252/psb.v6i1.15638

Dewatisari, W.F. et al., 2021. The potency of *Sansevieria trifasciata* and *S. cylindrica* leaves extracts as an antibacterial against *Pseudomonas aeruginosa. Biodiversitas,* 22(1), pp.408–415. doi: 10.13057/biodiv/d220150

Dey, B. et al., 2014. Mechanistic explorations of antidiabetic potentials of *Sansevieria trifasciata. Indo Global Journal of Pharmaceutical Sciences,* 4(2), pp.113–122. doi: 10.35652/IGJPS.2014.115

Ekeanyanwu, R.C. & Njoku, O.U., 2014. Acute and subacute oral toxicity study on the flavonoid rich fraction of *Monodora tenuifolia* seed in albino rats. *Asian Pacific Journal of Tropical Biomedicine,* 4(3), pp.194–202. doi: 10.1016/S2221-1691(14)60231-8

Ellis, A.G. et al., 1996. Inhibition of etoposide elimination in the isolated perfused rat liver by Cremophor EL and Tween 80. *Cancer Chemotherapy Pharmacol*ogy, 38(1), pp.81–87. doi: 10.1007/s002800050451

Etame, R.M.E. et al., 2017. Acute and sub-acute toxicity of *Harungana madagascariensis* LAM (Hypericaceae) stem bark methanol extract. *Journal of Applied Pharmaceutical Science,* 7(3), pp.160–167. doi: 10.7324/JAPS.2017.70326

Fitria, L. et al., 2019. Nilai rujukan untuk evaluasi fungsi hati dan ginjal pada tikus (*Rattus norvegicus* Berkenhout, 1769) Galur Wistar. *Jurnal Pendidikan Matematika dan IPA,* 10(2), pp.243–258. doi: 10.26418/ jpmipa.v10i2.34144

FSCJ, 2007. *Polysorbates (Polysorbates 20, 60, 65, and 80), evaluation report of food addictives,* Food Safety Commission of Japan.

Greaves, P., 2012. *Histopathology of Preclinical Toxicity Studies*. In London: Academic Press/Elsevier B.V. pp 433–535.

Ifebi, H.M.N. et al., 2021. Evaluation of antidiabetic property of *Sansevieria liberica* Gerald and Labroy (Dracaenaceae) leaf using alloxan-induced diabetes model. *Nigerian Journal of Pharmaceutical Research,* 16(5), pp.73–84. doi: 10.4314/njpr.v16i2.9S

Ighodaro, O.M. et al., 2017. Toxicity status and antiulcerative potential of *Sansevieria trifasciata* leaf extract in Wistar rats. *Journal of Intercultural Ethnopharmacology*, 6(2), pp. 234–239. doi: 10.5455/jice.20170421103553

Ikewuchi, C.C. et al., 2011. Weight reducing and hypocholesterolaemic effect of aqueous leaf extract of *Sansevieria senegambica* Baker on sub-chronic salt-loaded rats: Implication for the reduction of cardiovascular risk. *Research Journal of Pharmacy and Technology,* 4(5), pp.725–729. doi: 10.4314/IJBCS.V5I2.72131

Ikewuchi, C.C., 2012a. Hepatoprotective effect of an aqueous extract of the leaves of *Sansevieria liberica* Gerome and Labroy against carbon tetrachloride-induced liver injury in Wistar rats. *Pacific Journal of Science and Technology,* 13(1), pp.512–518.

Ikewuchi, C.C., 2012b. Hypocholesterolemic effect of an aqueous extract of the leaves of *Sansevieria senegambica* Baker on plasma lipid profile and atherogenic indices of rats fed egg yolk supplemented diet. *EXCLI Journal,* 11, pp.346–356.

Jain, A. & Singhai, A.K., 2010. Nephroprotective activity of *Momordica dioica* Roxb. in cisplatin-induced nephrotoxicity. *Natural Product Research,* 24(9), pp.846–854. doi: 10.1080/14786410903132589

Johnkennedy, N. et al., 2014. Hypolipidemic effects of aqueous extract of *Sansevieria liberica* leaves extracts in hypercholesterolaemia rats. *International Journal of Traditional System of Medicine,* 1(1), pp.22–25.

Khan, R.A. et al., 2012. Hepatoprotection with a chloroform extract of *Launaea procumbens* against CCl_4-induced injuries in rats. *BMC Complementary and Alternative Medicine,* 12, 114. doi: 10.1186/1472-6882-12-114

Liu, H. et al., 2019. Efficacy of terpenoid in attenuating aortic atherosclerosis in apolipoprotein-E deficient mice: A meta-analysis of animal studies. *BioMed Research International,* Article ID 2931831, 12. doi: 10.1155/2019/2931831

Liu, J. et al., 2020. Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. *Journal of Translational Medicine,* 18, 206. doi: 10.1186/s12967-020-02374-0

Maner, B.S. & Moosavi, L., 2021. *Mean Corpuscular Volume.* In Treasure Island: StatPearls Publishing.

Mantskava, M. et al., 2018. The influence of polysorbate Tween 80 on somatic health rats in experiment. *MOJ Anatomy & Physiology,* 5(2), 152. doi: 10.15406/mojap.2018.05.00180

Morita, J. et al., 2017. Effects of reduced food intake for 4 weeks on physiological parameters in toxicity studies in dogs. *The Journal of Toxicological Sciences,* 42(1), pp.31–42. doi: 10.2131/jts.42.31

Mulyati et al., 2019. Kidney function test of female Wistar rat (*Rattus norvegicus* Berkenhout, 1769) of subchronic toxicity test of *Arthrospira maxima* and *Chlorella vulgaris*. *Journal of Tropical Biodiversity and Biotechnology*, 4(3), pp.119 –123. 10.22146/jtbb.42306

NC3RS, 2017. *Study looking at natural behaviours of lab rats wins international 3Rs prize,* National Centre for the Replacement Refinement & Reduction of Animal Research.

NRC, 2011. *Guide for the care and use of laboratory animals*. In Washington DC: National Research Council. The National Academies Press.

OECD, 2002. *Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure.* In Paris: The Organisation for Economic Co-operation and Development (OECD) Publishing.

OECD, 2008. *Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents.* In Paris: The Organisation for Economic Co-operation and Development (OECD) Publishing.

Ota, A. & Ulrih, N.P., 2017. An overview of herbal products and secondary metabolites used for management of type two diabetes. *Frontiers in Pharmacology,* 8, 436. doi: 10.3389/fphar.2017.00436

Pagare, S. et al., 2015. Secondary metabolites of plants and their role: Overview. *Current Trends in Biotechnology and Pharmacy,* 9(3), pp.293–304.

Parasuraman, S., 2011. Toxicological screening. *Journal of Pharmacology and Pharmacotherapeutics*, 2(2), pp.74–79. doi: 10.4103/0976-500X.81895

Parker, J.E. et al., 2021. Phytochemical and toxicological studies of methanol and chloroform fractions of *Acanthus montanus* leaves. *Journal of Biological Sciences,* 21(2), pp.52–58. doi: 10.3923/jbs.2021.52.58

Poitout-Belissent, F.M. & McCartney, J.E., 2010. Interpretation of hematology data in preclinical toxicological studies. In Ames: *Schalm's Veterinary Hematology.* Blackwell Pub. Ltd., a John Wiley & Sons, Ltd. Pub. pp. 78–84.

Qomariyah, N. et al., 2012. Antidiabetic effects of a decoction of leaves of *Sansevieria trifasciata* in alloxan-induced diabetic white rats (*Rattus norvegicus* L.). *ITB Journal of Science,* 44(4), pp.308–316. doi: 10.5614/itbj.sci.2012.44.4.2

Rogers, A.B. & Dintzis, R.Z., 2018. Hepatobiliary system. In London: *Comparative Anatomy and Histology.* Academic Press/Elsevier Inc. pp 229–239.

Salehi, B. et al., 2021. Phytosterols: From preclinical evidence to potential clinical applications. *Frontiers in Pharmacology,* 11, 1819. doi: 10.3389/fphar.2020.599959

Sanad, F.A.A., 2020. Evaluation of the hypolipidemic and antioxidant activities of chloroformic extract from *Vangueria infausta*leaves in rats with hypercholesterolemia. *Current Science International,* 9(3), pp.462–471. doi: 10.4103/pm.pm_402_16

Santos-Lopez, J.A. et al., 2010. Antisecretory activity of methanol and chloroform extracts from aerial parts and flowers of *Phytolacca icosandra* L. *Revista CENIC Ciencias Biológicas,* 41, pp.1–5.

Song, M. et al., 2021. Neutrophil-to-lymphocyte ratio and mortality in the United States general population. *Scientific Reports,* 11, 464. doi: 10.1038/s41598-020-79431-7

Sukandar, E.Y. & Sheba, S.H., 2019. Acute and sub-chronic toxicity studies of combination of Physalis *angulata* L. (cecendet) extract and methylprednisolone on animals. *International Journal of Integrated Health Sciences,* 7 (1), pp. 48–55. doi: 10.15850/ijihs.v7n1.1619

Sutrisni, N.N.W. et al., 2019. Acute and subchronic (28-day) oral toxicity studies on the film formulation of k-carrageenan and konjac glucomannan for soft capsule application. *Scientia Pharmaceutica,* 87(2), 9. doi: 10.3390/scipharm87020009

Thamir, S.N. et al., 2013. Investigation the immunoadjuvant activity for polysorbate 80. *Asian Journal of Pharmacy, Nursing and Medical Sciences,* 1(1), 22.

Tien, S.L., 1995. Validating the platelet count. *Singapore Medical Journal,* 36(3), pp.255–256.

United Nations, 2011. *Globally Harmonized System of Classification and Labelling of Chemicals (GHS).* In New York and Geneva: United Nations. pp.20, 109 -119.

Vilchez, C., 2020. Examination of the peripheral blood film and correlation with the complete blood count. In *Rodak's Hematology.* St. Louis: Saunders/Elsevier Inc. pp.201–218.

Wang, W. et al., 2019. Acute and subacute toxicity assessment of oxyclozanide in Wistar rats. *Frontiers in Veterinary Science,* 6, 294. doi: 10.3389/fvets.2019.00294

Yumna, M. et al., 2018. Effect of mother-in-law's tongue leaves (*Sansevieria trifasciata*) extract's solvent polarity on anti-diabetic activity through in vitro α-glucosidase enzyme inhibition test. *E3S Web of Conferences,* 67, 03003. doi: 10.1051/e3sconf/20186703003

Zakaria, Z.A. et al., 2015. Gastroprotective activity of chloroform extract of *Muntingia calabura* and *Melastoma malabathricum* leaves. *Pharmaceutical Biology,* 54, pp.1–15. doi: 10.3109/13880209.2015.1085580