Borneo Journal of Pharmacy
Vol 6 Issue 1 February 2023
Pages 1 – 7

https://journal.umpr.ac.id/index.php/bjop/article/view/2099
DOI: https://doi.org/10.33084/bjop.v6i1.2099
e-ISSN: 2621-4814

Research Article

Acute Toxicity of Self-Nanoemulsifying Drug Delivery System of *Ipomoea reptans* Poir Leaves Extract on Female Wistar Rats

Cynthia Astiti Putri*

Farida Hayati

Lutfi Chabib

Muhammad Iqbal Pangestu

Department of Pharmacy, Universitas Islam Indonesia, Sleman, Special Region of Yogyakarta, Indonesia

*email: cynthia.astiti@uii.ac.id

Keywords:
Acute toxicity
Ipomoea reptans leaves
LD_{50}
OECD 423
SNEDDS

Abstract

Ipomoea reptans Poir has many health benefits, such as decreasing blood glucose and as an antioxidant. Self-nano emulsifying drug delivery systems (SNEDDS) are an innovation in pharmaceutical technology that minimizes drug molecules and maximizes surface area, thus increasing drug absorption. This study aimed to investigate SNEDDS of *I. reptans* acute toxicity in female Wistar rats. An acute toxicity test was done using a limit test of OECD 423. Female Wistar rats were divided into control and treatment groups, with three animals for each group being used per step. No animals died after they were given SNEDDS of *I. reptans* leaves extract at a dose of 2000 mg/kg. No toxic effect was detected at clinical examination and histopathology of the organ. SNEDDS of *I. reptans* leaves extract had an LD_{50} cut-off value of 5000 mg/kg.

Received: February 7th, 2021
1st Revised: February 26th, 2022
2nd Revised: April 4th, 2022
Accepted: February 26th, 2023
Published: February 28th, 2023

INTRODUCTION

Ipomoea reptans Poir, known as *kangkung* in Indonesia, is widely distributed in tropical countries in Asia, Africa, and Australia. This plant is usually consumed as a green leafy vegetable[1]. In Indonesia, this plant is planted in aquatic or land environments, as well as known as *Ipomoea aquatica*[2]. Moreover, this plant is traditionally used to cure some diseases[3]. *Ipomoea reptans* was confirmed to have antidiabetic, antioxidant, anticancer, anti-inflammatory, antiarthritic, antiulcer, antimicrobial, hypolipidemic, diuretic, and central nervous system activity[4,5].

The safety of *I. reptans* extract has been confirmed. Acute oral toxicity test exhibited that pseudo-LD_{50} was more than 9375 mg/kg, and no toxic effect was detected in both sexes of Wistar rats[6]. In Sivaraman *et al*. study[7], no mortality and clinical signs changed at the maximum 2000 mg/kg dose. Administration of ethanolic extract of *I. reptans* for 14 days on female mice showed that AST and ALT values increased significantly at doses of 759 and 1200 mg/kg. However, there were no harmful changes in the histology of the kidney and liver organs[8]. Nevertheless, *I. reptans* has low water solubility. Therefore, modification of extract preparation is required.

Self-nano emulsifying drug delivery system (SNEDDS) is one alternative method to improve the bioavailability of the extract[9]. It comprises a surfactant, oil phase, active pharmaceutical ingredient, and hydrophilic co-surfactant[10]. The SNEDDS is anhydrous preconcentrates of nanoemulsions with droplet sizes ranging from 20-600 nm[11]. This technology has many advantages, such as improved lipophilic drug solubilization, improved physical stability, provided greater surface area and absorption, and decreased first-pass metabolism[12,13]. However, no acute oral toxicity is observed for SNEDDS of *I. reptans* extracts. Therefore, this study aimed to evaluate the effect of *I. reptans* leaves extract SNEDDS on clinical and organ histology examination.

How to cite: Putri CA, Hayati F, Chabib L, Pangestu MI. Acute Toxicity of Self-Nanoemulsifying Drug Delivery System of Ipomoea reptans Poir Leaves Extract on Female Wistar Rats. Borneo J Pharm. 2023;6(1):1-7. doi:10.33084/bjop.v6i1.2099

MATERIALS AND METHODS

Materials

The materials used were *I. reptans* Poir. leaves (collected from Sidorejo, Muruh, Gantiwarno, Klaten, Central Java, Indonesia), aqua pro injection, Tween 20 (Vivantis Inc.), polyethylene glycol 400 (Brataco), and Capryol 90 (Gattefose). The tools utilized were micropipette (Thermo Scientific), Particle size analyzer (PSA) (HORIBA SZ 100), Ultrasonicator (Model 300 VT Biologics, Inc), Analytical scale (Metler Toledo XS205 Dual Range), Centrifugator (Hanil MF 80), Climacell Chamber, Waterbath shaker (Memmert), and Rotary evaporator (Heidolph).

Methods

Preparation of extract

The Laboratory of Plant Systematic, Faculty of Biology, Universitas Gadjah Mada (01058/S.Tb./V/2017) has validated the plant. Harvesting the leaves was done on day 30 in the morning. The standardized extract of *I. reptans* has been acquired based on the extraction procedures by Hayati *et al*[14].

Preparation of SNEDDS

The formulation of I. reptans leaf extract was obtained from Jumaryatno *et al*. study[9]. The compositions were Capryol 90, Tween 20, and PEG 400 with a ratio of 1 : 7 : 2 consecutively.

Observation of stability

Determination of stability of SNEDDS used centrifugation test, heating cooling cycle test, and freeze-thaw cycle test continually, as mentioned in Jumaryatno *et al*. study[9].

Determination of size, polydisperse index (PDI) and zeta potential

The measurement carried out using PSA was to know the formula's size and distribution. A total of 1 mL SNEDDS was mixed with distilled water to 100 mL, stirred until nanoemulsion was obtained, and set to PSA.

Toxicity test

A limit test of Acute Toxic Class Method OECD 423 was performed to determine LD_{50} of SNEDDS on 2-2.5-month-old female Wistar rats[15]. The animals were supplied by the Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia. The rats were kept under controlled conditions (room temperature (25±2)°C; 12 hours light/dark cycle) and allowed free access to standard food and water. The animals were acclimatized for seven days before the experiment. Ethical clearance for the experiments was obtained from the Ethical Committee Faculty of Medicine, Universitas Islam Indonesia, with number 843/KE/XII/2017.

The animals were divided into the control group (vehicle/SNEDDS base) and treatment group (SNEDDS of *I. reptans* extract 2000 mg/kg). Each group consisted of three animals per step. There were no test animals that died unwanted during the trial period. The scheme of the limit test can be seen in **Figure 1**. The animals were given a dose of 2000 mg/kg for the limit test. If there were 2-3 dead animals, the dose was reduced to 300 mg/kg, and the main test should be performed. However, if 0-1 animal died, the dose administration still used 2000 mg/kg. The results (LD_{50}) were dependent on the number of dead animals.

The vehicle or extract SNEDDS was given as a single dose. For the first four hours, every 30 min after administration, rats' clinical examination (tremor, hyperactivity, eyes change, convulsion, and mortality) was observed thoroughly. The rats were examined periodically during the first 24 hours and daily after that for 14 days. Body weight was measured daily. At the end of the experiment or when the animals were found dead during the investigation, their organs (heart, liver, kidney, lung) were harvested and evaluated histopathologically.

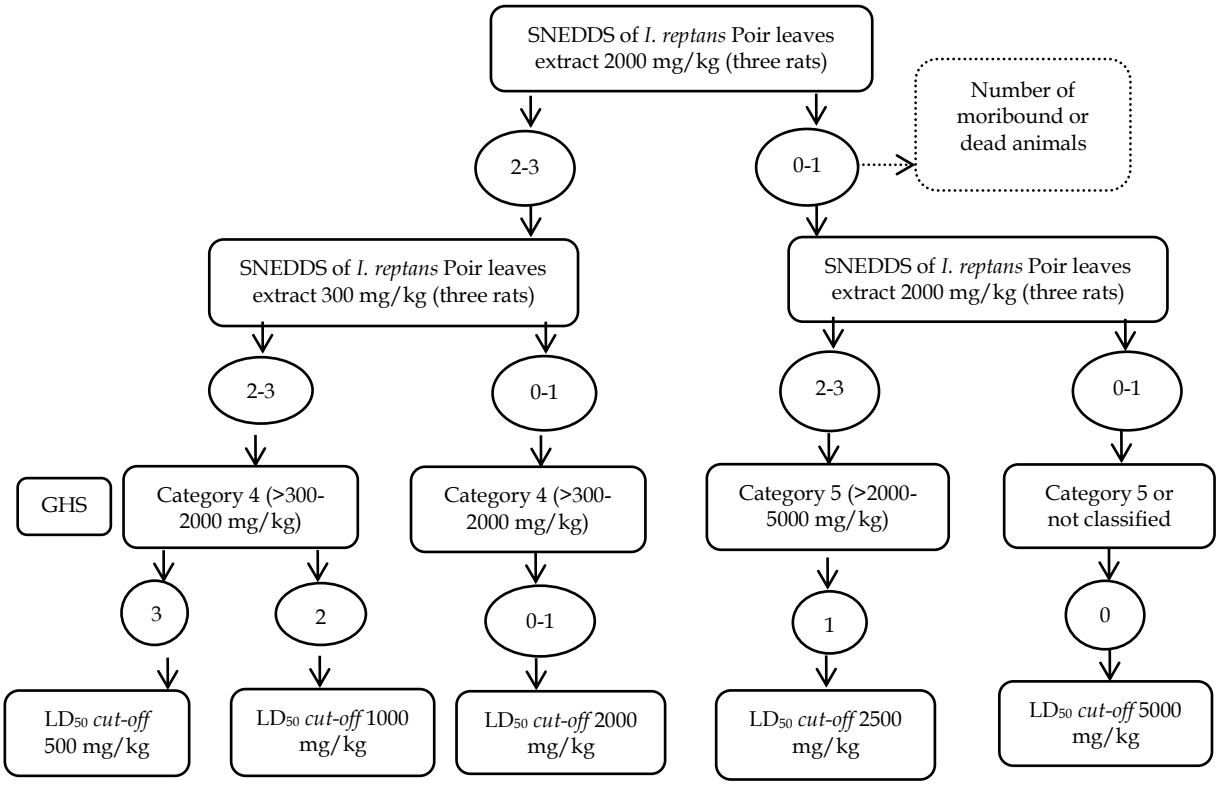

Figure 1. Scheme of toxicity test (OECD 423)[15].

RESULTS AND DISCUSSION

Stability, particle size, PDI, and zeta potential of I. reptans leaves extract SNEDDS

SNEDDS of *I. reptans* leaves extract formulation consists of 2000 mg/kg *I. reptans* as the active ingredient, 1 mL Capryol 90 as the oil phase, 7 mL Tween 20 as the surfactant, and 2 mL PEG 400 as co-surfactant (**Figure 2**). Stability determination showed that SNEDDS was stable, indicated by no phase separation, at 40-45°C. Reduction of droplet size and Brownian motion keep the formula from gravity force. The temperature changes also can not separate the phase of the nanoemulsion[9,16].

Figure 2. SNEEDS of *I. reptans* leaves extract.

The test result using PSA can be seen in **Table I**. The droplet size of nanoemulsion is varying 20-600 nm. The small size of the *I. reptans* extract SNEDDS droplet made the formula will not break through dilution. Therefore, it will enhance the surface area and absorption of the active ingredient[17]. As a consequence, the bioavailability of *I. reptans* will escalate. Measurement of PDI implies the homogeneity of particle size in SNEDDS. Higher values indicate a broader distribution and smaller droplet size uniformity[18]. Zeta potential ranging ± 30 mV suggests the stability of the nanoemulsion preserved. A negative value implies that there are free fatty acids in the emulsion[19].

Table I. Various test on SNEDDS of *I. reptans* leaf extract

Test	Result
Stability test	No phase separation
Particle size (nm)	227.5
Polydispersity index	0.460
Zeta potential (mV)	-26.7
Acute oral toxicity test	LD_{50} *cut-off* 5000 mg/kg

Acute oral toxicity

OECD guideline No. 423 is an alternative for conventional acute oral toxicity (OECD 401), published in 1987. The former regulation was criticized heavily regarding animal welfare because it used many animals of both sexes. In the recent guideline, the animal used was limited (three animals per step). In addition, the subject of the toxicity test was female rats only. This sex is considerably more sensitive[20]. OECD 423 applies a fixed dose method (5, 50, 300, 2000 mg/kg). The limit test could be employed if previous data about the safety showed that the compounds were probably nontoxic[21].

In a study by Hayati *et al.*[6], quasi-LD_{50} of *I. reptans* leaves ethanol extract was more than 9375 mg/kg. This plant was classified as a practically nontoxic compound based on Loomis criteria. Based on the Sivaraman *et al.* study[7], the animal's given hydroalcoholic leaf extract of *I. aquatica* at a dose of 2000 mg/kg did not show any toxicity signs or mortality. In this study, no mortality was found in both steps. Therefore, SNEDDS of *I. reptans* leaves ethanol extract were categorized 5 or unclassified in the Globally Harmonized Classification System (GHS) for Chemical Substances and Mixtures[22]. OECD 423 do not encourage testing animal in dose above 2000 mg/kg for welfare reason, except for the substances that defend human or animal health[23].

There are no apparent toxic effects observed during this research. In the previous study, the animal showed no harm after being administered *I. reptans* ethanol extract in a single high dose (>9375 mg/kg)[6]. Body weight measurement for 14 days can be found in **Figure 3**. There is no significant difference in weight gain between the control and treatment groups. The average daily gain in the treatment group obtained was 0.86 g/day; the control group was 1.32 g/day. The previous research exhibited 0.62±0.22 g weight gain per day in *I. reptans* extract at a dose of 9375 mg/kg group[6].

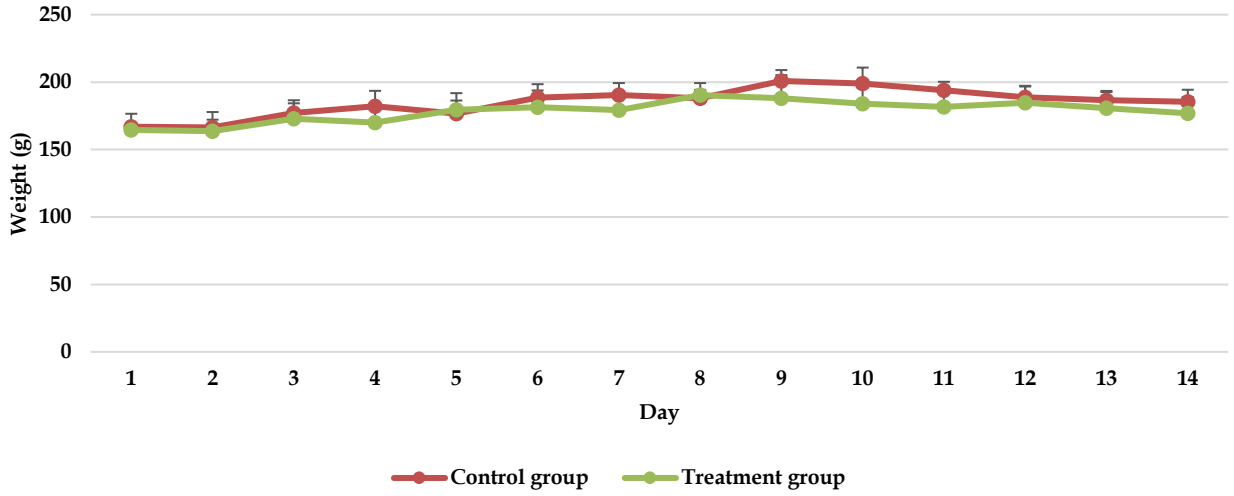

Figure 3. Body weight of control and treatment group.

A histopathology organ test was run to investigate the effect of *I. reptans* extract SNEDDS on the organ. Cellular changes may occur, although no toxic symptoms were observed during the clinical examination. **Figure 4** showed that administering *I. reptans* extracts SNEDDS did not make any difference in the organ. A study by Hayati *et al.*[8] demonstrated the same result in kidney and liver organs in female mice, although AST and ALT value rise after administration of *I. reptans* extracts for 14 days. Therefore, *I. reptans* extract SNEDDS was safe for animal and human.

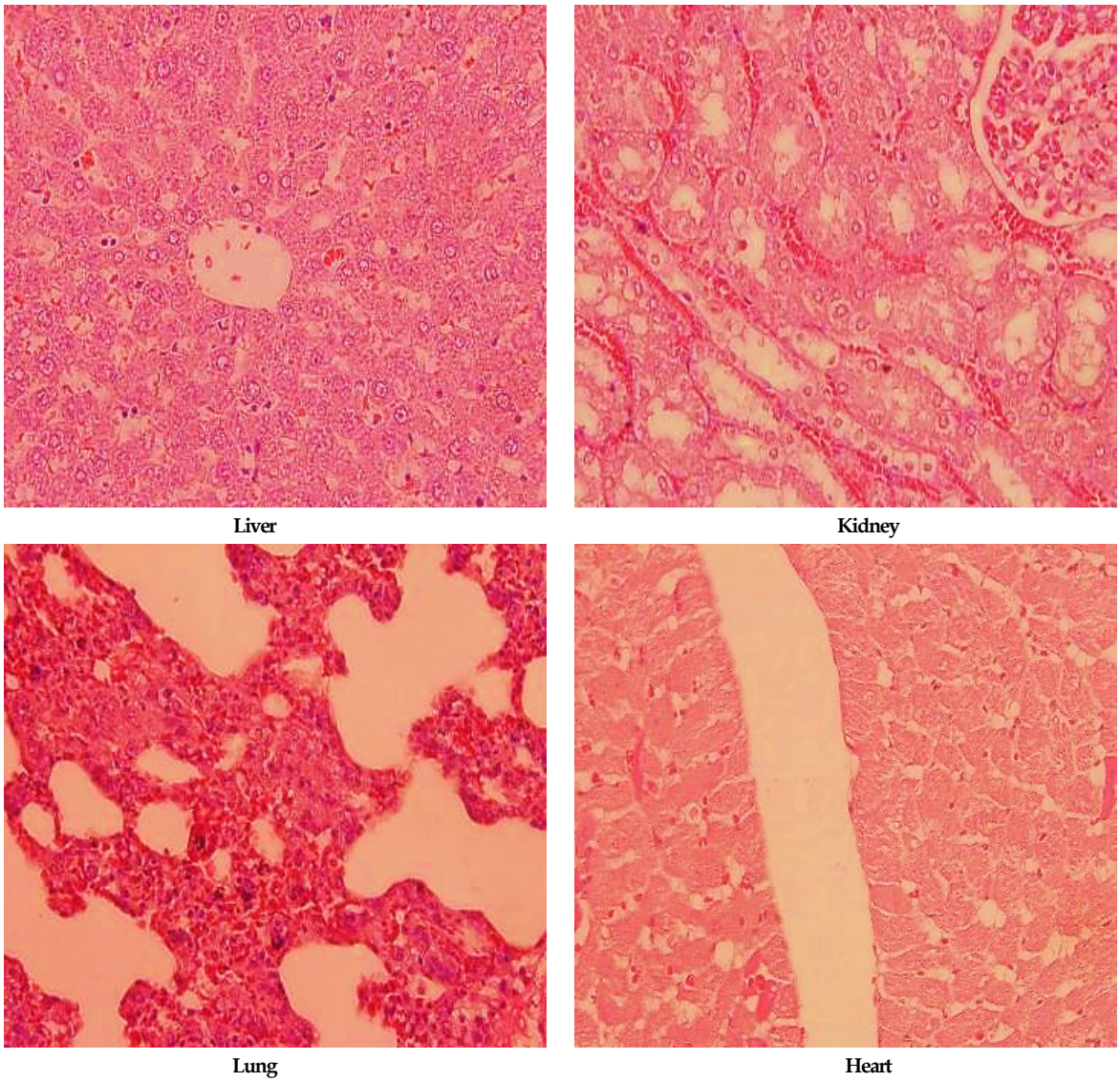

Figure 4. Organ histology of treatment group.

CONCLUSION

SNEEDS of *I. reptans* leaves extract were stable and dispersed perfectly. It also had an LD_{50} cut-off value of 5000 mg/kg.

ACKNOWLEDGMENT

None.

AUTHORS' CONTRIBUTION

All authors have an equal contribution in carrying out this study.

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Shi L, Jin M, Shen M, Lu C, Wang H, Zhou X, et al. Using Ipomoea aquatic as an environmental-friendly alternative to Elodea nuttallii for the aquaculture of Chinese mitten crab. PeerJ. 2019;7:e6785. doi:10.7717/peerj.6785

2. Mariani R, Perdana F, Fadhlillah FM, Qowiyyah A, Triyana H. Antioxidant activity of Indonesian water spinach and land spinach (Ipomoea aquatica): A comparative study. J Phys Conf Ser. 2019;1402:055091. doi:10.1088/1742-6596/1402/5/055091

3. Sharifi-Rad M, Fokou PVT, Sharopov F, Martorell M, Ademiluyi AO, Rajkovic J, et al. Antiulcer Agents: From Plant Extracts to Phytochemicals in Healing Promotion. Molecules. 2018;23(7):1751. doi:10.3390/molecules23071751

4. Kurniawan H, Dacamis ES, Simamora A, Tobing PSDL, Hanapiah A, Santoso AW. Antioxidant, Antidiabetic, and Anti-obesity Potential of Ipomoea reptans Poir Leaves. Borneo J Pharm. 2020;3(4):216-26. doi:10.33084/bjop.v3i4.1583

5. Manvar MN, Desai TR. Phytochemical and pharmacological profile of Ipomoea aquatica. Indian J Med Sci. 2013;67(3):49–60. doi:10.4103/0019-5359.121115

6. Hayati F, Istikharah R, Arifah S, Nurhasanah D. Effect of LD50 of ethanolic leaf extract from Ipomoea reptans Poir. in rats. In Zairina E, Khotib J, Ardianto C, Sulaiman SAS, Sands C III, Welty T, editors. Unity in Diversity and the Standardisation of Clinical Pharmacy Services. London: CRC press; 2017. p. 95–8.

7. Sivaraman D, Panneerselvam P, Muralidharan P, Prabhu TP. Preclinical Toxicological Profile of Hydroalcholic Leaf Extract of Ipomoea aquatica Forsk an Indian Medicinal Plant. Pharmacologia. 2013;4(2):107–16. doi:10.17311/pharmacologia.2013.107.116

8. Hayati F, Murwanti R, Zabrina G. Keamanan Pemberian Berulang Ekstrak Kangkung Darat (Ipomoea reptans,Poir) Terstandar terhadap Fungsi Ginjal dan Hepar Mencit Betina. J Ilmiah Farmasi. 2013;10(1):1–6. doi:10.20885/jif.vol10.iss1.art1

9. Jumaryatno P, Chabib L, Hayati F, Awaluddin R. Stability study of Ipomoea reptans extract self-nanoemulsifying drug delivery system (SNEDDS) as anti-diabetic therapy. J Appl Pharm Sci. 2018;8(9):11–4. doi:https://doi.org/10.7324/JAPS.2018.8903

10. Rehman FU, Shah KU, Shah SU, Khan IU, Khan GM, Khan A. From nanoemulsions to self-nanoemulsions, with recent advances in self-nanoemulsifying drug delivery systems (SNEDDS). Expert Opin Drug Deliv. 2017;14(11):1325-40. doi:10.1080/17425247.2016.1218462

11. Nair AB, Singh B, Shah J, Jacob S, Aldhubiab B, Sreeharsha N, et al. Formulation and Evaluation of Self-Nanoemulsifying Drug Delivery System Derived Tablet Containing Sertraline. Pharmaceutics. 2022;14(2):336. doi:10.3390/pharmaceutics14020336

12. Nasr A, Gardouh A, Ghorab M. Novel Solid Self-Nanoemulsifying Drug Delivery System (S-SNEDDS) for Oral Delivery of Olmesartan Medoxomil: Design, Formulation, Pharmacokinetic and Bioavailability Evaluation. Pharmaceutics. 2016;8(3):20. doi:10.3390/pharmaceutics8030020

13. Jaiswal M, Dudhe R, Sharma PK. Nanoemulsion: an advanced mode of drug delivery system. 3 Biotech. 2015;5(2):123-7. doi:10.1007/s13205-014-0214-0

14. Hayati F, Wibowo A, Jumaryatno P, Nugroho AT, Amalia D. Standardisasi Ekstrak Daun Kangkung Darat (Ipomoea reptans Poir) Hasil Budi Daya di Wilayah Sardonoharjo, Sleman dan Potensinya sebagai Antioksidan. J Ilmu Kefarmasian Indones. 2015;13(2):151–7.

15. Organisation for Economic Co-operation and Development. OECD Guideline for Testing of Chemicals Test No 423. Acute oral toxicity. France: OECD; 2001. p. 1-26.

16. Baloch J, Sohail MF, Sarwar HS, Kiani MH, Khan GM, Jahan S, et al. Self-Nanoemulsifying Drug Delivery System (SNEDDS) for Improved Oral Bioavailability of Chlorpromazine: In Vitro and In Vivo Evaluation. Medicina. 2019;55(5):210. doi:10.3390/medicina55050210

17. Wang CY, Yen CC, Hsu MC, Wu YT. Self-Nanoemulsifying Drug Delivery Systems for Enhancing Solubility, Permeability, and Bioavailability of Sesamin. Molecules. 2020;25(14):3119. doi:10.3390/molecules25143119

18. Izham MNM, Hussin Y, Aziz MNM, Yeap SK, Rahman HS, Masarudin MJ, et al. Preparation and Characterization of Self Nano-Emulsifying Drug Delivery System Loaded with Citraland Its Antiproliferative Effect on Colorectal Cells In Vitro. Nanomaterials. 2019;9(7):1028. doi:10.3390/nano9071028

19. Patel J, Kevin G, Patel A, Raval M, Sheth N. Design and development of a self-nanoemulsifying drug delivery system for telmisartan for oral drug delivery. Int J Pharm Investig. 2011;1(2):112-8. doi:10.4103/2230-973x.82431

20. Walum E. Acute Oral Toxicity. Environmental Health Perspectives. 1998;106(Suppl 2):497-503. doi:10.2307/3433801

21. Erhirhie EO, Ihekwereme CP, Ilodigwe EE. Advances in acute toxicity testing: strengths, weaknesses and regulatory acceptance. Interdiscip Toxicol. 2018;11(1):5-12. doi:10.2478/intox-2018-0001

22. Yazid MFHA, Ta GC, Mokhtar M. Classified Chemicals in Accordance with the Globally Harmonized System of Classification and Labeling of Chemicals: Comparison of Lists of the European Union, Japan, Malaysia and New Zealand. Saf Health Work. 2020;11(2):152-8. doi:10.1016/j.shaw.2020.03.002

23. Bishop PL, Manuppello JR, Willett CE, Sandler JT. Animal use and lessons learned in the U.S. High Production Volume Chemicals Challenge Program. Environ Health Perspect. 2012;120(12):1631-9. doi:10.1289/ehp.1104666