Toxicity Studies of Centella Asiatica for Drug Development: Mini Review

Gayuk Kalih Prasesti [1,2] (ID) **, Neng Fisheri Kurniati** [1,*] (ID)

1   Department of Pharmacology-Clinical Pharmacy, School of Pharmacy, Institut Teknologi Bandung (ITB), Bandung, Indonesia; nfkurniati@fa.itb.ac.id (N.F.K), gayukkp@gmail.com (G.K.P);

2   Faculty of Pharmacy, Mulawarman University, Samarinda, East Borneo, Indonesia; gayukkp@gmail.com (G.K.P);

*   Correspondence: nfkurniati@fa.itb.ac.id (N.F.K);

Abstract: Toxicity studies' quality control of drug plant-based products is an important aspect of pharmacological research. The purpose of this literature review is to extract about toxicity test on *Centella asiatica*. The plant component utilized, the test animals used, the type of toxicity test, evaluation, the findings, and conclusions of each test are all included in this review—the database used in PubMed. Most of the literature results obtained from this review indicate high safety in *C. asiatica* plants. The acute toxicity test is that the most frequently used toxicity test. The use varies from plant parts to whole plants, with minimal side effects reported and high in safety, so it can be concluded that *C. asiatica* is very prospective to be developed as a medicine.

**Keywords**: toxicity; *Centella asiatica* safety; drug development.

1. Introduction

Drug safety is one of the three requirements for a drug, apart from the effectiveness and quality of the drug [1]. In addition to drug conventional, herbal medicines must also have safety requirements [2]. In the drug development process, these safety requirements can be shown from the result of the toxicity test at the preclinical stage. If the results of the toxicity test state that the toxic level is low, then the drug substance can be continued at the next stage in the drug development process. In general, this information can be obtained from experiments using test animals as models designed in a series of toxicity tests which include oral acute toxicity tests, oral subchronic toxicity, chronic oral toxicity, teratogenicity, skin sensitization, eye irritation, acute dermal irritation, vaginal mucosal irritation, acute dermal toxicity, subchronic dermal toxicity, and carcinogenicity. The aim of conducting a toxicity test is as supporting evidence of the safety of test preparation. The choice of the kind toxicological test depends on the purpose of using a substance and the possible risk of exposure to humans [3]. *Centella asiatica* is a common herb found in China, Japan, Italy, Sri Lanka, Iran, India, Madagascar, the United States, Australia, South Africa, Indonesia, and Malaysia, widely developed as both oral and topical drugs [4]. Considering the importance of toxicity tests to ensure safety and the high potential for Centella to be developed, this review aims to collect information on the toxicology studies of *C. asiatica*. The key information of this review is the type of toxicology test, the type of animals used, and the reported parameters as a whole or several parts from *C. asiatica*. With this structured and systematic information of the *C. asiatica* plant, it can be used as an

additional reference for researchers to develop medicines based on *C. asiatica* as medicines, traditional medicines, quasi-drugs, health products, and cosmetics

2. Toxicity Studies

Toxicity studies are tests to evaluate the toxic effect of a substance on a biological system and obtain typical dose-response data from the test preparation. The data obtained can be used to provide information about the degree of danger of the test preparation in case of exposure to humans so that the dosage for use can be determined for human safety. The toxicity test using test animals as a model is useful for seeing the presence of biochemical, physiological, and pathological reactions in humans to test preparation. Toxicity studies results cannot be used to prove the safety of a substance/preparation in humans but can provide signs of relative toxicity and help determine the toxic effects that occur if exposed to humans [3]. Drug toxicology regulations aim to find, develop, and manufacture novel, effective remedies for the market while also safeguarding customers from unsafe pharmaceutical products [5,6]. The schema of the importance of the toxicity studies can be seen in (Figure 1). Toxicity of substances can be evaluated by (a) accidental exposures to a substance (b) cells/ cell lines, *in vitro* (c) effect substance in experimental animals, *in vivo* [7]. The dose level recommended for the treatment of the disease was preceded by a toxicity test in the animal models [8].
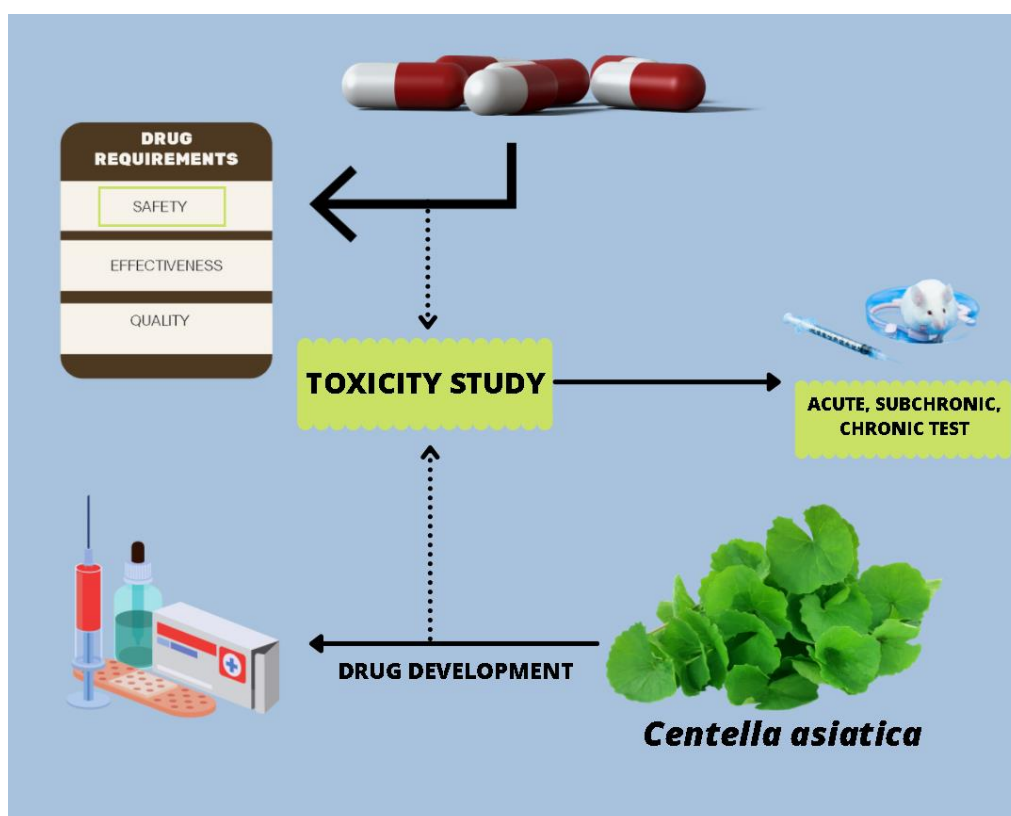
Figure 1. The importance of toxicity studies in drug development. In the process of developing drugs from natural sources, it is very important to do toxicity studies to ensure the safety requirements of a finished drug before it reaches humans. Because toxicity tests are not possible at the clinical stage, the role of experimental animals such as mice and rats is very high to support the toxicity test of a natural source.

2.1. Acute oral toxicity test

Acute oral toxicity assesses the effects of a single dose of a chemical or numerous doses given within 24 hours. The acute oral toxicity test principle is that the test is prepared at many

levels of doses, given to several groups of tested animals with one dose per group. Then the existence of harmful influence/toxic effects and death is seen. The goals of the oral acute toxicity test are to determine a substance's toxicity, determine target organs, species sensitivity, obtain hazard information after acute exposure to a substance, obtain initial information that can be used to determine dose levels, design further toxicity tests, obtain an LD50 value for ingredients/preparations, and determine the classification and labeling of ingredients/preparations [3].

2.2. Subchronic oral toxicity test.

Subchronic oral toxicity is a test that evaluates the effects that arise when test animals are given repeated doses of the test preparation orally. The test preparation in multiple-dose levels is given every day to several groups of animals with one dose per group in 28 or 90 days, according to the subchronic oral toxicity test principle. Animals will be assessed for the existence of harmful effects while the test preparation is being delivered. If an animal dies during the test preparation phase and has not reached the rigor mortis (stiff) stage, the organs and tissues are promptly dinecropsed and examined macropatologically and histopathologically. All living animals were dyneciopsed at the end of the test preparation time, and macroecological observations were made on each organ and tissue. Hematological, clinical biochemical, and histopathological tests were also performed [9].

The goal of subchronic oral toxicity testing is to evaluate data about toxic effects of substances that were not detected in acute toxicity tests; data about the possibility of a toxic effect after repeated exposure to the test preparation over a period of time; data about the dosage that does not cause toxic effects (No Observed Adverse Effect Level / NOAEL); and studying the total effects and reversible effects of these substances [9].

2.3. Chronic oral toxicity test.

Chronic oral toxicity is a test that evaluates harmful effects that arise after the test preparation is given repeatedly throughout the course of the test animal's life. The chronic toxicity test is similar to the subchronic toxicity test in theory, but the test preparation must last at least: a. 9 months for widely recognized as safe test materials; or b. 12 months for a pure chemical or test substance with a hazardous potential [10].

2.4. Teratogenicity test.

Teratogenicity testing is a procedure for determining prenatal anomalies caused by administering test preparations during the creation of fetal organs (organogenesis period). This information comprises morphology (outside the fetus), soft tissue, and fetal skeletal abnormalities. The administration of preparation at various dose levels in different groups of pregnant animals for at least the organogenetic phase of pregnancy, one dose per group, is the basis of teratogenicity testing. The mother is surgically removed one day before birth, the uterus is removed, and the fetus is evaluated [11].

2.5. Skin sensitization test.

The skin sensitization test is used to determine if a chemical is capable of causing skin sensitivity. The skin sensitization test is based on animals being induced with and without Freund's Complete Adjuvant (FCA) via intradermal and/or topical injection to create an

immune system, followed by a challenge test. The Magnusson and Kligman scales were used to grade the severity of skin responses [12].

2.6. Eye irritation test.

The eye irritation test detects harmful effects in test animals (albino rabbits) following exposure to the test preparation in the eyes. The eye irritation test works on the idea of exposing a single dosage of the test preparation into one eye of numerous test animals who have previously been administered systemic analgesics and local anesthetics, with the untreated eye serving as a control. Injury to the conjunctiva, cornea, and iris was scored at certain time intervals to determine the degree of irritation/corrosion. The purpose of the eye irritation test is to learn about the potential dangers that might occur if the test preparation is exposed to the eyes and mucous membranes of the eyes [13].

2.7. Dermal irritation/corrosion test.

The acute dermal irritation/corrosion test is performed on animals (albino rabbits) to detect harmful effects that develop after up to 4 hours of skin exposure to the test preparation. The acute cutaneous irritation/corrosion test works on the idea of exposing the test preparation to the skin of the test animal in a single dosage, with the untreated skin region serving as a control. The acute dermal irritation/corrosion test is used to identify whether or not a chemical has an irritating impact on the skin, as well as to analyze and evaluate the properties of a material when it is exposed to the skin [14].

2.8. Vaginal mucosal irritation test.

The vaginal mucosal irritation test is used to evaluate a test preparation that directly touches vaginal tissue and cannot be evaluated otherwise. The vaginal mucosal irritation test is based on the test preparation's assumption that an extract in 0.9 percent NaCl solution or olive oil is made. The extract is then exposed to the test animals' vaginal mucosal lining for at least 5 with a 24-hour interval between exposures. The vaginal mucosal tissue was examined and evaluated for erythema, exudate, and edema during exposure. The test animals were slaughtered when the exposure was completed, and their vaginal mucosal tissue was removed for histological investigation. The purpose of the vaginal mucosal irritation test is to determine if medical devices that come into touch with the vaginal mucosa are safe [15].

2.9. Acute dermal toxicity test.

The acute dermal toxicity test is used to identify a hazardous impact that occurs within a short period of time following exposure to a test preparation via the dermal route in a single dosage. The acute dermal toxicity test is based on the idea that numerous groups of tested animals of one sex are exposed to the test preparation at a certain dose, with the first dose chosen based on the preliminary test findings [16].

2.10. Subchronic dermal toxicity test.

The subchronic dermal toxicity test is used to identify harmful effects that occur when repeated doses of the test preparation are given to test animals via the dermal route over a portion of their lives, but not more than 10% of their total lifetime. The premise of the

subchronic dermal toxicity test is that the test preparation is administered to many groups of tested animals every day at various dosages and exposed to their skin [17].

2.11. Carcinogenicity test.

The carcinogenic test is a procedure for detecting and identifying the test preparation's carcinogenic qualities after administering the test preparation to the test animal in many doses over the course of the animal's life in line with the desired route of administration. Toxicity and the development of neoplastic tumors were extensively monitored in the animals. The goal of a carcinogenicity test is to collect information about a chemical that causes a rise in the frequency of neoplasms, an increase in the proportion of malignant neoplasms, or a decrease in the appearance of neoplasms; determine the carcinogenicity of target organs; determine the timing of neoplasm emergence; characterize tumor dose-response correlations; determine the NOAEL (no adverse effect level) or the baseline Benchmark Dose (BMD); extrapolating carcinogenic effects to low-dose human shelf levels; data selection to test ideas about action mechanism [18].

3. *Centella asiatica*

3.1. Plant description.

C. asiatica, commonly used in Southeast Asian countries, is a traditional Chinese medicine with broad medicinal value [19,20]. *C. asiatica* from Apiaceae plant family is a small, perennial, herbaceous creeper. The genus Centella consists of 50 species distributed in tropical and subtropical regions of the world [21]. The species is original to tropical countries such as India, Sri Lanka, China, Indonesia, Malaysia, South Africa, and Madagascar [22]. It is native to the warmer regions of both hemispheres. This plant can be found along the sides of rivers, streams, ponds, and irrigated fields and grows wild in moist, gloomy locations up to 7000 feet. In India and Sri Lanka, it grows among stone walls or other rocky regions at the height of around 2000 feet [23]. The plant has several synonyms such as *C. coriacea* Nannfd., *Hydrocotyle asiatica* L., *H. lunata* Lam., and *Trisanthus cochinchinensis* Lour [24]. The systematic classification of *C. asiatica* is shown in Table 1.

Table 1. Taxonomy of *Centella asiatica.*

| Classification	Name
Kingdom	*Eukaryota*
Division	*Spermatophyta*
Class	*Dicotyledoneae*
Order	*Araliales*
Family	*Apiaceae*
Genus	*Centella*
Species	*Centella asiatica*

3.2. Pharmacological activity.

C. asiatica is a medicinal plant that has been used to treat skin disorders in the past [25–29], illness of the nervous system [30–32], disorders of the endocrine system [33–35], disorders of the cardiovascular system [36–42], disorders of the digestive diseases [43,44], respiratory diseases [45,46], gynecological diseases [47–49], effect on rheumatoid arthritis [50,51] and antiaging effect [52]. *C. asiatica* and its triterpenoids can be used in many diseases because they have anti-apoptotic and anti-inflammatory effects [41,53], improve mitochondrial

function, and relieve oxidant stress [19]. Lip wrinkling was minimized by using asiaticoside as a lipstick for 8 days. *C. asiatica*, but not tretinoin, significantly increased skin moisture [54]. *C. asiatica* showed anti-inflammatory action against dextran sulfate sodium (DSS)-induced colitis, which could be due to the restoration of mucosa barrier and gut microbiota balance. This suggests that *C. asiatica* could be used to treat ulcerative colitis clinically [55].

3.3. Phytochemicals.

The major chemical class in *C. asiatica* are the pentacyclic triterpenoids [56–58] present either in their free or glycosidic forms, asiaticoside [59], madecassoside, asiatic acid, and madecassic acid [60]. *C. asiatica* has phytosterol (campesterol, sitosterol, and stigmasterol), phenolic compounds like flavonoids (quercetin and kaempferol), ferulic acid, chlorogenic acid and gulonic acid [61]. Apart from triterpenoids, flavonoids, phenolic acids and sterols have been reported as essential oils [62].

Mostly, asiaticoside and madecassoside are mainly plentiful triterpenoids found in *C. asiatica*, and the concentrations depend on the environment, origin, and time of harvest or the preparation methods used [60]. *C. asiatica* and its triterpenoids have wide therapeutic agents [19]. The characteristic mechanism mainly involves the following four aspects: (1) anti-apoptosis; (2) anti-fibrosis (3) anti-inflammatory; and (4) antioxidant [19,63].
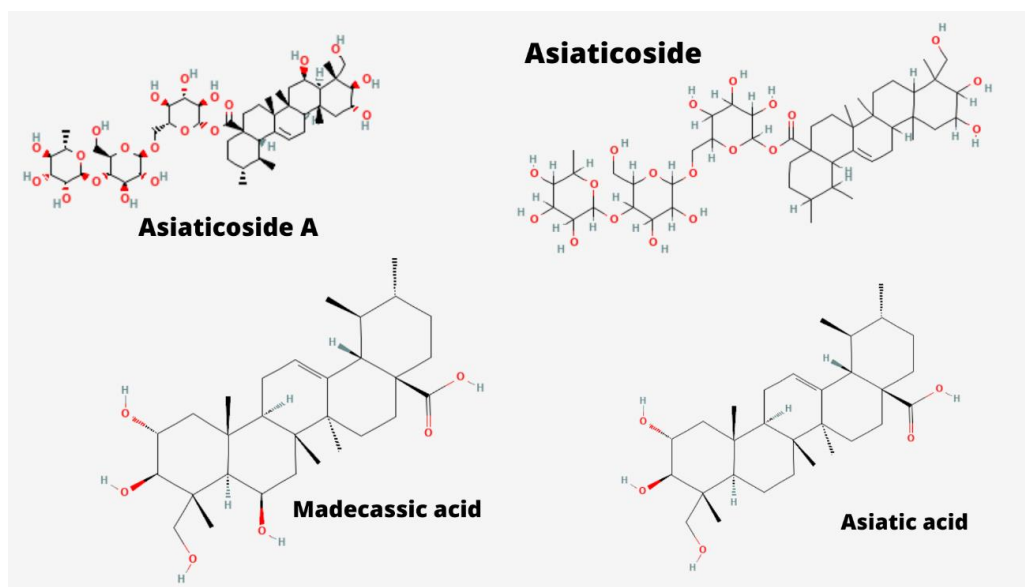
Figure 2. Triterpenoid found in *Centella asiatica.*

Standardized extracts of *C. asiatica* such as titrated extract of *C. asiatica*, TTFCA, and total triterpenic fraction are marketed under different names such as Madecassol, Centellase, and Blastoestimulina. The compositions of these highly purified extracts are the same, in which 40% of the extracts are asiaticoside, and the remaining 60% are a mixture of asiatic acid and madecassic acid [26,64]. Other standardized extracts of *C. asiatica* include ECa 233, which contains at least 80% asiaticoside and madecassoside [65], and Centellicum, which contains 35% of triterpenes [66].

4. Toxicity Studies of *Centella asiatica*

Most of the toxicity tests performed on *C. asiatica* were acute toxicity tests (Table 2); there was one study that reported a single compound toxicity test on *C. asiatica in vitro* using

zebrafish and one study using cells to see cell viability. Generally, the test animals used are mice and rats. Toxicity test on *C. asiatica* in the form of leaf extract, standardized leaf extract, and whole plant juice. The resume of the acute toxicity test on *C. asiatica* is shown in table 2; Subacute toxicity test *Centella asiatica* in Table 3 and Subchronic toxicity test *Centella asiatica* in Table 4.

Table 2. Acute toxicity test on *C. asiatica.*

No	Sample	Dose	Time	Observation	Subject	Parameters	Result	Conclusion	Ref.
1	ECa 233 (standardization *C. asiatica*)	single dose of 10 g/kg	Once	14 days	Mice	toxic signs, mortality and gross pathological lesions	There was no evidence of toxicity or fatality in male or female mice, and no severe pathological abnormalities were seen in any organs.	ECa 233 is a safe compound that should be explored further into pharmaceuticals or dietary supplements.	[67]
2	Leaf acetone extract *C. asiatica*	100, 500, 1000, 2000 and 4000 mg/kg	Once	3 hours continuously, followed up to 24 hours after giving the extract	female and male Swiss mice	Psychological habits and activities	In male and female mice, there were no significant changes in behavior, respiration, cutaneous effects, sensory nerve system responses, or gastrointestinal effects. There will be no death if water and food consumption are not reduced, no adverse effect of *C. asiatica*	The toxicity of *C. asiatica* appears to be low. The mean lethal dosage (LD50) for male and female mice is greater than 4000 mg/kg, which puts this plant's therapeutic usage in traditional medicine in jeopardy.	[68]
3	Juice *C. asiatica* (whole plant powder)	3, 5, and 7 g/kg body weight	Once	14 days	Female and male Albino Swiss mice	body weight, food, and water consumption	All dosages had no effect on the animals' body weight, food intake, or water consumption.	Plant powder from *C. asiatica* had no toxicity in mice.	[69]
4	Leaf ethanol extract *C. asiatica*	Cons. 2500, 1250, 625, 312.5, and 156.5 mg/L	Once	2, 6, 24, 48, 72 and 96 hours	Zebrafish	Mortality, The LC50 (test concentration that kills 50% of the fishes)	At dosages of 312.5 and 156.5 mg / L, no zebrafish died; at 1250 mg / L, half of the zebrafish died (LD50); and at 2500 mg / L, 100 percent of the zebrafish died.	Adult zebrafish survival was impacted by a leaf ethanolic extract of *C. asiatica* at doses more than 1000 mg/kg BW for up to 96 hours.	[60]
5	Four- active compound *C. asiatica*	Cons. 10, 50, 100, and 500 mg/kg	Once	2, 6, 24, 48, 72 and 96 hours	Zebrafish	Mortality, The LC50 (test concentration that kills 50% of the fish)	The adult, wild-type zebrafish did not die after ninety-six hours of exposure to each chemical at test dosages of 10 to 500 mg/kg.	asiatic acid, madecassic acid, asiaticoside and madecassoside did not cause toxicity effect	[60]
6	Leaf ethanol extract *C. asiatica*	Dose-response 1-1000 μg/ml between the percentage of cell viability and conc. of the extracts were constructed Hemolysis: Nine different conc.	Once	-	PBMC (in-vitro)	Cell Viability, hemolysis	Cell viability: Lethal concentration 50 (LC50) value for *Centella asiatica* extract was reported to be 69.17 ±3.2 μg/ml Hemolysis: LC50 476.19 ±5.9 μg/ml.	-	[70]

No	Sample	Dose	Time	Observation	Subject	Parameters	Result	Conclusion	Ref.
		(0,5,10,30,50, 100,200,300, 400μg/ml)							
7	Standardization extract (INDCA)	0-2000 mg/kg BW	Once	14 days	Rat	mortality and clinical signs of toxicity; histology	LD50 more than 2000 mg/kg	no significant toxic effects, excellent safety margins of INDCA	[71]

Table 3. Subacute toxicity test *Centella asiatica.*

	Sample	Dose	Time	Observation	Subject	Parameters	Result	Conclusion	Ref.
1	Leaf acetone extract *C. asiatica*	100, 500, 1000, 2000 and 4000 mg/kg	15 days (every morning)	Every 3 days (weight measurement), measurement parameters at the end of the experiment	female and male Swiss mice	Bodyweight, organ weight, hematology (control group, doses 1000 and 4000 mg), biochemistry	There were no significant changes between the experimental group and the control group (body weight, organ macroscopic analysis), hematological, or biochemistry.	*C. asiatica* appears to have few harmful effects, which could compromise the medicinal use of this plant in folk medicine	[68]

Table 4. Subchronic toxicity test *Centella asiatica.*

No	Sample	Dose	Time	Observation	Subject	Parameters	Result	Conclusion	Ref.
1	ECa 233 (standardization *C. asiatica*)	10, 100 and 1,000 mg/kg/day	90 days	body weight, food consumption, and animal health were measured weekly. Other parameters at the end of the treatment.	Rat	Effects on body weight, food consumption, and animal health; gross pathology and organs weight; hematological parameters, clinical chemistry, Histopathology	There were no hazardous signs in any of the measures, except for the 1,000 mg dosage, which had a considerably larger number of white blood cell (WBC) than the control group.	ECa 233 is a safe compound that should be explored further into pharmaceuticals or dietary supplements.	[67]
2	Shade dried, powdered, muslins, aerial parts were used along with gum acacia (binding agent) for oral administration in rats.	250, 500, 1,000 mg/kg.	30 days	10 days (interval) for body weight and on 32nd for all parameters	Albino rats	clinical signs, gross behavioral changes, morbidity, and mortality once daily. Hematology, marker enzyme level, vital organ.	• There was no evident toxicity or alterations in body weight growth. • a considerable rise in spleen weight in rats given *C. asiatica* at 1,000 mg/kg • An increase in the level of ALT and AST • Increase in BUN and CREATININE • Hematological values differ significantly from those in the control group. • *C. asiatica* at 1,000 mg/kg induced a marginal increase in apoptosis in hepatic tissue and a minor increase in apoptosis in renal tissue.	Based on the findings, it was determined that giving *C. asiatica* to rats at a dose of 1,000 mg/kg for 30 days might induce severe liver tissue damage. *C. asiatica*-induced apoptosis in the liver provides a viable avenue for further research into its involvement in tumor therapy.	[72]

The chronic toxicity test for *C. asiatica* yielded no results. A mutagenic toxicity study was found [71], which discovered mutagenic toxicity research in which the tester strains met the quality check standards. Up to a concentration of 5000 g/plate, the bacterial background lawn was equivalent to that of the respective viable count (VC) plate. In the presence or absence of metabolic activation, no significant increases in the revertant colony count were seen in any of the five strains at any of the test dosages. The revertant count increased significantly when positive controls were used. The frequencies of spontaneous reversion in both the negative and positive controls were within historical ranges. In any of the five tester strains preincubated with the test item, no physiologically meaningful rise in revertant numbers was detected. The findings imply that standard extract INDCA did not cause gene mutation in the genomes of the strains tested through pair alterations or frameshifts under the experimental circumstances.

5. Safety and Toxicity of *Centella asiatica*

Toxicity assessment is an important aspect of pharmacological research and the quality control of plant-based health products. The most-reported toxicity test is acute toxicity. The most tested part was leaves. There was 1 study using whole plants as the test sample [69]. The solvent used is mostly ethanol; one experiment used acetone as the solvent [68]. The test animals used include mice, rats, and zebrafish. Most of the research indicates the level of safety. LD50 value 1250 mg/kg [60], and more than 2000 mg/kg [71]. But with asetone extract LD50 is higher 4000mg/kg [68]. In whole-plant trials (*C. asiatica* juice), a dose of 7000 mg/kg still did not show signs of toxicity [69].

In clinical studies, *C. asiatica* extract dosages of 250 mg and 500 mg were well tolerated in single and repeated oral doses. According to modern pharmacological testing, the interaction potential of *C. asiatica* physiologically active substances with cytochrome (CYP) isoenzymes is insignificant, and the extract's heavy metal concentration is within the allowed range [73].

According to another case report, three women who got jaundice after using *C. asiatica* for 30, 20, and 60 days were diagnosed with granulomatous hepatitis, and their symptoms improved once the medication was withdrawn. Although preclinical investigations of *C. asiatica* have discovered that it has a wide variety of pharmacological effects and shown its safety, given the negative reports in a few clinical instances, a thorough study is advised to discover clinical doses with the greatest safety. Hepatotoxic chemicals are produced by several plants. Germander, Skullcap, and Glycyrrhizin contain di- or triterpenic active principles that promote apoptosis and change cell membranes, causing liver damage. These pathways may have resulted in *C. asiatica*-related injuries [74].

6. Conclusions

Centella asiatica is an herb used in traditional Chinese medicine. Mainly components that potenial are asiaticoside, asiatic acid, madecassoside, and madecassic acid. *C. asiatica* and its triterpenoids have a broad range of medicinal values. *C. asiatica* is a medicinal plant with reported uses for treating skin problems, neurological diseases, endocrine diseases, cardiovascular diseases, digestive diseases, respiratory diseases, gynecological diseases, and its effect on rheumatoid arthritis. *C. asiatica* and its triterpenoids can be used in many medical situations because they have anti-inflammatory and anti-apoptotic effects, relieve oxidant stress, and improve mitochondrial function. Most of the findings from this literature study indicate that the *C. asiatica* plant is quite safe. However, because there are negative case reports

due to consumption of *C. asiatica* in patients with liver disorders, additional investigations are needed regarding the impact of using *C. asiatica* on other disorders.

Funding

Acknowledgments

Conflicts of Interest

The authors declare no conflict of interest.

References

1. World Health Organization *Effective medicines regulation : ensuring safety, efficacy and quality*; World Health Organization, **2003**.
2. Mehla, J.; Gupta, P.; Pahuja, M.; Diwan, D.; Diksha, D. Indian Medicinal Herbs and Formulations for Alzheimer's Disease, from Traditional Knowledge to Scientific Assessment. *Brain Sci* **2020**, *10*, E964, https://doi.org/10.3390/brainsci10120964.
3. OECD *Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method*; Organisation for Economic Co-operation and Development: Paris, **2002**.
4. Idris, F.N.; Mohd Nadzir, M. Comparative Studies on Different Extraction Methods of Centella Asiatica and Extracts Bioactive Compounds Effects on Antimicrobial Activities. *Antibiotics* **2021**, *10*, 457, https://doi.org/10.3390/antibiotics10040457.
5. Fischer, I.; Milton, C.; Wallace, H. Toxicity Testing Is Evolving! *Toxicology Research* **2020**, *9*, 67–80, https://doi.org/10.1093/toxres/tfaa011.
6. Movia, D.; Bruni-Favier, S.; Prina-Mello, A. In Vitro Alternatives to Acute Inhalation Toxicity Studies in Animal Models—A Perspective. *Frontiers in Bioengineering and Biotechnology* **2020**, *8*, 549, https://doi.org/10.3389/fbioe.2020.00549.
7. Parasuraman, S. Toxicological Screening. *J Pharmacol Pharmacother* **2011**, *2*, 74–79, https://doi.org/10.4103/0976-500X.81895.
8. Toxicity Studies. *Prog Drug Res* **2016**, *71*, 81–87, Toxicity Studies. https://pubmed.ncbi.nlm.nih.gov/26939270/
9. OECD *Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents*; Organisation for Economic Co-operation and Development: Paris, 2018.
10. OECD *Test No. 452: Chronic Toxicity Studies*; Organisation for Economic Co-operation and Development: Paris, 2018.
11. Reproductive Toxicity – Developmental Studies (Teratogenicity) (Two Species) | Pesticide Registration Toolkit | Food and Agriculture Organization of the United Nations Available online: https://www.fao.org/pesticide-registration-toolkit/registration-tools/data-requirements-and-testing-guidelines/study-detail/en/c/1186762/ (accessed on 13 October 2021).
12. OECD ILibrary | Test No. 406: Skin Sensitisation Available online: https://www.oecd-ilibrary.org/environment/test-no-406-skin-sensitisation_9789264070660-en (accessed on 13 October 2021).
13. OECD *Test No. 405: Acute Eye Irritation/Corrosion*; Organisation for Economic Co-operation and Development: Paris, 2021.
14. OECD ILibrary | Test No. 404: Acute Dermal Irritation/Corrosion Available online: https://www.oecd-ilibrary.org/environment/test-no-404-acute-dermal-irritation-corrosion_9789264070622-en (accessed on 13 October 2021).
15. ISO 10993-10:2010(En), Biological Evaluation of Medical Devices — Part 10: Tests for Irritation and Skin Sensitization Available online: https://www.iso.org/obp/ui/fr/#iso:std:iso:10993:-10:ed-3:v1:en (accessed on 13 October 2021).
16. Test No. 402: Acute Dermal Toxicity | En | OECD Available online: https://www.oecd.org/env/test-no-402-acute-dermal-toxicity-9789264070585-en.htm (accessed on 13 October 2021).
17. OECD *Test No. 411: Subchronic Dermal Toxicity: 90-Day Study*; Organisation for Economic Co-operation and Development: Paris, 1981.

18. Test No. 451: Carcinogenicity Studies | En | OECD, https://rb.gy/xnp0h3 (accessed on 13 October 2021).

19. Sun, B.; Wu, L.; Wu, Y.; Zhang, C.; Qin, L.; Hayashi, M.; Kudo, M.; Gao, M.; Liu, T. Therapeutic Potential of Centella Asiatica and Its Triterpenes: A Review. *Front. Pharmacol.* **2020**, *11*, https://doi.org/10.3389/fphar.2020.568032.

20. Torbati, F.A.; Ramezani, M.; Dehghan, R.; Amiri, M.S.; Moghadam, A.T.; Shakour, N.; Elyasi, S.; Sahebkar, A.; Emami, S.A. Ethnobotany, Phytochemistry and Pharmacological Features of Centella Asiatica: A Comprehensive Review. *Adv Exp Med Biol* **2021**, *1308*, 451–499, https://doi.org/10.1007/978-3-030-64872-5_25.

21. Azerad, R. Chemical Structures, Production and Enzymatic Transformations of Sapogenins and Saponins from Centella Asiatica (L.) Urban. *Fitoterapia* **2016**, *114*, 168–187, https://doi.org/10.1016/j.fitote.2016.07.011.

22. Orhan, I.E. Centella Asiatica (L.) Urban: From Traditional Medicine to Modern Medicine with Neuroprotective Potential **2021,** *2012*, https://doi.org/10.1155/2012/946259. .

23. Chandrika, U.G.; Kumara, P.A.P. Gotu Kola (Centella Asiatica): Nutritional Properties and Plausible Health Benefits. *Advances in food and nutrition research* **2015**, *76*, 125–157, https://doi.org/10.1016/bs.afnr.2015.08.001.

24. Gray, N.E.; Zweig, J.A.; Caruso, M.; Zhu, J.Y.; Wright, K.M.; Quinn, J.F.; Soumyanath, A. Centella Asiatica Attenuates Hippocampal Mitochondrial Dysfunction and Improves Memory and Executive Function in β-Amyloid Overexpressing Mice. *Mol Cell Neurosci* **2018**, *93*, 1–9, https://doi.org/10.1016/j.mcn.2018.09.002.

25. Venesia, N.F.; Fachrial, E.; Lister, I.N.E. Effectiveness Test of Centella Asiatica Extract on Improvement of Collagen and Hydration in Female White Rat (Rattus Norwegicus Wistar). **2020**, *65*, 10, https://asrjetsjournal.org/index.php/American_Scientific_Journal/article/view/5658.

26. Bylka, W.; Znajdek-Awiżeń, P.; Studzińska-Sroka, E.; Brzezińska, M. Centella Asiatica in Cosmetology. *Postepy Dermatol Alergol* **2013**, *30*, 46–49, https://doi.org/10.5114/pdia.2013.33378.

27. Buranasudja, V.; Rani, D.; Malla, A.; Kobtrakul, K.; Vimolmangkang, S. Insights into Antioxidant Activities and Anti-Skin-Aging Potential of Callus Extract from Centella Asiatica (L.). *Sci Rep* **2021**, *11*, 13459, https://doi.org/10.1038/s41598-021-92958-7.

28. Damayanti; Umborowati, M.A.; Anggraeni2, S.; Prakoeswa, C.R.S. The Role of Aloe Vera and Centella Asiatica to the Improvement of Skin Barrier Function in Indonesian Batik Workers. *Indian Journal of Forensic Medicine & Toxicology* **2021**, *15*, 2805–2811, https://doi.org/10.37506/ijfmt.v15i3.15731.

29. Indria, I.; Rahayu, A. Effectiveness of Gotu Kola Extract Lotion (Centella Asiatica) in Reducing Stretch Marks. *JHS* **2021**, *14*, 196–201, https://doi.org/10.33086/jhs.v14i3.2066.

30. Song, D.; Jiang, X.; Liu, Y.; Sun, Y.; Cao, S.; Zhang, Z. Asiaticoside Attenuates Cell Growth Inhibition and Apoptosis Induced by Aβ1-42 via Inhibiting the TLR4/NF-KB Signaling Pathway in Human Brain Microvascular Endothelial Cells. *Front Pharmacol* **2018**, *9*, https://doi.org/10.3389/fphar.2018.00028.

31. Gelders, G.; Baekelandt, V.; Van der Perren, A. Linking Neuroinflammation and Neurodegeneration in Parkinson's Disease **2018**, *2018,* https://www.hindawi.com/journals/jir/2018/4784268.

32. Mohammadi, A.; Amooeian, V.G.; Rashidi, E. Dysfunction in Brain-Derived Neurotrophic Factor Signaling Pathway and Susceptibility to Schizophrenia, Parkinson's and Alzheimer's Diseases. *Curr Gene Ther* **2018**, *18*, 45–63, https://doi.org/10.2174/1566523218666180302163029.

33. M.; M.; Abas, F.; Khatib, A.; Perumal, V.; Suppaiah, V.; Ismail, A.; Hamid, M.; Lajis, N.H. Metabolic Alteration in Obese Diabetes Rats upon Treatment with Centella Asiatica Extract. *Journal of Ethnopharmacology* **2016**, *180*, https://doi.org/10.1016/j.jep.2016.01.001.

34. Oyenihi, A.B.; Chegou, N.N.; Oguntibeju, O.O.; Masola, B. Centella Asiatica Enhances Hepatic Antioxidant Status and Regulates Hepatic Inflammatory Cytokines in Type 2 Diabetic Rats. *Pharmaceutical Biology* **2017**, *55*, 1671–1678, https://doi.org/10.1080/13880209.2017.1318293.

35. Masola, B.; Oguntibeju, O.; Oyenihi, A. Centella Asiatica Ameliorates Diabetes-Induced Stress in Rat Tissues via Influences on Antioxidants and Inflammatory Cytokines. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **2018**, *101*, 447–457, https://doi.org/10.1016/j.biopha.2018.02.115.

36. Fong, L.Y.; Ng, C.T.; Zakaria, Z.A.; Baharuldin, M.T.H.; Arifah, A.K.; Hakim, M.N.; Zuraini, A. Asiaticoside Inhibits TNF-α-Induced Endothelial Hyperpermeability of Human Aortic Endothelial Cells. *Phytother Res* **2015**, *29*, 1501–1508, https://doi.org/10.1002/ptr.5404.

37. Ly, F.; Ct, N.; Yk, Y.; Mn, H.; Z, A. Asiatic Acid Stabilizes Cytoskeletal Proteins and Prevents TNF-α-Induced Disorganization of Cell-Cell Junctions in Human Aortic Endothelial Cells. *Vascul Pharmacol* **2018**, *117*, 15–26, https://doi.org/10.1016/j.vph.2018.08.005.

38. Jing, L.; Haitao, W.; Qiong, W.; Fu, Z.; Nan, Z.; Xuezheng, Z. Anti Inflammatory Effect of Asiaticoside on Human Umbilical Vein Endothelial Cells Induced by Ox-LDL. *Cytotechnology* **2018**, *70*, 855–864, https://doi.org/10.1007/s10616-018-0198-4.

39. Dai, Y.; Wang, Z.; Quan, M.; Lv, Y.; Li, Y.; Xin, H.-B.; Qian, Y. Asiatic Acid Protests against Myocardial Ischemia/Reperfusion Injury via Modulation of Glycometabolism in Rat Cardiomyocyte. *Drud, Design, Development and Therapy* **2018**, *2018*, 3573-3582, https://doi.org/10.2147/DDDT.S175116.

40. Setyaningsih, W.A.W.; Arfian, N.; Fitriawan, A.S.; Yuniartha, R.; Sari, D.C.R. Ethanolic Extract of Centella Asiatica Treatment in the Early Stage of Hyperglycemia Condition Inhibits Glomerular Injury and Vascular

Remodeling in Diabetic Rat Model. *Evidence-Based Complementary and Alternative Medicine* **2021**, *2021*, e6671130, https://doi.org/10.1155/2021/6671130.

41. Seo, M.-G.; Jo, M.-J.; Hong, N.I.; Kim, M.J.; Shim, K.S.; Shin, E.; Lee, J.J.; Park, S.-J. Anti-Inflammatory and Anti-Vascular Leakage Effects by Combination of Centella Asiatica and Vitis Vinifera L. Leaf Extracts. *Evidence-Based Complementary and Alternative Medicine* **2021**, *2021*, e7381620, https://doi.org/10.1155/2021/7381620.

42. Li, H.; Tian, X.; Ruan, Y.; Xing, J.; Meng, Z. Asiatic Acid Alleviates Ang-II Induced Cardiac Hypertrophy and Fibrosis via MiR-126/PIK3R2 Signaling. *Nutrition & Metabolism* **2021**, *18*, 71, https://doi.org/10.1186/s12986-021-00596-7.

43. Zheng, H.-M.; Choi, M.-J.; Kim, J.M.; Cha, K.H.; Lee, K.W.; Park, Y.H.; Hong, S.-S.; Lee, D.H. Centella Asiatica Leaf Extract Protects Against Indomethacin-Induced Gastric Mucosal Injury in Rats. *J Med Food* **2016**, *19*, 38–46, https://doi.org/10.1089/jmf.2015.3464.

44. Choi, M.-J.; Zheng, H.-M.; Kim, J.M.; Lee, K.W.; Park, Y.H.; Lee, D.H. Protective Effects of Centella Asiatica Leaf Extract on Dimethylnitrosamine-induced Liver Injury in Rats. *Mol Med Rep* **2016**, *14*, 4521–4528, https://doi.org/10.3892/mmr.2016.5809.

45. Cui, Q.; Ren, J.; Zhou, Q.; Yang, Q.; Li, B. Effect of Asiatic Acid on Epithelial-mesenchymal Transition of Human Alveolar Epithelium A549 Cells Induced by TGF-β1. *Oncology Letters* **2019**, *17*, 4285–4292, https://doi.org/10.3892/ol.2019.10140.

46. Sh, D.; Yw, L.; F, W.; Hz, T.; Zd, H. Asiatic Acid Ameliorates Pulmonary Fibrosis Induced by Bleomycin (BLM) via Suppressing pro-Fibrotic and Inflammatory Signaling Pathways. *Biomed Pharmacother* **2017**, *89*, 1297–1309, https://doi.org/10.1016/j.biopha.2017.03.005.

47. Kong, D.; Fu, P.; Zhang, Q.; Ma, X.; Jiang, P. Protective Effects of Asiatic Acid against Pelvic Inflammatory Disease in Rats. *Experimental and Therapeutic Medicine* **2019**, *17*, 4687–4692, https://doi.org/10.3892/etm.2019.7498.

48. Fard, S.E.; Tafvizi, F.; Torbati, M.B. Silver Nanoparticles Biosynthesised Using Centella Asiatica Leaf Extract: Apoptosis Induction in MCF-7 Breast Cancer Cell Line. *IET Nanobiotechnol* **2018**, *12*, 994–1002, https://doi.org/10.1049/iet-nbt.2018.5069.

49. Cao, S.-Y.; Wang, W.; Nan, F.-F.; Liu, Y.-N.; Wei, S.-Y.; Li, F.-F.; Chen, L. Asiatic Acid Inhibits LPS-Induced Inflammatory Response in Endometrial Epithelial Cells. *Microb Pathog* **2018**, *116*, 195–199, https://doi.org/10.1016/j.micpath.2018.01.022.

50. Wang, T.; Wei, Z.; Dou, Y.; Yang, Y.; Leng, D.; Kong, L.; Dai, Y.; Xia, Y. Intestinal Interleukin-10 Mobilization as a Contributor to the Anti-Arthritis Effect of Orally Administered Madecassoside: A Unique Action Mode of Saponin Compounds with Poor Bioavailability. *Biochem Pharmacol* **2015**, *94*, 30–38, https://doi.org/10.1016/j.bcp.2015.01.004.

51. Yu, W.-G.; Shen, Y.; Wu, J.-Z.; Gao, Y.-B.; Zhang, L.-X. Madecassoside Impedes Invasion of Rheumatoid Fibroblast-like Synoviocyte from Adjuvant Arthritis Rats via Inhibition of NF-KB-Mediated Matrix Metalloproteinase-13 Expression. *Chin J Nat Med* **2018**, *16*, 330–338, https://doi.org/10.1016/S1875-5364(18)30064-5.

52. Karsono, A.H.; Tandrasasmita, O.M.; Berlian, G.; Tjandrawinata, R.R. <p>Potential Antiaging Effects of DLBS1649, a <em>Centella Asiatica</Em> Bioactive Extract</P>. *JEP* **2021**, *13*, 781–795, https://doi.org/10.2147/JEP.S299547.

53. Damayanti, S.; Fabelle, N.R.; Yooin, W.; Insanu, M.; Jiranusornkul, S.; Wongrattanakamon, P. Molecular Modeling for Potential Cathepsin L Inhibitor Identification as New Anti-Photoaging Agents from Tropical Medicinal Plants. *J Bioenerg Biomembr* **2021**, *53*, 259–274, https://doi.org/10.1007/s10863-021-09893-5.

54. Kongkaew, C.; Meesomperm, P.; Scholfield, C.N.; Chaiwiang, N.; Waranuch, N. Efficacy and Safety of Centella Asiatica (L.) Urb. on Wrinkles: A Systematic Review of Published Data and Network Meta-Analysis. *J Cosmet Sci* **2020**, *71*, 439–454, https://pubmed.ncbi.nlm.nih.gov/33413787/.

55. Li, H.; Chen, X.; Liu, J.; Chen, M.; Huang, M.; Huang, G.; Chen, X.; Du, Q.; Su, J.; Lin, R. Ethanol Extract of Centella Asiatica Alleviated Dextran Sulfate Sodium-Induced Colitis: Restoration on Mucosa Barrier and Gut Microbiota Homeostasis. *J Ethnopharmacol* **2021**, *267*, 113445, https://doi.org/10.1016/j.jep.2020.113445.

56. Zhang, D.; Arunachalam, K.; Wang, Y.; Zhang, Y.; Yang, J.; Hein, P.P.; Mon, A.M.; Li, J.; Inta, A.; Yang, X. Evaluation on Antidiabetic Properties of Medicinal Plants from Myanmar. *ScientificWorldJournal* **2021**, *2021*, 1424675, https://doi.org/10.1155/2021/1424675.

57. Wahyuningtyas, D.; Saputra, H.F.; Sunarsih, S. Optimasi Pengambilan Triterpenoid Dari Daun Pegagan (Centella Asiatica) Dengan Metode Ekstraksi Bertingkat. *Jurnal Pengendalian Pencemaran Lingkungan (JPPL)* **2021**, *3*, 20–26, https://doi.org/10.35970/jppl.v3i1.538.

58. Ren, B.; Luo, W.; Xie, M.; Zhang, M. Two New Triterpenoid Saponins from Centella Asiatica. *Phytochemistry Letters* **2021**, *44*, 102–105, https://doi.org/10.1016/j.phytol.2021.06.012.

59. Zulkipli, N.N.; Zakaria, R.; Long, I.; Abdullah, S.F.; Muhammad, E.F.; Wahab, H.A.; Sasongko, T.H. In Silico Analyses and Cytotoxicity Study of Asiaticoside and Asiatic Acid from Malaysian Plant as Potential MTOR Inhibitors. *Molecules* **2020**, *25*, E3991, https://doi.org/10.3390/molecules25173991.

60. Zakaria, F.; Ibrahim, W.N.W.; Ismail, I.S.; Ahmad, H.; Manshoor, N.; Ismail, N.; Zainal, Z.; Shaari, K. LCMS/MS Metabolite Profiling and Analysis of Acute Toxicity Effect of the Ethanolic Extract of Centella Asiatica on Zebrafsh Model. *Pertanika Journal of Science & Technology* **2019**, *27*, http://psasir.upm.edu.my/id/eprint/68709/.

61. Razali, N.N.M.; Ng, C.T.; Fong, L.Y. Cardiovascular Protective Effects of Centella Asiatica and Its Triterpenes: A Review. *Planta Med* **2019**, *85*, 1203–1215, https://doi.org/10.1055/a-1008-6138.

62. Francis, S.C.; Thomas, M.T. Essential Oil Profiling of Centella Asiatica (L.) Urb.-a Medicinally Important Herb. *South Indian Journal of Biological Sciences* **2016**, *2*, 169–173, http://dx.doi.org/10.22205/sijbs%2F2016%2Fv%2Fi1%2F100387.

63. Muchtaromah, B.; Habibie, S.; Ma'arif, B.; Ramadhan, R.; Savitri, E.S.; Maghfuroh, Z. Comparative Analysis of Phytochemicals and Antioxidant Activity of Ethanol Extract of Centella Asiatica Leaves and Its Nanoparticle Form. *Tropical Journal of Natural Product Research* **2021**, *5*, 465–469, http://dx.doi.org/10.26538/tjnpr/v5i3.9.

64. James, J.T.; Dubery, I.A. Pentacyclic Triterpenoids from the Medicinal Herb, Centella Asiatica (L.) Urban. *Molecules* **2009**, *14*, 3922–3941, https://doi.org/10.3390/molecules14103922.

65. Anukunwithaya, T.; Tantisira, M.H.; Tantisira, B.; Khemawoot, P. Pharmacokinetics of a Standardized Extract of Centella Asiatica ECa 233 in Rats. *Planta Med* **2017**, *83*, 710–717, https://doi.org/10.1055/s-0042-122344.

66. Cotellese, R.; Hu, S.; Belcaro, G.; Ledda, A.; Feragalli, B.; Dugall, M.; Hosoi, M.; Ippolito, E. Centella Asiatica (Centellicum®) Facilitates the Regular Healing of Surgical Scars in Subjects at High Risk of Keloids. *Minerva Chir* **2018**, *73*, 151–156, https://doi.org/10.23736/S0026-4733.18.07666-6.

67. Chivapat, S.; Chavalittumrong, P.; Tantisira, M.H. Acute and Sub-Chronic Toxicity Studies of a Standardized Extract of Centella Asiatica ECa 233. *Thai J Pharm Sci* **2011**, *35*, 55–64, https://www.researchgate.net/publication/233996776_Acute_and_sub-chronic_toxicity_of_a_standardized_extract_of_Centella_asiatica_ECa_233.

68. Chauhan, P.K.; Singh, V. Acute and Subacute Toxicity Study of the Acetone Leaf Extract of Centella Asiatica in Experimental Animal Models. *Asian Pacific Journal of Tropical Biomedicine* **2012**, *2*, S511–S513, https://doi.org/10.1016/S2221-1691(12)60263-9.

69. Pingale, S.S. Acute Toxicity Study for Centella Asiatica Whole Plant Powder. *Newsletter, Pharmacologyonline* **2008**, *3*, 80–84, https://pharmacologyonline.silae.it/files/newsletter/2008/vol3/10.Pingale1r.pdf.

70. Ghosh, K.; Indra, N. Phytochemistry, in Vitro Free Radical Scavenging, Chelating and Toxicity of Centela Asiatica L.(Apiaceae) Ethanolic Leaf Extract. *International Journal of Pharmaceutical Sciences Review and Research* **2014**, *29*, 328–334, https://www.researchgate.net/publication/268576879_Phytochemistry_in_vitro_Free_Radical_Scavenging_Chelating_and_Toxicity_of_Centela_asiatica_L_Apiaceae_Ethanolic_Leaf_Extract.

71. Deshpande, P.O.; Mohan, V.; Thakurdesai, P. Preclinical Safety Assessment of Standardized Extract of Centella Asiatica (L.) Urban Leaves. *Toxicology international* **2015**, *22*, 10, https://doi.org/10.4103/0971-6580.172251.

72. Oruganti, M.; Roy, B.K.; Singh, K.K.; Prasad, R.; Kumar, S. Safety Assemment of Centella Asiatica in Albino Rats. *Pharmacognosy Journal* **2010**, *2*, 5–13.

73. Kar, A.; Pandit, S.; Mukherjee, K.; Bahadur, S.; Mukherjee, P.K. Safety Assessment of Selected Medicinal Food Plants Used in Ayurveda through CYP450 Enzyme Inhibition Study. *Journal of the Science of Food and Agriculture* **2017**, *97*, 333–340, https://doi.org/10.1002/jsfa.7739.

74. Jorge, O.A.; Jorge, A.D. Hepatotoxicity Associated with the Ingestion of Centella Asiatica. *Rev Esp Enferm Dig* **2005**, *97*, 115–124, https://doi.org/10.4321/s1130-01082005000200006.