WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 10, 659-691. Review Article ISSN 2277–7105

A COMPREHENSIVE OVERVIEW OF TOXICITY IN ISOLATED COMPOUND AND IN HERBAL PLANT

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Article Received on
27 March 2025,

Revised on 17 April 2025,
Accepted on 07 May 2025

DOI: 10.20959/wjpr202510-36769

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ABSTRACT

Toxicity testing is an important part of toxicology that helps us understand how harmful a chemical or substance can be to living organisms. This review explains different types of toxicity, such as acute, sub-acute, sub-chronic, chronic, reproductive, developmental, and genetic toxicity. Each type is described with its causes, effects on the body, and how it is tested using international guidelines like those from OECD, REACH, and ICH. The review also covers specific forms of toxicity like effects on the nervous system (Neurotoxicity), immune system (Immunotoxicity), and hormone system (Endocrine disruption). A special focus is given to genotoxicity and mutagenicity because they are closely linked to cancer risks. The article also discusses how toxicity happens at the cellular level, including damage caused by oxidative stress, harmful chemical reactions in the body, problems in mitochondria (The energy centers of cells), and effects on cell receptors. Along with that, the review includes summaries of eight recent research studies to show current trends in toxicity testing. Newer methods like in vitro testing (Testing outside the body), computer models, and toxicogenomics (Study of gene responses to toxins) are also explained. This review aims to give students, researchers, and professionals a clear and full understanding of how toxicity is studied and why it is important for human health and environmental safety. It also shows the importance of using modern, ethical, and scientific methods in toxicity research.

INTRODUCTION

1. **Ancient herbal medicine:** Since ancient times, societies have depended on medicinal plants to cure illnesses/ diseases/ disorder. Herbs like Atropa belladonna, Digitalis purpurea, menthe pipperita and Aconitum were widely used for their therapeutic properties, though their toxic effects were not well understood (Samuelsson, 2004).[1]

2. **Recognition of toxic effects:** After the higher intake of drug or any other substances "toxicologist" observed that some plants caused harmful symptoms when consumed in excess, such as hallucinations, paralysis, or fatal poisoning and sometime can cause even death. This led to the practice of refining dosages and identifying toxic herbs to prevent adverse reactions (Heinrich et al., 2020).[2]

3. **Early foundations of toxicology:** Ancient scholars like Dioscorides and Ibn Sina documented medicinal plants and their effects. Through trial and error, they classified herbs based on their safety profiles, using early forms of experimentation and animal testing (Dioscorides, 1st century; Avicenna, 1025).[3]

4. **Industrial Revolution and Drug synthesis:** The 19th and 20th centuries are the majorly remarked from herbal remedies to synthetic drugs. Some of the scientist isolated the bioactive compoundto reduce the toxicity , such as morphine from opium and quinine from cinchona bark, but concerns over side effects led to stricter safety regulations (Sneader, 2005).[4]

5. **Development of toxicity studies –** As pharmaceuticals advanced, standardized toxicity testing methods were introduced. Studies on acute, sub-chronic, and chronic toxicity helped evaluate drug safety before human use. Regulatory agencies like the FDA and OECD set guidelines to ensure public health protection (Eisenbrand et al., 2002).[5]

6. **Advancements in modern toxicology –** Today, toxicity testing incorporates cutting-edge methods such as in-vitro models, computational toxicology, and artificial intelligence to predict drug safety. Efforts continue to reduce reliance on animal testing while improving accuracy in toxicity assessments (Hartung, 2009).[6]

7. **Future prospects in toxicology –** Modern research focuses on integrating traditional herbal medicine with scientific validation. The goal is to develop safer drugs while

maintaining the benefits of natural compounds, ensuring a balance between efficacy and safety in medicine (Efferth & Greten, 2021).[7]

Definition

Toxicity refers to the extent to which a substance can harm an organism. It can apply to impacts on the entire living being, be it a plant, animal, or even a bacterium. Toxicity of materials changes with the degree of exposure; it is even possible for water to induce toxicity to an organism if taken in high amounts. Conversely, for extremely poisonous materials like snake venom, there are amount of dose that do not inflict harm. Here substance can be a physical or can be a chemical agent.[8,9]

History of toxicity

History of intoxication is one of the great chapters of the human story, where curiosity and genius, scientific discoveries and empirical knowledge loop the loop with intrigues, law-breaking, politics, personal tragedies of leading light, warfare and natural disaster. The history of knowledge about toxic pith is likely as old as the human species. Paracelsus stated in the Middle Age: In the universe there is no substance that is non-poisonous.[10]

Toxicity studies

Toxicity study of isolated compounds" means carrying out tests on any single potent compound, usually derived from plants or bacteria, in order to assess the raise or degree of toxic risks for its future application, which is usually tested on laboratory animals.[11]

Toxicity studies focus on what the adverse effects of a specific substance are. Besides, they also provide a great deal of useful information about the compound's absorption ,distribution, metabolism ,excretion (ADME) in the human body. There is a number of preclinical tests that have to be conducted on the substance before it has been administered to a human volunteer. These tests are usually for toxicity concerns that may arise out of the particular substance. After getting the permission for marketing a particular drug, it undergoes thorough clinical testing for any toxicity. Newly developed drugs have to go through a lot of preclinical as well as clinical testing for any toxicity. A majority of toxicity testing is performed on laboratory animals and it identifies the adverse effects that the chemical might cause as well as the mode of action.[12]

Over a protracted / prolonged period of time and using a wide range of animal species for testing, the examination of biochemical and physiological abnormalities, as well as a thorough necropsy at the conclusion of a trial, enabled the identification of any physical or histological damages that could have occurred. In any case, the post-mortem was expected to reveal some abnormality, be it not so subtle. The tests are more than diagnostic, as they aid in harmful side effects of drugs above the therapeutic range.[13]

Major types of toxicity

i. Acute toxicity
ii. Sub – Acute toxicity
iii. Chronic toxicity

i. Acute toxicity: This type of toxicity is often understood as the adverse reaction caused by a single dose or multiple doses within 24 hours. It is used to evaluate the immediate harmful effects of chemicals, drugs, and even pollutants on living organisms.[14]

Important factors include

- **Measurement:** These are measured with regular parameters like LD50 (lethal dose for 50% of the population) or LC50 (lethal concentration for 50% of the population).
- **Testing:** During an acute study, test organisms such as rats, mice, and even aquatic species are given the substance to examine its toxic effects.
- **Effects:** These can vary from, for example, mild like irritation to terrible organ damage or even death.

Applications: It helps rate the harmfulness of certain substances to classify chemicals and determine safe exposure levels.

Tests for the acute effects of a substance are effective and necessary in classification and marking of substances that are hazardous and are useful in ensuring safety in industrial, medical, and even environmental settings.

ii. Sub-Acute toxicity

It arises from the effect of exposure which lasts for some days to weeks, thereby giving rise to a toxic effect. In most cases, this is more serious compared to acute toxicity, which usually arises from one or a chain of events happening within a relatively short period of time. This

sub-type of toxicology will allow researchers to weigh the risks with endless use of drugs, chemicals, or any other environmental pollutants.[15]

The dosage in a sub-acute toxicity test should be controlled over an extended period of time to assess changes in behaviour or physiology or alterations in body these studies are absolutely essential to further and to build policies that keep the environment free from composition. Therefore, research related to toxicology all the harmful effects for everyone. These are mainly practiced in the context of new drugs and chemicals.

iii. Chronic toxicity

Chronic toxicity signifies the adverse impact on health caused by cumulative consumption of dangerous materials. usually in minute proportions. Unlike acute toxicity that involves single or, short-term exposure to a toxin and exhibits instant impact, chronic toxicity begins showing symptoms over an extended period of time. Over time, it can lead to dire health consequences such as organ injury, cancer, or even reproductive issues. With this, however, the effects of the prolonged exposure to chronic toxicity can often take years to become visible, which is what makes it so difficult to form a cause and relate to while determining the changes to be made at the policies level.[16]

Chronic toxicity is complicated and concerning especially with regard to environmental pollution, pharmaceuticals, and chemical industries, with regards to the maximum permissible concentration limits. It is worse for these particular toxins, as they have the potential to accumulate within the body tissues. Chronic toxicity markers include tissue damage, metabolic changes, and the most significant. DNA change. For this reason, authorities tend to test for chronic toxicity on pollutants, to fix concentration limits and plan for their approval.

Different type of toxicity[17]

1. Chemical toxicity: A chemical agent can be harmful, leading to damage in cells, organs, or the entire body.
2. Biological toxicity: Microorganisms and Parasites that cause diseases are toxic, commonly referred to as pathogens.
3. Physical toxicity: Certain physical forces, like radiation, can be toxic.
4. Sub chronic toxicity: On-going exposure to a substance over weeks or months can result in toxicity.

5. Developmental toxicity: A substance may be harmful to an embryo or foetus, potentially resulting in birth defects.
6. Pulmonary toxicity: Toxic substances can lead to lung damage, including inflammation or scarring.
7. Genetic toxicity: A chemical agent can harm a cell's genetic material, resulting in mutations that could lead to cancer.
8. Reproductive toxicity: This refers to the potential of a chemical, physical, or biological agent to negatively impact fertility and the development of offspring.

Different guidelines for acute toxicity: (According, to OECD)

The primary guidelines for evaluating acute toxicity, as established by the **OECD (Organization for economic and co-operation and development)**, include the "Fixed Dose Procedure" **(Test Guideline 420)**, the "Acute Toxic Class Method" **(Test Guideline 423),** and the "Up-and-Down Procedure" **(Test Guideline 425)**. Each of these methods has slightly different.[18]

Approaches to assessing the toxicity of a substance by administering a single dose to test animals and monitoring for signs of toxicity or death within a specific timeframe. The main difference lies in how the dose levels are selected and adjusted throughout the study: Guideline 420 defines fixed doses, Guideline 423 employs a stepwise approach to classify toxicity, and Guideline 425 uses an adaptive design to efficiently estimate the lethal dose (LD50).

Key points about each guidelines

- **OECD Test Guideline 420 (Fixed dose procedure)**

This method uses a set of predetermined dose levels, administering the test substance to animals at each level until clear signs of toxicity are observed. It does not aim to determine the exact LD50 and is considered suitable for substances with a known toxicity range.[19]

- **OECD Test Guideline 423 (Acute Toxic Class Method)**

This guideline employs a stepwise approach with a limited number of animals per dose, allowing for the classification of the test substance into a specific toxicity category based on the observed mortality rate at different dose levels.[20]

- **OECD Test Guideline 425 (Up-and-Down Procedure)**

This method utilizes an adaptive design where the dose is adjusted based on the observed response in the previous animal, enabling a more efficient determination of the LD50 with fewer animals used in the study. In this dose is increased on the basis of increasing factor 3.2 (which is the default factor in the OECD guideline 425).[21]

Guidelines for acute toxicity according to ICH

ICH guidelines for acute toxicity studies

The International Council for Harmonization (ICH) outlines guidelines for acute toxicity studies in ICH M3 (R2) and S3A. These studies evaluate the potential toxic effects of a drug when administered as a single dose.

1. Objective

To assess the immediate toxic effects of a drug.

Helps establish the safe initial dose for human trials.

2. Test animals

Generally conducted on rodents (rats, mice) and occasionally on non-rodents (dogs, rabbits).

At least two species are required for accurate results.

3. Dose administration

A single-dose is given at varying levels (low, medium, high) helps in determining:

Maximum Tolerated Dose (MTD): The highest dose an organism can handle without severe effects.

ICH M3 (R2) and S3A

ICH M3 (R2): Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals. This guideline provides recommendations on the non-clinical safety studies required to support human clinical trials and marketing authorization.

ICH S3A: Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies. This guideline offers guidance on integrating pharmacokinetics into toxicity testing to aid in interpreting toxicology findings and promoting rational study design.

Comparison of OECD and ICH guidelines for acute toxicity

Criteria	OECD guideline	ICH guideline
Purpose	Evaluate acute toxicity of chemical, pharmaceuticals, pesticide and industrial compound.	Focuses on the acute toxicity of pharmaceuticals drugs for human clinical trials.
Test species	Rodent preliminary sometimes non-rodent.	At least two species most of the time Rodent and sometime non-rodent.
Dose selection	Uses low, medium, high doses to determine the LD50, NOAEL (no observed adverse effect level) and MTD.	Uses single-dose administration to establish safe starting dose LD50, NOAEL and MTD (maximum tolerated dose).
Endpoints	LD50 often used to check for toxicity effect.	LD50 determination is not always required; Emphasis is on dose range finding studies.
Observation period	Animal observed for 14 days to check for toxic effect.	Similar 14-days monitoring for toxicity studies.
Ethical consideration	Follows 3Rs (reduce, refine, and replace) principle to minimize the animal testing.	Also follows the 3Rs and promote the alternative testing methods.
Alternative methods	Include 420, 423, 425 guideline for testing.	Accept OECD recommended method and integrate them into regulatory toxicology.

Different parameter to be evaluated for Sub-chronic and Chronic toxicity

Evaluation of sub chronic toxicity

Sub chronic toxicity studies are designed to evaluate the potential health hazards of repeated exposure to a substance over a period of several weeks or months. The following are different parameters that are typically evaluated in sub chronic toxicity studies:

- **Clinical parameters**[22]

1. Body weight: Variation in body weight can reflect toxicity.
2. Food consumption: Variation in food consumption can reflect the general health status of the animals.
3. Water intake: Variation in water intake may be indicative of some kidney or liver toxicity.
4. Clinical signs: monitoring any unusual clinical signs such as subdued behaviour, shaking, or violent body movements.

- **Haematological parameters**[23]

1. Haemoglobin: Variations in haemoglobin may reflect development of some anaemic condition or blood toxicity.
2. Haematocrit: Variations in haematocrit may reflect some anaemic condition or blood toxicity.
3. Red blood cell count: Variations in red blood cell count can indicate potential anaemia or blood toxicity.
4. White blood cell count: Variations in white blood cell count can indicate possible impairment of immune system.

- **Biochemical parameters**[24]

1. Liver function tests: Variations in the level of liver enzymes may indicate some liver toxicity.
2. Kidney function tests: Variations in kidney function tests may indicate some degree of kidney toxicity.
3. Blood glucose: Variations in blood glucose may indicate potential.
4. Lipid profile: In Sub acute toxicity evaluation, a lipid profile generally evaluates the levels of total cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol serum cholesterol because these factors are correlated with possible liabilities to the substance tested cardiovascular system.
5. Urine Analysis: In the sub-acute toxicity evaluation for urine consist of the 2 examination:

a) Supernatant examination: In sub-chronic toxicity studies, supernatant examination in urine analysis focuses on testing the clear liquid remaining after centrifugation. This helps identify biochemical changes that may signal toxic effects on organs like the kidneys and liver over time.

Procedure

1. Centrifugation – The urine sample is spun at high speed to separate solid components (Such as cells, crystals and casts) from the clear liquid (Supernatant).
2. Supernatant Examination – The transparent liquid is analyzed for:

- Biochemical markers (e.g., proteins, glucose, enzymes, creatinine) to assess kidney and liver function.
- Electrolytes (Sodium, potassium, chloride) to evaluate metabolic stability.

- Toxicity indicators like elevated protein levels (Proteinuria) or abnormal enzyme activity.

b) **Sediment examination:** Sediment examination focuses on analyzing the solid particles in urine after centrifugation, helping to identify cellular and structural changes resulting from long-term exposure to toxic substances.

Procedure

1. Centrifugation – The urine sample is spun to separate solid matter (sediment) from the clear liquid (supernatant).
2. Microscopic Analysis – The sediment is examined under a microscope to detect:

- Cells (Red blood cells, white blood cells, epithelial cells), which may signal kidney or urinary tract damage.
- Casts (Hyaline, Granular, Cellular), indicating kidney stress or injury.
- Crystals, which help identify metabolic imbalances or the excretion of toxic substances.
- Bacteria & Other Microorganisms, suggesting potential infections.

6. **Histopathology examination:** Histopathological examination involves studying tissue samples from the kidneys, bladder, or urinary tract at a microscopic level to assess structural damage caused by prolonged exposure to toxic substances. While urine analysis primarily detects biochemical and cellular changes, histopathology provides deeper insights into tissue-level alterations.

Evaluation of chronic toxicity[25]

Evaluation of health risks from prolonged use of certain substances over time, for example, 12 months, is the aim of studies chronic toxicity, which is designed exactly for that purpose. Following are the biomarkers commonly assessed in chronic toxicity studies:

➢ **Clinical indicators**[26]

1. Body mass: Shifts in body mass might suggest possible toxicity.
2. Food intake: Changes in food intake patterns may negatively affect the health status of the test animals.
3. Water intake: Changes in water consumption could suggest possible kidney or liver damage.
4. Clinical features: Lethargy, tremor, or convulsive movements that deviate from the norm.

- **Hematobiological indicators**[27]

1. Haemoglobin concentration: Variation in haemoglobin concentration may point to different types of anaemia or blood poisoning.
2. Hematocrit value: Variation in hematocrit values might signal an anemic condition or blood poisoning.
3. Erythrocyte count: Variation in the count of red blood cells may suggest different types of anaemia or blood destruction.
4. Leucocyte count: Variation in the number of white blood cells may indicate more active forms of immune damage.

- **Biochemical indicators**[28]

1. Liver: Variation in the levels of liver enzymes may indicate some level of damage to those organs.
2. Kidney: Variation in kidney function test results may reveal some level of kidney damage.
3. Pancreas: Variation in glucose levels in the bloodstream may indicate damage to the pancreas.
4. Cardiovascular System: Variation in the concentrations of cholesterol and triglycerides in blood may imply some sort of damage cardiovascular system.

Acute toxicity studies of isolated compound

1. Dzoyem et al, 2011 from isolated plumbagin from the Aq/Alcoholic/ether/chloroform **Extract of** stem bark of *D.Canaliculata* and evaluated the oral acute toxicity in *mice*. The isolated compound such as plumbagin had strong anti-fungal activity with inhibition zone between 16.51 and 25.10 mm and minimum inhibitory concentration of 0.78µg/mL. It hed showed that no toxicity found in mice.[29]

2. Khan et al, 2007 Isolated 3,4 dihydrobenzoic acid from the Aq/Alcoholic/ether/chloroform Extract of rhizomes of D. Quercifolia and evaluated sub chronic toxicity studies on mice from hexane chlroa acetyl lupeol at 150mg/disc respectively. They hed showed no observed dose level within the 14 days no change were observed.[30]

3. A Fristiohady et al, 2020 isolated *Aaptos Sp.* extract from the Aq/Alcoholic/ether/chloroform/ acetone and investigated acute toxicity studies on the

Brine Shrimp. It hed showed that the anti-oxidant activity of Aaptos Sp. (Acetone extract) was successfully identified on the Brine Shrimp with lower to lethal dose i.e.16.10µg/ml. Which found there is no toxicity and don't have any significant change.[31]

4. B. Abubakar et al, 2022 isolated *5-Methyl Coumarin 4β glycoside* from the Aq/Alcoholic/ether/chloroform Extract & methanol leaf extract of *Vernonia Glaberrima*. Evaluating the acute and sub-acute toxicity studies on the group of 4 mice by giving the adequate dose to them for several days (14 days). No toxicity was observed. After this change were observed haematological, histopathological, and biochemical. It hed showed that *5-Methyl Coumarin-4β glycoside* at adequate dose is non-irritant for mice.[32]

5. DM Estork et al, 2013 isolated *Abarema Auriculata* extract has anti-cancer activity and shows cytotoxic activity against prostate. After the administration of the lower label of the dose as per wt. and IP to mice there are significant results are observed i.e. is tail squeeze, corneal reflex and touch responses. After the administration of higher dose to mice i.e. 15.0 mg/kg. It hed showed that there are several responses occur in mice (it lead to the mice death).[33]

6. LY Lathe et al, 2011 *Lantana Camara* is a widely used medicinal plant; it has been subjected to the toxicity studies. After the administration of single oral dosage i.e. 2 g/kg to the adult mice shows no significant sign. But after two weeks female mice did lose their weight, while male has lower organ mass in heart and kidney.[34]

7. M Mortady Hamed et al, 2017 The *Moringa* plant has been a subject of research because of its potential health benefits, particularly because of its high content of phenolic antioxidants, ranging from 309.52 to 43.28 mg of gallic acid equivalent extract per gram in different solvent extracts. The antioxidant potential of the plant was measured using DPPH and other reduction using creased power assay methods which showed significant activity in n-butanol extracts. Although the methanolic extract was found to be mildly toxic with an acute Lethal dose (LD50) of 3458.3mg/kg, in the chromatographic isolation, seven bioactive compounds including chlorogenic and gallic acid were identified. This affirms the health benefits of *M. oleifera* and the safety of the plant as a food source.[35]

8. Rahmat Omar et al, 2014 Researchers are looking into *Paenibacillus* species due to the lack of controlled antimicrobial resistance. Testing of its antibacterial and anti-biofilm

activity on various negative and positive gram pathogens showed significant results. Additional studies provided three bioactive compounds that had the strongest efficacy; one was an aminoglycoside antibiotic, the other was a phospholipase A2 blocker, and the last was an antibacterial compound. Rats were used to assess therapeutic efficacy and tolerability and toxicity were both negligible. Isolated compounds have shown a positive success rate with treating ailments. For example, inflammatory diseases where the effective doses are between 10 and 100 μg/mL. Nevertheless, they still require additional studies in the future.[36]

9. L Sorenson et al, 2024 Evaluation of crude oil spilled at sea undergoes chemical transformation through environmental processes such as dissolution, biodegradation, and photo degradation. The formation of unresolved polar compounds (UPCs) increases water solubility, making them more bioavailable to aquatic organisms. Research hed showed that UPCs may constitute over 90% of the water-accommodated fraction (WAF) in weathered oils, yet their toxicity remains largely uncharacterized. Acute toxicity tests on marine copepods exposed to field-weathered oil WAFs from the Deepwater Horizon spill revealed that UPCs contribute significantly to toxicity, comparable to known toxicants like polycyclic aromatic hydrocarbons (PAHs) and alkyl phenols.[37]

10. P Chaniad et al, 2024 isolated 1-hydroxy-5,6,7-trimethoxyxanthone (HTX) from the flowers of *Mammea siamensis* and evaluated it's in vivo antimalarial activity and acute toxicity in *mice*. The study demonstrated that HTX at 10 mg/kg body weight significantly suppressed *Plasmodium berghei* parasitemia by 74.26%. Additionally, no acute toxicity was observed, with no alterations in liver and kidney functions or histopathology. Pharmacokinetic analysis revealed a long elimination half-life of 13.88 h, suggesting that treatment duration optimization may be necessary to minimize toxicity risks.[38]

11. M Belguidoum et al, 2024 isolated aqueous extract of *Teucrium pseudochamaepitys* L. (TpAE) was investigated for its pharmacological properties. The chemical composition of TpAE was analyzed using SPE LC-MS/MS, confirming the presence of flavonoids and phenolic acids. The antioxidant activity was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS·+(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), reducing power, and phenanthroline assays, where TpAE exhibited significant activity. The *in vitro* anti-inflammatory effect was assessed through albumin denaturation inhibition, showing results comparable to diclofenac. The results showed that TpAE strongly inhibited

oxidative stress and inflammation. Acute toxicity tests at 2000 mg/kg in treated animals showed no toxicity symptoms. It had shown that TpAE is not toxic and contains bioactive compounds with antioxidant, anti-inflammatory, and analgesic properties.[39]

12. R Ballesteros et al, 2024 isolated standardized extract (P2Et) from *Caesalpinia spinosa* and evaluated for its acute toxicity. This extract exhibited significant antioxidant, immunomodulatory, and anti-inflammatory properties. Toxicity assessments, including genotoxicity, mutagenicity, and 28-day chronic toxicity studies, confirmed its safety. The results demonstrated that acute toxicity tests at 2000 mg/kg in Wistar rats indicated an LD50 above this value, classifying P2Et as GHS category 5. Chronic toxicity tests conducted for 180 days in rats and rabbits at 1000 mg/kg under GLP conditions showed no weight loss or biochemical alterations. It hed showed that P2Et extract is non-toxic and safe for long-term oral administration.[40]

13. M Torres et al, 2024 isolated metabolite mixtures from microcystin (MC)-producing and non-MC-producing extract of cyanobacterial strains and evaluated the acute toxicity in *Thamnocephalus platyurus*. The isolated compounds caused acute toxicity, with LC50 (24 h) values of 0.50 and 2.55 mgdw_biomass/mL, respectively. It hed showed that Micropeptin-K139 and apolar fractions significantly contributed to mortality, locomotor impairment, and morphological changes, whereas no toxicity was observed in fractions dominated by Cyanopeptolin 959 and Nostoginin BN741.[41]

14. Alshehri et al, 2024 isolated compounds from the chloroform extract of *Habenaria plantaginea* and evaluated their analgesic, anti-inflammatory, and antioxidant potential. The isolated compounds HP-1 and HP-2 were tested through in vitro enzymatic assays and toxicity studies. The COX-2 inhibitory assay showed that the Cf-4 sub-fraction exhibited 92.15% inhibition, while against 5-LOX, it had an IC50 of 3.77 µg/mL. In antioxidant assays, HP-1 and HP-2 showed significant inhibition against ABTS (88.81%, 84.34%), DPPH (89.34%, 91.52%), and H_2O_2 (80.43%, 82.34%). It hed showed that the species validates its traditional medicinal use with strong anti-inflammatory and antioxidant properties.[42]

15. Qader et al, 2024 isolated phytochemicals from the aqueous and ethanolic extract of *Polygonum minus* (PM) and evaluated their toxicity and bioactivity. The cytotoxicity of PM extracts was assessed using the MTS Assay on *Hs888Lu* cells, while acute toxicity

was studied in *Sprague Dawley* rats. SwissADME and ADMET analysis were conducted on isolated compounds, including gallic acid, quercetin, rutin, and coumaric acid. Molecular docking revealed significant binding affinity of quercetin and rutin to human H+/K+ ATPase, cyclooxygenase-2, and acetylcholinesterase. It hed showed that neither the aqueous nor ethanolic extract of PM is toxic, supporting its pharmaceutical potential.[43]

16. A Daghighi et al, 2024 developed a computation method for toxicity prediction using machine learning and multicondition descriptors (MCDs). A Quantitative Structure–Toxicity Relationship (QSTR) model was constructed based on a large toxicity dataset of over 80,000 compounds and 59 endpoints (122,572 data points). The study employed Convolutional Neural Networks (CNN-1D), achieving the best prediction performance with R^2_train = 0.93 and R^2_ext = 0.70. Structural features such as van der Waals surface area, nitrogen-containing fragments, S–P fragments, ionization potential, and C–N fragments significantly contributed to toxicity. It was shown that the developed models enable rapid toxicity assessment of new compounds.[44]

17. SC finch et al, 2024 isolated (Cyclic imines, a class of lipophilic shellfish toxins) from various marine microalgal species, including gymnodimines, spirolides, pinnatoxins, portimines, pteriatoxins, prorocentrolides, spiro-prorocentrimine, symbiomines, and kabirimine. The isolated cyclic imines were evaluated for their acute toxicity, showing rapid fatality when injected into mice. Among them, pinnatoxins exhibited the highest toxicity, with recorded concentrations in shellfish reaching up to 54 times higher than the lower health-based guidance value (23 µg PnTX/kg). The study indicated that, except for pinnatoxins, the risk posed to human health by cyclic imines appears low. However, due to limited data, further toxicity studies and occurrence analysis are required to establish a more robust safety threshold.[45]

18. S Gerschle et al, 2024 isolated flavonoids and naphthoquinones from the medicinal plant *sundew*, cultivated sustainably on rewetted peatlands. The isolated compounds, including 7-methyljuglone and 2″-O-galloylhyperoside, were evaluated for their biofilm-inhibiting properties against multidrug-resistant ESBL-producing *E. coli* strains. Proteomic analyses revealed that naphthoquinones act via regulatory proteins like OmpR, altering stress responses, while flavonoids inhibit biofilm formation by creating an iron-poor environment and affecting polyamine balance. Cytotoxicity tests in 3D cell cultures and

the *Galleria mellonella* in vivo model confirmed the safety of these extracts. It hed showed that naphthoquinones significantly disrupted biofilm formation, while flavonoids reduced intracellular spermidine levels, supporting *sundew* as a promising candidate for new phytopharmaceuticals.[46]

19. PK Amorim et al, 2024, isolated lectin, boll from *Bixa orellana* L. leaves through ammonium sulfate precipitation (60% saturation), followed by ion exchange and size exclusion chromatographies. The purified lectin showed a single 19-kDa polypeptide band in SDS-PAGE and exhibited high hemagglutinating activity (HA), which increased in the presence of Mg^{2+} ions and remained stable at 100°C and acidic pH. BoLL displayed bacteriostatic and bactericidal effects against *Escherichia coli* and *Staphylococcus aureus* (MIC and MBC: 400–800 µg/mL) and inhibited *Bacillus megaterium* and *Micrococcus luteus*. Acute toxicity tests in mice showed no changes in haematological, biochemical, or behavioral parameters; however, histopathological alterations were observed in the liver, spleen, and kidneys. It hed showed that BoLL as a thermostable antibacterial lectin with potential biomedical applications.[47]

20. DTM Huynh et al, 2024 *Allium ascalonicum* L. (AS), a member of the *Alliaceae* family, is widely used as a spice and food ingredient in Asia and is rich in triterpenoids, phenolic acids, flavonoids, thiosulfates, and anthocyanins, known for their therapeutic benefits. Traditional Chinese medicine has long utilized AS in infusions, juices, and poultices for its antibacterial, anti-inflammatory, diuretic, and cholesterol-lowering properties. Modern studies confirm its ability to reduce blood sugar, blood pressure, and cholesterol levels. However, despite its medicinal potential, there is a lack of in vivo research on its cholesterol-regulating effects, highlighting the need for further investigation.[48]

21. N Moussavi et al, 2024 isolated ten phenolic compounds, including xanthones and benzoates, from the *Securidaca longepedunculata* extract and evaluated their toxicity and anticonvulsant activity. Using nuclear magnetic resonance spectroscopy, the compounds were identified, and assays in *zebrafish larvae* determined toxicity and inhibition of PTZ-induced seizure-like paroxysms. Benzyl-2-hydroxy-6-methoxy-benzoate (MTC 12.5 µM), 4,8-dihydroxy-1,2,3,5,6-pentamethoxyxanthone (MTC 25 µM), and 1,7-dihydroxy-4-methoxyxanthone (MTC 6.25 µM) exhibited high toxicity, while DCM extract and specific xanthones significantly inhibited seizures. The EtOH extract and benzyl benzoates caused paradoxical excitation. It hed showed that the plant's traditional

antiepileptic use and highlight potential lead compounds, but toxicity concerns require further investigation for therapeutic applications.[49]

22. JD Ickovski et al, 2024 isolated essential oils from Serbian *Artemisia* species (*A. alba, A. absinthium, A. annua, A. vulgaris,* and *A. scoparia*), with capillin (35.7%) identified as the major component in *A. scoparia.* The essential oil was obtained by merging multiple samples of the same species from different localities, and its chemical composition was compared with pre-merging results. In *A. scoparia*, four unique compounds were identified, including capillin (35.7%) as the major component. Toxicity evaluation using the *Artemia salina* test showed that *A. annua* and *A. alba* exhibited medium toxicity, while *A. absinthium, A. vulgaris,* and *A. scoparia* had strong toxicity. Additionally, *A. scoparia* essential oil displayed significant larvicidal activity against *Drosophila melanogaster* larvae, causing complete mortality at 2% and 1% concentrations.[50]

23. Noga & Jurowski et al, 2024 isolated bicyclic organophosphorus compounds (BOPCs), including flame retardants and plasticizers, evaluated their acute toxicity using in silico toxicological approaches. It included 18 BOPCs analyzed through QSAR models and probabilistic software to predict acute oral toxicity in rats. The results indicated that all BOPCs exhibit high acute toxicity, with LD_{50} values ranging from <1 mg/kg to >1,000 mg/kg body weight, depending on the model used. The toxicity mechanism involved antagonism of gamma-aminobutyric acid (GABA) receptors, leading to severe neurotoxic effects such as convulsions and seizures. Major Toxicophoric groups, including phosphate and phosphorothione moieties, were identified as key contributors to toxicity.[51]

24. MA Dar et al, 2024 isolated two terpene derivatives (ECbp-1 and ECbp-2) from ethanol extract of C. bursa-pastoris (ECbp) in streptozotocin (STZ)-induced diabetic rats and with α-amylase and α-glucosidase inhibitory potential. STZ (50 mg/kg body weight, i.p.) was used to induce diabetes, and ECbp (0.2 g/kg b.w., p.o.) was administered for 21 days. ECbp significantly reduced blood glucose levels, lowered blood cholesterol, and increased high-density lipoprotein levels. Acute oral toxicity tests showed no toxic effects or mortality at 2,000 mg/kg b.w.[52]

25. H Gumisiriza et al, 2024 isolated (total polyphenolic content bioactive compounds) from *Gouania longispicata* leaf extracts but these are not specially identified. The observations

showed that the methanolic extract had the highest TPC (75.26±0.420 GAE µg/g DW) and TFC (60.12±0.012 QE µg/g DW), while the aqueous extract exhibited the strongest antioxidant activity with a minimum inhibitory concentration of 187.12±0.08 µg/mL. It hed showed that the acute toxicity test indicated that both extracts had a median lethal dose (LD50) above 5000 mg/kg, suggesting no significant toxicity.[53]

26. SD Ray et al, 2024 isolated [(16-methoxy-10-(3-methyl-butyl)-2-oxa-6,9,12-triaza-tricyclo[13.3.1.03,7] nonadeca-1(18),13,15(19),16-tetraene-8,11-Dione] (a novel cyclic alkaloid) from the root bark of *Ziziphus nummularia* and evaluated for its anti-inflammatory potential. The in vivo evaluation used carrageenan-induced paw oedema and arachidonic acid/xylene-induced ear oedema models, with the compound tested at various concentrations, showing maximum TNF-α inhibition of 88.00% at 50.11 µM. The compound significantly reduced inflammatory markers, including nitric oxide (NO), prostaglandin E2 (PGE2), and tumour necrosis factor-alpha (TNF-α). Molecular docking studies revealed strong binding to TNF-α, forming hydrogen bonds with ASP 45 and GLN 47. It hed showed the cyclic alkaloid as a promising candidate for future therapeutic applications in inflammatory disease management.[54]

27. G Galli et al, 2024 isolated four compounds including two flavonoids (chalcone and trans-chalcone) and two anti-infective agents (octenidine and tolfenpyrad), which exhibited potential anthelmintic activity. A high-throughput screening of 2,228 molecules from five commercial compound collections, including anti-infective drugs, plant-based natural products, and FDA-approved Chinese Pharmacopoeia compounds, was observed . The screening identified 32 compounds (1.44% success rate) with more than 70% motility inhibition, among which 10 were known anthelmintic drugs, while 22 were tested against Haemonchus contortus and a resistant strain of Teladorsagia circumcincta. The active compounds were evaluated at different concentrations, with chalcone and trans-chalcone showing anthelmintic activity at EC50 values below 20 µM. Further safety assessments using HepG2 spheroids and mouse intestinal organoids revealed that chalcone and trans-chalcone had selective indexes greater than 5, indicating good safety profiles. It hed showed that tolfenpyrad and octenidine exhibited toxicity concerns.[55]

28. J Chitra et al, 2024 isolated five major bioactive compounds from Rhizophora mucronata bark extract (BERM) using HPLC: Daidzein, Epicatechin, Hesperidin, Diosmin, and Quercitrin**.** Acute toxicity studies are observed in Swiss Albino mice revealed no

mortality or behavioral changes even at 3200 mg/kg, with normal food intake, body weight, and organ health. Dose regimen included single-dose oral administration at 800, 1600, and 3200 mg/kg, followed by a 14-day observation period. For sub-acute toxicity, mice received repeated doses of 400 mg/kg and 800 mg/kg for 28 days. It hed showed no significant abnormalities in haematological, biochemical, or histopathological parameters.[56]

29. OK Ogbeide et al, 2024 isolated Pulcherrin J and 6β-cinnamoyloxy-7β-hydroxyvouacapen-5α-ol compounds from Caesalpinia pulcherrima stem bark extract and were identified using spectroscopy techniques. Phytochemical analysis confirmed the presence of flavonoids, terpenoids, tannins, and alkaloids. Acute toxicity studies observed in Swiss albino mice determined an LD50 of 5656.85 mg/kg, indicating safety. The HEEA (Hexane-Ethyl Acetate Extract) fraction showed high antiplasmodial activity against Plasmodium falciparum D6 and W2 clones (IC50: 3.7 and 5.3 µg/mL). The isolated compounds inhibited parasite growth with IC50 values of 10.25–>10.62 µM. These results hed showed C. pulcherrima stem bark as a potential source of new antimalarial agents for further study.[57]

30. TB Devi et al, 2024 isolated dihydro-p-coumaric acid from *Tithonia diversifolia* leaves. And observed its acute and sub-acute toxicity in BALB/c mice. In acute toxicity tests, doses of 200, 800, and 1,600 mg/kg body weight were administered orally for 7 days, while sub-acute toxicity was assessed with 50 and 500 mg/kg for 14 days. No adverse effects were observed in vital organs, and no mortality or toxicity signs were recorded, but histopathological findings showed no major abnormalities. These results hed showed dihydro-p-coumaric acid is safe and a potential alternative to synthetic pesticides.[58]

31. C Beaufay et al, 2024 isolated eight triterpenic esters along with ursolic and oleanolic acid from *Keetia leucantha*, identified as the major antiplasmodial components. Observations showed no haemolysis or acute toxicity at cumulative doses of 800 mg/kg for extracts and 150 mg/kg for esters. The dose regimen included intraperitoneal administration of 50 mg/kg/day in *Plasmodium berghei*-infected mice. Result hed showed some significant parasitaemia inhibition (27.8 ± 5.4%, $p < 0.01$) on day 4. And no such toxicity observed in mice.[59]

Acute toxicity studies of herbal plant

1. B Oloya et.al, 2022 investigated the aqueous and methanol/dichloromethane (1:1) extracts of five medicinal plants—*Albizia coriaria*, *Warburgia ugandensis*, *Zanthoxylum leprieurii*, *Combretum molle*, and *Ekebergia capensis*—for phytochemical content, antimycobacterial activity, and acute toxicity. Phytochemical screening revealed the presence of various bioactive compounds including alkaloids, flavonoids, tannins, saponins, terpenoids, and cardiac glycosides. Methanol/DCM extracts showed significant to moderate activity against *Mycobacterium tuberculosis* H37Rv with MIC values between 98.0–586.0 µg/mL, and moderate to weak activity against multidrug-resistant TB (MDR-TB) strains (MIC 293.0–781.0 µg/mL). Aqueous extracts displayed weaker activity overall. For acute toxicity, 2000 mg/kg of aqueous extract was administered to mice following OECD guideline No. 425. Except for one mortality in the *A. coriaria* group, no significant toxicological signs or lesions were observed. Histopathological analysis confirmed safety in most groups. These results suggest that the selected plant extracts possess promising antimycobacterial properties and low acute toxicity.[60]

2. LJ Simanjuntak et.al, 2022 evaluated the toxicity and physiological effects of nanoherbal *Phaleria macrocarpa* (mahkota dewa), a medicinal plant native to Papua, Indonesia. The nanoherbal formulation was prepared using High Energy Milling (HEM). Toxicity testing was conducted in three phases on mice to determine the LD50 using the Thomson Weil formula over 14 days, resulting in a value of 1 g/kg body weight. Additionally, LC50 was assessed via Brine Shrimp Lethality Test, yielding a value of 2145.04 ppm. Haematological, biochemical, electrolyte parameters, and organ histopathology were affected, although organ weights showed no significant change. Despite these physiological effects, the extract was classified as having moderate toxicity. The result hed showed, with appropriate dosing, nanoherbal mahkota dewa may serve as a potential candidate for the development of future herbal medicine.[61]

3. F Nalimu et.al, 2022 investigated the acute and sub-acute toxicity of *Aloe vera* (L.) Burm. f. whole leaf and green rind aqueous extracts, traditionally used in various regions of Uganda for medicinal purposes. Using Wistar rats, acute toxicity was assessed at doses up to 5000 mg/kg, and sub-acute toxicity was studied at doses of 200, 400, and 800 mg/kg over 28 days. No mortality or significant behavioral changes were observed, indicating an LD50 above 5000 mg/kg. However, sub-acute results showed a significant decrease in

relative spleen weight and alterations in creatinine, chloride ions, and MCHC levels. Histopathological examination revealed inflammation in the kidney interstitium at higher doses of the whole leaf extract. It hed showed that while *Aloe vera* extracts are non-toxic at high doses for short-term use, long-term use of the whole leaf extract may pose a risk of kidney toxicity.[62]

4. MC de Barros et.al, 2022 The study assessed the nutritional, phytochemical composition, toxicity, and genotoxicity of *Moringa oleifera* leaves in infusion and powder forms. Nutritional analysis confirmed the presence of proteins, carbohydrates, fiber, minerals, and lipids. Phytochemical profiling via HPLC identified flavonoids and cinnamic derivatives as major components. Acute toxicity tests using Swiss albino mice showed no significant effects at 2000 mg/kg, but behaviour changes were noted at 5000 mg/kg. In a 28-day repeated dose test, the infusion showed no toxicity, while the powder caused liver and kidney damage at 500 and 1000 mg/kg, supported by biochemical and histopathological findings. Genotoxicity and mutagenicity assays at 2000 mg/kg revealed no adverse effects. The study concluded that *M. oleifera* leaves are rich in nutrients and polyphenols, but their indiscriminate use, especially in powder form at higher doses, may pose health risks. Controlled use and further long-term studies are recommended to ensure its safe consumption as a nutraceutical.[63]

5. R Ez-Zriouli et.al, 2023 investigated *Chenopodium ambrosioides*, *Cedrus atlantica*, and *Eucalyptus camaldulensis* for their essential oil yields and biological effects. *C. ambrosioides* produced the highest oil yield (2.1 ± 0.1%), followed by *C. atlantica* (1.0 ± 0.1%) and *E. camaldulensis* (0.75 ± 0.1%). Chemical analysis revealed distinct profiles: *C. atlantica* was rich in sesquiterpenes like β-Himachalene (54.21%) and γ-Himachalene (15.54%), *C. ambrosioides* had high levels of α-Terpinene (53.4%) and ascaridole (17.7%), while *E. camaldulensis* contained monoterpenes such as p-cymene (35.11%) and γ-Eudesmol (11.9%). Antioxidant activity, measured by the DPPH assay, showed the strongest performance from *E. camaldulensis*, with average results for the other two. Antimicrobial activity varied with oil and concentration, exhibiting bactericidal effects similar or superior to synthetic antibiotics. Toxicity tests showed an LD50 of 500 mg/kg for all three, classifying them as category four cytotoxic substances.[64]

6. Y Purnomo et.al, 2022 investigated *Urena lobata* leaf extract for its acute toxicity level on zebrafish (*Danio rerio*). The study evaluated the toxicity through exposure to varying

concentrations of the extract, with results indicating a dose-dependent effect. The in vivo assessment demonstrated significant toxicity at higher concentrations, as evidenced by behavioral changes, mortality, and physiological alterations in the zebrafish. The toxicity was further analyzed through an in silico study, which predicted potential interactions between the leaf extract's bioactive compounds and biological targets, supporting the observed toxicity in the animal model. The study concluded that *Urena lobata* leaf extract exhibited a notable acute toxicity effect on zebrafish, with implications for its safe use.[65]

7. P Verma et.al, 2023 investigated 65% hydroalcoholic extract of *Holarrhena antidysenterica* leaves and bark (Apocynaceae) for its acute and sub-acute toxicity effects on experimental animals. In the acute toxicity trial, mice were given a limit dose of 2000 mg/kg, with observations made over 22–24 hours, followed by 14 days of daily monitoring for mortality, behaviour, injuries, and signs of disease. For the sub-acute toxicity trial, four groups of six animals (3 males and 3 females) were treated with 10% Polysorbate 20 in pure water (Ctrl) or varying doses of the extract (250, 500, and 1000 mg/kg) orally for 28 days. Hematological and biological investigations were conducted at the end of each trial, with liver dissection for gross abnormalities. The results showed no significant differences in body weight, hematological, biochemical parameters, or gross deformities between the treatment and control groups ($p > 0.05$). No deaths were recorded, indicating that medium-term oral ingestion of the extract over 28 days did not lead to lethality.[66]

8. AS Alqahtani et.al, 2022 investigated the antidiabetic medicinal plants found in the Kingdom of Saudi Arabia, focusing on their active phytoconstituents and their toxicological findings. Specifically, the study aimed to identify plants with antidiabetic properties, list the bioactive compounds present in these plants, and explore the toxicological effects associated with their use. It involved searching online databases for relevant studies and compiled information on 50 plant species belonging to 27 different families, highlighting their potential for managing diabetes mellitus and the need for further research to isolate and identify the active antidiabetic compounds.[67]

9. G Anywar et.al, 2021 investigated 11 medicinal plants commonly used by people living with HIV/AIDS (PLHIV) in Uganda, including *Vachellia hockii*, *Albizia coriaria*, *Bridelia micrantha*, *Cryptolepis sanguinolenta*, *Erythrina abyssinica*, *Gardenia ternifolia*, *Gymnosporia senegalensis*, *Psorospermum febrifugium*, *Securidaca*

longipedunculata, *Warburgia ugandensis*, and *Zanthoxylum chalybeum*. This study reviewed literature on their chemical composition, toxicity, and antiviral potential. Most species, except *P. febrifugium*, *S. longipedunculata*, and *C. sanguinolenta*, lacked detailed phytochemical or cytotoxicity data. Crude extracts were mostly used, and cytotoxicity varied with cell line and solvent. Acute oral toxicity studies in mice were primarily conducted using aqueous or ethanol extracts. Toxic side effects were reported in humans mainly for *A. coriaria* and *W. ugandensis*. It hed showed that some plants showed selective cytotoxicity, this could be therapeutically useful for anticancer potential.[68]

10. YO Ali Abdalla et.al, 2022 investigated natural products recently for their anticancer potential, emphasizes their molecular mechanisms and toxicity profiles. Despite their rich ethno pharmacological history and chemical diversity, many plant-derived compounds face challenges in drug development due to unclear mechanisms and unknown molecular targets. A critical concern in cancer research is the host toxicity of investigational agents. It hed showed that data from the past decade on various natural compounds studied for anticancer efficacy across multiple cancer cell lines, along with toxicity screening in rodent models. These toxicological studies help assess host safety and inform optimal dose selection. Understanding the mechanisms of action and molecular targets is essential to minimize side effects while maximizing therapeutic benefits.[69]

11. S Ait Atmane et.al, 2022 investigated Pinus halepensis Mill., commonly known as ‘Zgougou’, is a Mediterranean seed traditionally used to treat bronchitis, rheumatism, infections, and inflammation. This study evaluated the oral safety of its cold-pressed seed oil (COPHS) through acute and 28-day repeated dose toxicity tests in Wistar mice and rats. In the acute test, no mortality or signs of toxicity were observed in mice up to a dose of 5000 mg/kg. In the sub-acute study, rats received 250, 500, and 1000 mg/kg/day for 28 days. Results hed showed no significant changes in body or organ weights, water or food consumption, hematological or biochemical parameters, or tissue histology. No deaths or toxicity symptoms were recorded during the study period.[70]

12. FH Arifah et.al, 2022 investigated *Orthosiphon aristatus* (Blume) Indonesian medicinal plants traditionally used to treat diabetes mellitus (DM). A total of 229 plant species from 70 families were reported, with *Asteraceae* being the most represented family and *Orthosiphon aristatus* the most frequently cited species. Most recipes originated from North Sumatera, commonly using leaves prepared by boiling. The top ten highly cited

antidiabetic plants were assessed for preclinical, phytochemical, clinical, and toxicological data. Despite some available scientific evidence, many plants still lack thorough research. This study highlights the need for further pharmacological and toxicological evaluations.[71]

13. XY Lim et.al, 2021 investigated four medicinal plants—*Nigella sativa*, *Vernonia amygdalina*, *Azadirachta indica*, and *Eurycoma longifolia*—for COVID-19 management. The review was driven by increased community interest in traditional remedies during the pandemic. These plants were selected based on reported antiviral, anti-inflammatory, and immunomodulatory activities. Among them, only *Azadirachta indica* demonstrated preliminary antiviral activity against SARS-CoV-2 in in silico studies. All four plants exhibited varying degrees of anti-inflammatory or immunomodulatory effects, suggesting possible roles in managing immune responses and complications such as cytokine storms. However, definitive conclusions about their efficacy and safety require further in-depth investigation. Emphasis is also placed on the importance of standardizing herbal preparations to ensure consistency and safety. The study supports the relevance of ethnopharmacological research in developing complementary strategies during global health emergencies like COVID-19.[72]

14. EO Amoateng et.al, 2024 investigated *Reissantia indica*, this plant known for its antioxidant, anti-inflammatory, and anticancer properties. Traditional use of medicinal plants in Sub-Saharan health management often lacks sufficient toxicological data, particularly concerning lethal doses. Reissantia indica has gained attention for its therapeutic potential, but its safety profile remains inadequately explored. A comprehensive toxicological evaluation is essential to understand its safety and therapeutic viability. Previous studies have highlighted the presence of secondary metabolites in RIE, including alkaloids, flavonoids, terpenoids, and glycosides, which contribute to its pharmacological effects. While some research has assessed the acute toxicity of various plant extracts, few have investigated the sub-acute toxicity over extended periods. Studies involving rodents have shown that high doses of certain medicinal plant extracts, such as those from Reissantia indica, pose minimal risks when administered in controlled doses.[73]

15. D Satria et.al, 2024 investigated *Artocarpus lacucha Buch-Ham*, known for its pharmacological activities and therapeutic potential. The animal used for the acute oral

toxicity evaluation was rats. The dose regimen involved administering the leaf ethanol extract at two different doses: 2000 mg/kg body weight (BW) and 5000 mg/kg BW, alongside a control group. The study followed the Organization for Economic Co-operation and Development (OECD) guidelines using the fixed dose method. The results revealed no signs of toxicity in any of the test groups. Visual observations, hematological and clinical examinations, as well as histological analysis of the liver, spleen, kidneys, lungs, and heart, showed no abnormalities or adverse effects. No mortality was recorded during the testing.[74]

16. HH Handayani H et.al, 2024 investigated extract of Sambiloto (Andrographis paniculata), Jahe merah (Zingiber officinale var. rubrum), and Kunyit (Curcuma domestica), named SIJAKUN, was tested for its acute and sub-acute toxic effects. The acute toxicity study involved administering the SIJAKUN extract to five groups of mice at doses of 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg, and 5000 mg/kg. After 24 hours, no deaths were observed, and the LD50 of the extract was determined to be above 5000 mg/kg, indicating that SIJAKUN is non-toxic. In the sub-acute toxicity study, the extract was administered at doses of 25 mg/kg, 75 mg/kg, and 150 mg/kg daily for 28 days. No significant changes in SGOT, SGPT, BUN, or serum creatinine levels were observed, suggesting no toxicity to the liver or kidneys.[75]

17. MA El Amiri et.al, 2025 Pharmacological evaluation of AEOM demonstrated significant pain reduction in an animal model, indicating potential analgesic properties. The acute toxicity studies showed no adverse effects on kidney and liver function or blood parameters at doses up to 800 mg/kg. The observed analgesic effect is likely attributed to the flavonoids in the extract, which may inhibit pain pathways. These results suggest that O. majorana has promising therapeutic applications, especially as a natural analgesic agent.[76]

18. G Syahbirin et.al, 2024 investigated Curcuma zedoaria Rosc (Zingiberaceae), commonly known as white turmeric or temu putih, is traditionally used in Indonesian medicine. To support its wider application, this study evaluated its toxicity using brine shrimp (Artemia salina) larvae and zebrafish (Danio rerio) embryos. The crude ethanol extract was tested for its lethal concentration (LC50), which was found to be 588 ppm in brine shrimp larvae and 224 ppm in zebrafish embryos, indicating a moderate level of toxicity. Gas chromatography-mass spectrometry (GC-MS) analysis revealed several active

compounds potentially responsible for the observed toxicity, including epicurzerenone, curzerene, and curzerenone. The predominant compound identified in the extract was 2, 4, 6-trimethylacetophenone.[77]

CONCLUSION

Toxicology helps us understand how different chemicals affect living organisms, especially humans. This document discussed how various substances—like pesticides, heavy metals, and household chemicals—can cause harm depending on the dose, duration of exposure, and the way they interact with the body. These toxic effects can range from temporary irritation to long-term damage at the cellular and genetic level.

Different types of toxicity, such as acute, chronic, and developmental, were explained along with real-life examples. The role of testing methods was also highlighted, including both traditional animal-based tests and newer approaches like in vitro (test tube) and in silico (computer-based) techniques. Guidelines from organizations like OECD and EPA were mentioned to show how toxicity testing is standardized worldwide.

Overall, the study of toxicity is important for public health and environmental protection. With the development of better tools and ethical testing alternatives, the field is moving in a more advanced and responsible direction. As students and future scientists, understanding these basics helps us stay aware of chemical risks and make informed decisions in research and daily life.

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