Toxicology Risk Summary of Neem and Giloy

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***Abstract*—The Neem tree (Azadirachta indica) and the Giloy (Tinospora cordifolia) are widely used medicinal plant known for their antimicrobial, anti-inflammatory, and immunomodulatory effects. In the treatment and prevention of several illnesses, it has been extensively utilized in Chinese, Ayurvedic, and Unani remedies around the globe, particularly in the Indian Subcontinent. As their usage has increased commercially, there is a need to evaluate the risk and safety profiles of these herbs. This project aims to assess the toxicological risk associated with the regular or high dose usage of Neem and Giloy, especially considering their growing popularity in self-medication and commercial formulations.The study also aims to support rational use by drawing attention to reported adverse events and regulatory concerns. Providing a risk summary that informs patients and healthcare providers about risk factors, safe dosage ranges, and contraindications is the aim of carefully reviewing toxicological literature. The ultimate purpose is to encourage the evidence-based use of herbal therapies by bridging the gap between traditional use and modern safety studies.**

***Index Terms*—Immunomodulatory, Anti-inflammatory, Anti-microbial, Herbal medicine, Toxicology, Self-medication, Adverse events, safe dosage ranges, herbal therapies, Safety studies.**

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11.	Reproductive and Developmental Toxicity Discusses fertility studies, teratogenicity outcomes, and impacts during pregnancy and lactation.	
12.	Clinical Toxicity & Pharmacovigilance Signals Presents human case reports, ADR databases, and real-world evidence of safety concerns.	
13.	Benefit-Risk Assessment and Comparative Toxicity Profile Weighs therapeutic potency against toxicity for Neem vs Giloy, identifying relative safety margins.	
14.	Regulatory Perspective and Safety Standards Includes WHO, AYUSH, OECD guidelines, standard permissible limits, and need for quality control.	
15.	Risk Mitigation Strategies & Future Research Needs Concludes with safety recommendations, monitoring parameters, and gaps requiring further toxicological investigation.	

Module 1

1. Overview Of Toxicological Risk Assessment in Herbal Medicine

1.1 Introduction

Toxicological risk assessment is the scientific method to evaluate the likelihood of side effects due to exposure of hazardous substances, including herbal products is a significant challenge in pharmaceutical industries. It involves identifying hazards, assessing the dose response relationship, estimating exposure levels, and characterizing the overall risk. This process is crucial for protecting human health and the environment.

1.2 Hazard vs. Risk

Hazard is a potential source of harm or damage, while risk is the likelihood that a hazard will cause harm, along with the severity of that harm. Both neem and giloy are used in herbal formulations and have least or no side effects but neem is possibly unsafe when taken orally for a longer period of time and might harm the kidneys and liver, though is not considered hazardous and is exempt from hazard classification by the OSHA Hazard Communication Standard. Whereas giloy is considered safe, but excessive or improper use of giloy can potentially harm the kidneys of a person with pre-existing kidney disorders. It can also stimulate auto-immune disorders. But if we look at the brighter side both neem and giloy are considered as safe and non-hazardous.

1.3 Why safety assessment is essential for plant-based therapies?

Safety assessments are crucial for plant-based therapies due to potential risks like contamination, variability in active compounds, and interactions with other medications or conditions. Thorough evaluations ensure the product's safety, efficacy, and quality, protecting consumers from adverse effects and promoting responsible use of herbal remedies. Toxicological evaluation helps identify safe dose ranges, predict likely adverse reactions, and ensure the rational and responsible integration of traditional herbs into modern healthcare.

Module 2

2. Ethnopharmacological Background Of Neem And Giloy

2.1 Introduction

Ethnopharmacological background refers to the historical and cultural context of traditional medicine practices, specifically focusing on how different ethnic groups use plants and other natural substances for medicinal purposes. It encompasses the knowledge, beliefs, and practices related to the use of these

remedies within a specific culture, including the methods of preparation, administration, and the perceived effects.

Neem tree was probably the best kept secret of Ancient India. Neem and Giloy both hold immense value in traditional ayurvedic system since they are used for centuries to treat patients for fever, skin diseases, fungal infections, respiratory ailments, metabolic disroders, and immune dysfunction. It is considered as “village pharmacy” or “the divine tree” due to its broad-spectrum therapeutic benefits such as anti-bacterial, anti-microbial, antiinflammatory, antipyretic and anti-parasitic properties. All parts of the plant are used in remedies such as the leaves, bark, seed, oil, etc. The neem tree holds profound historical significance, deeply intertwined with Indian culture and medicine for millennia. It's been revered as a source of healing, protection, and even divine origin. From ancient texts to modern applications, neem has consistently been valued for its medicinal properties and its role in agriculture and daily life.

Giloy revered as “Amrita” (root of immortality) highlights its reputation for rejuvenation and healing as it's been used for centuries to treat various ailments and promote overall wellbeing and is considered a powerful rejuvenator (rasayana) prescribed for chronic fever, diabetes, liver disorders, and general disability. Giloy leaves have been used for centuries to cure various ailments and diseases in the human body. leaves possess antioxidant activity and induce apoptosis of cells in the immune response.

Module 3

3.1 Taxonomy and Botanical Description

Taxonomy is defined as the practice and science of classification or categorization on the basis of shared characteristics. Accurate taxonomical and botanical identification is important as it helps to ensure the efficacy and safety of herbal preparations. Misidentification or substitution with morphologically similar but chemically different species can lead to serious toxicological consequences.

3.2Neem (Azadirachta indica)

Neem belongs to the family of Meliaceae, commonly known as Mahogany family. It is an evergrowing tree and is fast growing that can reach the height of 15 to 20m with a large crown up to 10-20m in diameter. It is native to Indian subcontinent and is widely cultivated across tropical and semi tropical regions of Asia, South America.

3.3 Morphology of neem:

- Neem is mainly evergreen but sometimes shed its leaves during the dry season. It has a deep taproot and is a mycorrhizal-dependent species.
- The bark is grey, becomes fissured and flakes in old trees.
- A sticky foetid sap exudates from old trees in humid climates .
- The leaves are alternate, petiolated, clustered at the end of the branches, unequally pinnate, glabrous and dark glossy green when matured .
- The leaflets are broad, sickle-shaped and slightly denticulate.
- The flowers are numerous, fragrant, white and borne in large clusters.
- Its fruits are 1-2 cm long drupes, smooth and green with white milky juice when unripe, turning to yellow to brown when mature.
- The fruits have a thin epicarp, a mucilagenous fleshy mesocarp and a hard endocarp that contain a variable number of ovoid oil seeds.

3.4 Uses of Neem:

- Antibacterial
- Anti-inflammatory
- Antifungal
- Antipyretic
- Anti-parasitic
- Pesticides and Insecticides

3.5 Giloy (Tinospora cordifolia)

Giloy belongs to the family of Menispermaceae that is a large deciduous climbing shrub. It grows mainly in tropical and sub-tropical regions, often climbing on larger trees like mango and neem. It is known as Gurcha in India.

3.6 Morphology of Giloy:

- Gurcha is a gregarious glabrous, twiner.
- Older stems are up to 2 cm in diameter along with a corky bark.
- Aerial roots arise from nodal scars of branches.
- Stem and branches are specked with white vertical lenticels.
- Bark is grey-brown or creamy white, papery thin, and peels off easily.
- Leaves are ovate, and acute.

- They are membranous when young but become more or less leathery when they matures.
- Flowers are yellow, unisexual, minute, and less than 2 mm in size.
- Male flowers are grouped in axillary racemes, while female flowers are solitary.
- Fruit is an ovoid and succulent drupe, lustrous, red in colour, and of the size of a large pea, having a single seed.
- Seed is fleshy and curved. Flowering occurs in May–June, while fruiting is witnessed in September–October.

3.7 Uses:

 General disability

- Immunomodulator
- Chronic fever
- Diabetes
- Liver disorders

Module 4

4.1 Phytochemical Composition – Neem (Azadirachta indica)

Phytochemicals are naturally occurring compounds that are present in or are extracted from the plant using physical or chemical methods.

S.No.	Test	Observation	Inference
Alkaloids	• 0.5g sample dissolved in hydrochloric acid, filtered. • 2ml filtrate treated with Dragendroff's reagent (potassium Bismuth iodide solution).	• Red precipitate formation confirms alkaloid presence.	• Hager's reagent treatment confirms alkaloid presence with yellow colour.
Saponins	• Boil 0.5g sample with 50ml distilled water. • Filter 5ml of filtrate with 3ml distilled water. Shake vigorously for 5 minutes.	• Confirm frothing formation, indicating presence of saponins.	• Presence of Saponins was confirmed.
Glycosides	• Dissolved sample in ethanol for 10 minutes and then filtered. • 0.3ml Fehlings solution A and B added to each filtrate.	• Alkaline solution indicates glycoside presence.	• Presence of Glycosides was confirmed.
Terpenoids	• Dissolved 0.5g sample in ethanol for 10 minutes. • Filtered and added acetic anhydride and concentrated H2S04.	• Change in colour from pink to violet indicates terpenoid presence.	• Presence of Terpenoids was confirmed.
Flavonoids	• Dissolve 0.5g sample in distilled water. • Filter to 5ml. Add 3ml lead ethanoate solution.	• Observance of pale yellow-brown flavonoid appearance.	• Presence of Flavonoids was confirmed.

Kingdom	Plantae
Division	Magnoliophyta
Order	Rutales
Suborder	Rutinae

Family	Meliaceae
Subfamily	Melioideae
Tribe	Melieae
Genus	Azadirachta
Species	Indica

TABLE4.1

Steriods	• Dissolved sample in distilled water. • Filtered to 4ml of filtrate. • Add 2ml acetic acid in it. • Cooled in refrigerator. • Added concentrated H2SO4 in the sample.	• Violet to bluish green colour indicated steroidal ring presence.	• Presence of Steroids was confirmed.
Phenol	• 0.5g sample boiled with 15ml distilled water. • Filtrate added 10% ferric chloride solution.	• Violet colour confirmed, indicating phenolic hydroxyl group.	• Presence of Phenol was confirmed.

A table showing major constituents of Neem and their pharmacological role :
TABLE4.2

Plant part	Major constituents	Pharmacological role	Toxicological Concerns
Leaves	Quercitin, Nimbin, Polyphenols	Anti-inflammatory, Antioxidant, Antimicrobial	Safer in dietary amounts but concentrated extracts may upset GIT.
Seeds	Azadirachtin, Nimbin, Salannin	Insecticidal, Antiparasitic	High doses may cause neurotoxocity, hepatotoxicity.
Bark	Nimbidin, Tannins, Polysaccharides	Anti-inflammatory, Anti-ulcer	Tannins may impair iron absorption in the body.
Oil	Azadirachtin, Fatty acids, Sulfur comounds	Antimicrobial, Wound healing	May be highly toxis if ingested undiluted.
Flowers	Flavonoids, Essential oils	Antioxidant, mild diuretic	They causes minimal toxicity.

This phytochemical profile of Neem shows a dose-dependent duality:

- At therapeutic doses, it delivers antimicrobial, anti-inflammatory, and immune-boosting benefits.
- At high or uncontrolled doses, particularly with seed oil or concentrated limonoids, it poses hepatic, neurological, and metabolic risks.

Module 5

5.1Phytochemical Composition – Giloy (Tinospora cordifolia)

Phytochemicals are naturally occurring compounds that are present in or are extracted from the plant using physical or chemical methods.

Giloy, also called *Amrita* in Ayurveda, owes its pharmacological versatility to a rich and diverse set of phytochemicals. These compounds, present in the

stem, leaves, and roots, are responsible for its immunomodulatory, antipyretic, antioxidant, and anti-inflammatory properties. However, certain components, particularly in high doses or prolonged use, have been implicated in liver toxicity, making a toxicology-oriented evaluation essential.

TABLE 5.1

Kingdom	Plantae
Division	Tracheophyta
Order	Ranunculales
Family	Menispermaceae
Genus	Tinospora
Species	Cordifolia

Major Classes of Phytochemicals

TABLE 5.2

S.no.	Test	Observation	Inference
1.Carbohydrates	• Molisch's test: Involves adding 20% alcoholic solution and concentrated sulphuric acid to a plant extract sample. • Benedict's test: Combines a small amount of plant extract with Benedict's solution. Boil and cool the solution. • Barfoed's test: Adds Barfoed's solution to a 0.5 ml solution and boils.	• Reddish violet and purple colour at junction between two liquids shows presence of carbohydrates. • Red precipitate is formed. • Red precipitate of copper oxide is formed.	• Presence of Carbohydrates is confirmed.
2.Alkaloids	• Dragendorff's test: Dissolve extract in water, add hydrochloric acid, and add potassium bismuth iodine solutions. • Wagner's test: Acidifies extract with hydrochloric acid, add Wagner's reagent. • Mayer's test: Add potassium mercuric iodine solution to extract.	• Orange red precipitate is formed. precipitate is formed. • Dull white precipitate Reddish brown is formed.	• Presence of Alkaloids is confirmed.
3.Glycosides	• Legal's test: Dissolves extract sample in pyridine, add sodium nitroprusside solution • Baljet's test: Adds sodium picrate solution • Borntrager's test: Add extract solution to dilute sulphuric acid solution and filter it then add chloroform and ether, separate organic layer with ammonia.	• Pink-red colour is produced. • Yellow-orange colour is produced. Pink, red, or violet colour is produced.	• Presence of Glycosides is confirmed.
4. Saponins	• 1ml alcoholic sample diluted with 20ml distilled water.	• Presence of a foam layer is seen in the sample.	

	Shake solution in graduated cylinder for 15 min. • Shinoda test: Involves adding hydrochloric acid	Reddish pink or brown colour indicates flavonoids presence.	• Presence of Saponin is confirmed.
	and magnesium to alcoholic sample extract. • Alkaline reagent test: Mixes plant extract with 2% NaOH solution, produces yellow colour.	• Yellow to colorless sample is obtained.	
5.Tanins	• Add ferric chloride solution to sample. • Potassium cyanide produces deep red color, indicating tannin presence. • Potassium dichromate forms yellow precipitate, indicating tannin presence.	• Dark blue or greenish black color indicates tannin presence.	• Presence of Tanin is confirmed.
6.Protein& Aminoacids	• Biuret's test: Mixing plant extract with sodium hydroxide and copper sulphate solutions. • Ninhydrin's test: Heating plant extracts with 0.2% Ninhydrin solution. • Xanthoprotein test: Adding nitric acid to extracts.	• A pinkish - violet and purple - violet colour is obtained. • White precipitate turns yellow upon heating. • Excess sodium hydroxide solution produces orange colour, indicating amino acid presence.	• Presence of Amino acids is confirmed.
7.Fats or Fixed oils	• Mixing extract with 1% copper sulphate solution and 10% sodium hydroxide solution. • Mixing plant extracts with 2% sodium carbonate solution	• Blue color indicates presence of glycerine. • Shaking, boiling, and separating soapy solution. • Addition of HCl reveals fatty separation and floatation.	• Presence of Fats is confirmed.

Class Class	Key Compound	Role	Toxicological Relevance
Alkaloids	Magnoflorine, Plamatine, Berberine, Jatrorrhizine	Immunostimulatory, antimicrobial, and anti-inflammatory effects.	Overuse can lead to hepatocellular damage, especially in patients with pre-existing liver conditions; berberine may

2. Distribution of Phytochemicals by Plant Part

TABLE 5.3

		Palmatine and berberine also contribute	interact with drugs metabolized via CYP450 enzymes.

		to antidiabetic and hepatoprotective activities.	
Diterpenoid Lactones	Tinosporide, Tinosporaside, Cordifolide, Cordifol	Anti-inflammatory, antioxidant, and hypoglycemic properties.	Prolonged exposure to high doses may disrupt liver enzyme balance, potentially leading to hepatotoxicity.
Glycosides	Tinosporaside, Cordioside	Enhance immunity, support liver function, and modulate inflammatory pathways.	Usually safe at recommended doses; concentrated extracts may cause mild gastrointestinal irritation.
Phenolic Compounds & Flavonoids	Quercetin, Apigenin, Luteolin	: Potent antioxidant activity, anti-allergic, and anti-inflammatory effects.	Generally safe, but high flavonoid concentrations may interfere with thyroid function in sensitive individuals.
Polysaccharides	Arabinogalactan, Glucans	Immunomodulatory effects by enhancing macrophage activity.	Minimal toxicity; may cause allergic reactions in rare cases.
Plant Part	Major Constituents	Pharmacological Role	Toxicological Concerns
Stem	Alkaloids, Diterpenoid lactones, Polysaccharides	Immunomodulation, antipyretic, anti-inflammatory	High-dose stem extracts linked to liver enzyme elevation
Leaves	Flavonoids, Alkaloids, Phenolic acids	Antioxidant, antimicrobial	Rare allergic reactions
Roots	Diterpenoids, Alkaloids	Anti-diabetic, hepatoprotective	High doses may affect liver function

5.2 Relevance to Toxicological Risk Assessment

Giloy's phytochemical diversity explains its wide therapeutic application, from fever reduction to immune enhancement. However, in recent clinical case reports, unsupervised or high-dose use—particularly in patients with underlying autoimmune or liver disease—has been linked to drug-induced liver injury (DILI). This underscores the importance of:

- Standardized extraction methods to control bioactive compound levels.
- Clear dosage guidelines to minimize adverse events.
- Patient screening before recommending prolonged use.

Module 6

6.1 Pharmacological Actions Linked to Toxicity Potential

Neem and Giloy are two fundamental botanicals in Ayurvedic medicine, each with a broad therapeutic spectrum supported by centuries of traditional use and modern pharmacological studies. While their mechanisms overlap in certain domains (e.g., anti-inflammatory and immunomodulatory effects), they also exhibit different bioactivities due to their differences in phytochemical profiles. Understanding these similarities and differences is critical for

accurate toxicological evaluation, safe clinical application, and the prevention of herb–herb or herb–drug interactions.

Overlapping Pharmacological Activities:

TABLE 6.1

Activity	Neem	Giloy	Relevance to risk assessment
Immunomodulatory	Enhances immune response via limonoids and polysaccharides.	Modulates immune pathways through diterpenoid lactones and alkaloids.	Risk of autoimmune flare-ups if used in susceptible individuals.
Anti-inflammatory	Inhibits COX & LOX pathways.	Down regulates proinflammatory cytokines.	Prolonged use could suppress necessary inflammatory responses.
Antioxidant	Rich in flavonoids & phenolic acids.	Contains quercitin, leteolin, phenolic compounds.	Generally beneficial, but excessive antioxidant intake may interfere with ROS- dependent immune mechanisms.
Antimicrobial	Strong antibacterial and antifungal effects.	Mild antibacterial and antiviral properties.	Potency of neem may disrupt gut microbiota with high doses.
Anti-diabetic	Azadirachtin & flavonoids regulate blood glucose.	Berberine & Tinosporaside lower glucose.	Risk of hypoglycaemia if combined with antidiabetic drugs.

Unique Pharmacological Features of Neem & Giloy:

TABLE6.2

Feature	Neem	Giloy
Antimalarial	Limonoids inhibit Plasmodium life cycle.	Not a primary activity.
Anticancer	Induces apoptosis in certain cancer cell lines via nimbolide.	Shows mild cytotoxicity in vitro.
Hepatoprotective vs hepatotoxicity	Traditionally used for liver health, but rare hepatotoxicity reported.	Recent clinical case reports of herb-induced liver injury.
Adaptogenic features	Mild adaptogen	Strong adaptogenic &antistress activity.

6.2 Mechanistic Comparison of Neem & Giloy:

- **Neem**:
  - Primarily it disrupts pathogen membrane integrity, supresses inflammatory enzymes, modulates immune signaling.
  - Secondarily enhances metabolic regulation and antioxidant protection.

Giloy:

  - Primarily affects the cytokine modulation, enhances phagocytic activity, antioxidant defense.
  - Secondarily it affects adaptogenic stress resistance, liver enzyme stabilization.

6.3 Implications for Combined Use of Neem & Giloy:

When Neem & Giloy are used together they may provide synergistic immunomodulation and increased antioxidant protection but,

- In Diabetic patients, overlapping hypoglycemic effects can cause dangerously low blood sugar levels.
- Additive immuno-stimulation may worsen autoimmune conditions.
- Potential hepatotoxicity risk if both are used in high doses or with hepatotoxic drugs.

6.4 Toxicological Significance

A comparative understanding allows for better clinical decision-making:

- In patients with chronic infection or immune suppression, combined use may be beneficial.
- In patients with autoimmune diseases, liver disorders, or on polypharmacy, monotherapy with close monitoring is safer.
- Standardization of extracts is essential to avoid unpredictable bioactive concentrations.

Module 7

7.1 Introduction

Acute toxicity studies as they provide first line of safety evaluation for any medicinal plant. In toxicology, the median lethal dose, **LD_{50}** is a toxic unit that measures the lethal dose of a given substance. The value of LD_{50} for a substance is the dose required to kill half the members of a tested population after a specified test duration. They help determine the LD_{50}, identify immediate toxic symptoms, and establish safe starting doses for further testing. For Neem (*Azadirachta indica*) and Giloy (*Tinospora cordifolia*), these studies are helpful to reveal important dose thresholds and early signs of adverse effects that are crucial for assessing their therapeutic window and preventing misuse.

Comparative Acute Toxicity Profile:

TABLE7.1

c. Toxicological Implication

Parameter	Neem(Azadirachta indica)	Giloy(Tinospora cordifolia)
LD_{50}(Oral, Rodents)	Leaves: >2,000 mg/kg Oil: 14-24 ml/kg	Aqueous: >5,000mg/kg Alcoholic: >2,000mg/kg
Primary concern	Oil toxicity(neurological & gastrointestinal symptoms).	Minimal acute risk at tasted doses.
Mortality at high dose	Yes, with neem oil ingestion.	No
Acute symptom severity	Moderate to severe symptoms	Mild sedation, soft stools

Giloy is considered practically nontoxic in acute settings, but effects on liver enzymes at extremely high doses should be monitored.

7.2 Acute Toxicity of Neem

a. LD_{50} and Dose Thresholds

- **Neem Seed Oil**: Reported LD_{50} in rats ranges between *14–24 mL/kg body weight* (oral).
- **Neem Leaf Extracts**: LD_{50} generally exceeds *2,000 mg/kg* in rodents, indicating relatively low acute toxicity.
- **Azadirachtin (active compound)**: LD_{50} values vary from *>3,540 mg/kg* (rats) to >5,000 mg/kg (mice) depending on purity.

b. Short-term Observations

- At high doses: drowsiness, respiratory distress, tremors, diarrhoea, and convulsions reported in animal studies.
- Very high oral doses of neem seed oil in young rats led to mortality within 24–48 hours.

c. Toxicological Implication

- Leaf-based preparations are safer than oil extracts.
- LD_{50} data supports the safe traditional use of leaves and bark at recommended doses but warns against excessive oil ingestion.

7.3 Acute Toxicity of Giloy

a. LD_{50} and Dose Thresholds

Aqueous Stem Extract: LD_{50} in rats > *5,000 mg/kg* (oral), suggesting a very wide safety margin.

Alcoholic Extract: LD_{50} > *2,000 mg/kg* in mice.

b. **Short-term Observations**

- At extremely high doses: mild sedation, decreased locomotor activity, and occasional loose stools reported.
- No mortality observed up to highest tested doses in most studies.

7.4 Toxicology Relevance

- Neem: Narrower safety margin when oil is used; dose control is critical.

- Giloy: Low acute toxicity but chronic safety still requires evaluation.
- Risk in Botanicals: Acute toxicity data forms the foundation for setting safe limits and designing sub-chronic and chronic studies.

Module 8: Sub-acute and Sub-chronic Toxicity Outcomes

8.1 Introduction

Acute toxicity describes the adverse effects of a substance that result either from a single exposure or from multiple exposures in a short period of time. While acute toxicity studies assess short-term safety, sub-acute (14–28 days) and sub-chronic (up to 90 days) studies help identify potential cumulative or delayed toxic effects from repeated exposure. These tests are particularly important for botanicals like Neem (*Azadirachta indica*) and Giloy (*Tinospora cordifolia*), which are often consumed daily over weeks or months in traditional medicine. Evaluations typically include organ weight analysis, hematological profiling, and biochemical markers to detect early organ-specific damage before visible clinical symptoms appear.

8.2 Sub-acute and Sub-chronic Toxicity of Neem:

a. Study Designs & Dose Ranges

Leaf Extracts: Oral administration in rats at 200–2,000 mg/kg for 28–90 days.

- Neem Oil: Administered at lower doses (≤0.5 mL/kg) due to known acute toxicity.

b. Observations in Animal Studies

- Organ Weights: No significant changes in liver, kidney, heart, or spleen at leaf extract doses ≤1,000 mg/kg.
- Hematology: Stable red blood cell (RBC) and white blood cell (WBC) counts at lower doses; occasional mild anemia at highest doses.
- Biochemical Markers:
  - Liver: Mild transient increase in ALT and AST at high doses (>1,500 mg/kg).
  - Kidney: Slight elevation of creatinine and urea in high-dose neem oil studies.
- Histopathology: Minor hepatic cellular swelling in high-dose neem oil groups; reversible on recovery.

c. Safety Conclusion

- Leaf extracts up to 1,000 mg/kg/day are generally safe for repeated use.
- Neem oil shows early signs of hepatotoxicity and nephrotoxicity if used daily in high doses.

8.3 Sub-acute and Sub-chronic Toxicity of Giloy:

a. Study Designs & Dose Ranges

- Aqueous Stem Extract: 250–1,000 mg/kg/day orally in rats for 28–90 days.
- Ethanolic Extract: 200–800 mg/kg/day for similar durations.

b. Observations in Animal Studies

- Organ Weights: No dose-dependent changes in liver, kidney, heart, or spleen.
- Hematology: Stable hemoglobin, RBC, and WBC counts; mild leukocytosis in some studies indicating immune stimulation.
- Biochemical Markers:

  - Liver: ALT [Alanine Aminotransferase (formerly called SGPT – Serum Glutamate Pyruvate Transaminase)], AST [Aspartate Aminotransferase (formerly called SGOT – Serum Glutamate Oxaloacetate Transaminase)], ALP (Alkaline Phosphatase) remain within normal limits in most cases.

  - Kidney: No significant changes in serum creatinine or urea.
  - Histopathology: Normal cellular architecture of major organs across dose groups.

c. Safety Conclusion

- Giloy demonstrates excellent subchronic tolerance up to 1,000 mg/kg/day without signs of major organ toxicity.
- Some studies suggest immunostimulatory activity, which could be beneficial but warrants further study in autoimmune conditions.

 Comparative Sub-acute & Sub-chronic Toxicity Summary:

TABLE8.1

Parameter	Neem (*Azadirachta indica*)	Giloy (*Tinospora cordifolia*)
Tested Dose Range	Leaves: 200–2,000 mg/kg Oil: ≤0.5 mL/kg	250–1,000 mg/kg
Organ Weight Changes	None at safe dose; minor changes at high oil doses	None observed
Hematology	Mild anemia at very high doses	Stable counts; occasional leukocytosis
Biochemical Changes	Slight ALT/AST elevation in high doses	No significant changes
Histopathology	Minor reversible hepatic alterations	Normal
Overall Safety	Safe up to 1,000 mg/kg/day for leaf extract; caution with oil	Safe up to 1,000 mg/kg/day

8.4 Toxicology Relevance:

Repeated-dose studies reveal that both herbs are generally safe at traditionally recommended doses, neem oil has a narrower margin of safety compared to leaf extracts, and Giloy maintains a high safety margin over weeks of use.This information is crucial for guiding dosage in herbal formulations and for regulatory approval processes.

Module 9 – Chronic Toxicity Evaluations (Long-term Use)

9.1Introduction

Chronic toxicity studies are designed to assess the effects of prolonged exposure to a substance, typically over three months to two years in experimental models. In the context of botanicals like Neem (*Azadirachta indica*) and Giloy (*Tinospora cordifolia*), these studies are essential for determining whether continuous or high-frequency consumption could lead to cumulative organ damage, carcinogenesis (cancer formation), or other systemic health issues.

9.2 Neem (*Azadirachta indica*)

Chronic administration studies in rodents at doses far exceeding typical human consumption have reported mild to moderate liver changes, alterations in reproductive hormones, and occasional kidney histopathology. However, at therapeutic doses, no significant organ damage has been documented. Concerns have also been raised over the potential long-term effects of azadirachtin-rich extracts, especially in reproductive health, but the evidence remains inconsistent.

9.3 Giloy (*Tinospora cordifolia*)

Long-term toxicity evaluations are comparatively limited. Animal studies with extended dosing (up to 6 months) suggest good overall tolerance, but isolated reports indicate possible hepatotoxicity in individuals with preexisting liver conditions, particularly when consumed in unregulated high doses or in combination with other hepatically metabolised herbs. The presence of diterpenoid lactones and alkaloids has prompted some researchers to investigate their potential role in oxidative stress–related liver injury, although findings are not conclusive.

Chronic toxicity assessments also explore carcinogenic potential. Current literature shows no strong evidence of carcinogenicity for either Neem or Giloy, but the absence of long-term human cohort data means this cannot be definitively ruled out. As part of responsible herbal safety evaluation, such studies help establish No Observed Adverse Effect Levels (NOAEL) and define safe upper limits for prolonged consumption, guiding both regulatory frameworks and public health recommendations.

Module 10 – Genotoxicity and Mutagenicity Testing

10.1Introduction

Genotoxicity is defined as the ability of a substance to damage the genetic material (i.e. DNA or chromosomes) within a cell, while mutagenicity focuses specifically on the induction of permanent, heritable genetic changes. These effects are particularly important to investigate in herbal

medicines because DNA damage is a key pathway to cancer development, reproductive problems, and hereditary disorders. For botanicals like Neem **(***Azadirachta indica***)** and Giloy **(***Tinospora cordifolia***)**, the perception of "natural equals safe" often overshadows the need for rigorous safety evaluation. However, given their widespread use in chronic conditions, assessing their genotoxic and mutagenic potential is essential for establishing long-term safety.

10.2 Key Test Methods Used in Evaluation

The genotoxicity and mutagenicity profile of plant extracts is usually assessed using a tiered approach:

1. Ames Test (Bacterial Reverse Mutation Assay) o Purpose: Detects point mutations in specific strains of *Salmonella typhimurium* or *Escherichia coli* by determining if the substance can reverse an existing mutation. o Neem Findings: Standardised aqueous and ethanolic extracts of neem leaves generally test negative for mutagenicity in the Ames assay, even at high doses. Neem seed oil at very high concentrations has sometimes produced weak positive results, likely due to the presence of azadirachtin and related limonoids, but these concentrations far exceed therapeutic exposure.
   - o Giloy Findings: Giloy extracts consistently show negative results in Ames tests, indicating low mutagenic risk. Interestingly, some studies have demonstrated *antimutagenic* effects, suggesting protective activity against chemical mutagens.
2. Chromosomal Aberration Test (In Vitro and In Vivo)
   - o Purpose: Detects structural or numerical changes in chromosomes, which can indicate clastogenic (chromosomebreaking) activity.
   - o Neem Findings: Rodent bone marrow studies and human lymphocyte cultures exposed to neem leaf extracts have shown no significant increase in chromosomal aberrations at medicinal doses. In vitro exposure to concentrated neem oil can cause minor aberrations, but only at toxic dose levels.
   - o Giloy Findings: Data from in vivo rodent models suggest no significant chromosomal damage after repeated administration of Giloy stem extracts.
3. Comet Assay (Single-Cell Gel Electrophoresis) o Purpose: Detects single- and double-strand DNA breaks at the individual cell level.
   - o Neem Findings: In animal hepatocytes and lymphocytes, neem leaf extract showed no evidence of DNA fragmentation at standard therapeutic doses; high doses caused mild, reversible DNA damage in isolated in vitro systems.
   - o Giloy Findings: Giloy extracts often display a DNA-protective effect in Comet assays, reducing strand breaks caused by known genotoxins such as hydrogen peroxide.

 Overall Interpretation:

TABLE 10.1

Test	Purpose	Neem	Giloy
Ames test	Helps to detect point mutation in specific strains of *Salmonella typhimurium* or *Escherichia coil*. Determines if the substance can reverse an existing mutation.	• Standardised extracts generally test negative for mutagenicity in Ames assay. • High concentrations of neem seed oil sometimes yield weak positive results. • These concentrations exceed therapeutic exposure.	• Consistently show negative results. • Demonstrates low mutagenic risk. • Some studies show antimutagenic effects.

Chromosomal Aberration test	Helps to detect structural or numerical changes in chromosomes.	• No significant increase in chromosomal aberrations at medicinal doses in rodent bone marrow and human lymphocyte studies. • Minor aberrations observed in vitro at toxic dose levels.	• Data from in vivo rodent models suggest no significant chromosomal damage after repeated administration.
Comet assay (Single-cell gel electrophoresis)	Helps to detect single and double stranded DNA break at individual cell level.	• No evidence of DNA fragmentation at standard doses. • High doses cause mild, reversible DNA damage.	• Displays DNA-protective effect in Comet assays. • Reduces strand breaks from genotoxins.

- Neem: The available body of evidence suggests neem extracts are largely nongenotoxic when used within traditional therapeutic ranges. Weak genotoxic signals appear only in highly concentrated, unprocessed seed oil or purified azadirachtin exposure, highlighting the importance of dose control and extraction method.
- Giloy: Demonstrates an even stronger safety margin, with multiple reports of DNA-protective effects due to its antioxidant and free radical scavenging properties. No mutagenic or clastogenic effects have been observed in standardised extracts at realistic exposure levels.

10.3 Regulatory and Safety Perspective:

Regulatory agencies such as OECD (Organisation for Economic Co-operation and Development) recommend at least a basic battery of genotoxicity tests — including a bacterial reverse mutation test and a mammalian chromosomal aberration test — for herbal products intended for chronic use. This ensures that even "natural" remedies meet the same safety standards as synthetic drugs.

For neem and giloy, the accumulated literature supports their low genotoxicity risk, but quality control (standardisation, proper identification, and removal of extraneous toxic plant parts) remains essential to maintain this safety profile.

Module 11 – Reproductive and Developmental Toxicity

11.1Introduction

Reproductive and developmental toxicity studies assess whether a substance can impair fertility, cause birth defects (teratogenicity), or adversely affect growth and development during gestation and lactation. This area of toxicology is especially critical for botanicals like Neem (*Azadirachta indica*) and Giloy **(***Tinospora cordifolia***)**, because they are often used in women of childbearing age, sometimes even during pregnancy, under the assumption that "natural equals safe." In reality, certain plant constituents can cross the placenta, interfere with hormone regulation, or be secreted in breast milk — potentially affecting both the mother and offspring.

11.2 Fertility and Contraceptive Effects:

- Neem:
  - *Traditional Use:* Neem has a longstanding history in traditional medicine as a natural contraceptive, particularly in Ayurveda, Unani, and folk practices. Neem oil has been used intravaginally as a spermicide.
  - *Scientific Evidence:* Animal studies in rats and rabbits have demonstrated that neem oil and leaf extracts can reduce sperm motility and viability, decrease testicular weight, and alter hormone levels when administered in high doses or via specific routes (e.g., intrauterine or intravaginal). These effects were generally reversible after

discontinuation, but they highlight a clear antifertility potential at certain concentrations.

- *Implication:* While neem's antifertility properties may be beneficial in controlled contraceptive use, they raise safety concerns for individuals trying to conceive.

• Giloy:

- Giloy is not traditionally linked to contraceptive effects, and animal studies show no significant impact on sperm count, motility, or female reproductive cycles at therapeutic doses.
- Some evidence suggests giloy's antioxidant and adaptogenic properties might protect reproductive tissues from oxidative stress, potentially supporting fertility rather than impairing it.

11.3 Teratogenicity (Birth Defects) Studies:

• Neem o High-dose neem oil administration in pregnant rats has been associated with embryotoxic effects, including increased resorption rates and growth retardation. However, these effects occurred at doses far exceeding typical medicinal use. o No consistent evidence of structural birth defects has been reported in controlled studies with standardised neem leaf extracts, but caution is warranted due to neem's hormonal and immunomodulatory activity.

• Giloy o Giloy extract administration during organogenesis in rats and rabbits has not shown teratogenic effects. In fact, some studies indicate a protective role against teratogens due to its antioxidant capacity.

o However, data on human pregnancy are limited, and absence of evidence does not guarantee safety — hence, medical supervision is advised during pregnancy.

11.4 Pregnancy and Lactation Safety:

• Neem o Due to its abortifacient potential at high doses (linked mainly to neem seed oil), neem is generally advised to be avoided during pregnancy, especially in the first trimester. o Small amounts in dietary or topical use (like toothpaste or mild herbal rinses) are unlikely

to be harmful, but systemic assessment during high-dose supplementation organogenesis. poses risks. o For botanicals, these guidelines are o There is minimal data on neem adapted to account for the constituents passing into breast complexity of plant mixtures milk, so precautionary (multiple active constituents). avoidance during lactation is ICH (International Council for recommended. Harmonisation):

• Giloy

• o ICH S5 (R3): Outlines

• o No reproductive toxicity or reproductive toxicity testing for lactation-related hazards have human pharmaceuticals, including been identified in available botanical preparations.

animal data. Giloy's o Requires a tiered approach**:** immunomodulatory nature

o could theoretically influence infant immune development, but no adverse outcomes have been documented in humans.

Given the lack of robust human studies, many herbal safety Fertility and early embryonic development (Segment I)

 Embryo–foetal development (Segment II)

 Pre- and postnatal development (Segment III)

references recommend

cautious use in pregnancy and o Special focus is placed on the need lactation. to identify NOAELs (No Observed

Adverse Effect Levels) for

11.5 Regulatory Perspective reproductive endpoints.

• US FDA (Food and Drug Reproductive and developmental toxicity Administration) – Botanical Drug assessment is considered non-negotiable for Development Guidance: any new botanical or phytopharmaceutical o FDA requires botanical drug product intended for human use. applicants to submit complete Internationally, several agencies have nonclinical reproductive toxicity established guidelines that must be followed data if there is potential for before such products are marketed, particularly exposure during pregnancy or if they are intended for long-term consumption lactation.

  • or may be used by women of childbearing age. o For herbal dietary supplements, strict toxicity studies may not be

1. Key Regulatory Frameworks**:** mandatory before marketing, but the manufacturer is responsible for

- OECD (Organisation for Economic ensuring product safety. Co-operation and Development): WHO (World Health Organization) – ₀ The OECD has developed Test Guidelines on the Safety Monitoring of Guidelines (e.g., TG 421, TG 422, Herbal Medicines

TG 414) for reproductive and o Emphasises the precautionary developmental toxicity testing. principle for pregnancy and

- TG 421 & TG 422: Screen for lactation: unless adequate effects on fertility, mating reproductive safety data exist, behaviour, gestation, and herbal products should be labelled early postnatal development as "not recommended during in rodents. pregnancy/lactation."
- TG 414: Specifically o Encourages post-marketing addresses prenatal surveillance for adverse developmental toxicity, reproductive outcomes. including teratogenicity

2. Relevance to Neem and Giloy:

- Neem (*Azadirachta indica*) o Given its known antifertility and abortifacient activity, neem products—especially concentrated oils and extracts—would likely trigger full reproductive toxicity testing requirements under OECD and ICH guidelines before being considered safe for women of childbearing potential. o Regulatory agencies may impose label restrictions such as:
- "Contraindicated in pregnancy"
- "May impair fertility in both sexes at high doses"

o In countries with strong herbal medicine regulation (e.g., Germany's Commission E), neem's internal use in pregnancy is not approved.

 Giloy (*Tinospora cordifolia*)

- o With no major evidence of reproductive toxicity, giloy may pass preliminary regulatory safety screens more easily, though confirmatory studies are still necessary to satisfy OECD/ICH standards.
- o Absence of harm in animals does not exempt it from pregnancy risk

4. Summary Table – Regulatory Red Flags: TABLE11.1

categorisation; in many jurisdictions, it would still carry a "Use with caution" or "Insufficient data" label.

3. Practical Implications for Product Development:

 If neem or giloy are intended as standalone herbal medicines or formulation components, regulatory authorities will expect:

1. Animal reproductive toxicity data covering all three ICH segments.
2. Standardisation of extracts to minimise batch-to-batch variability in active constituents.
3. Post-marketing pharmacovigilance to detect rare adverse reproductive effects in humans.

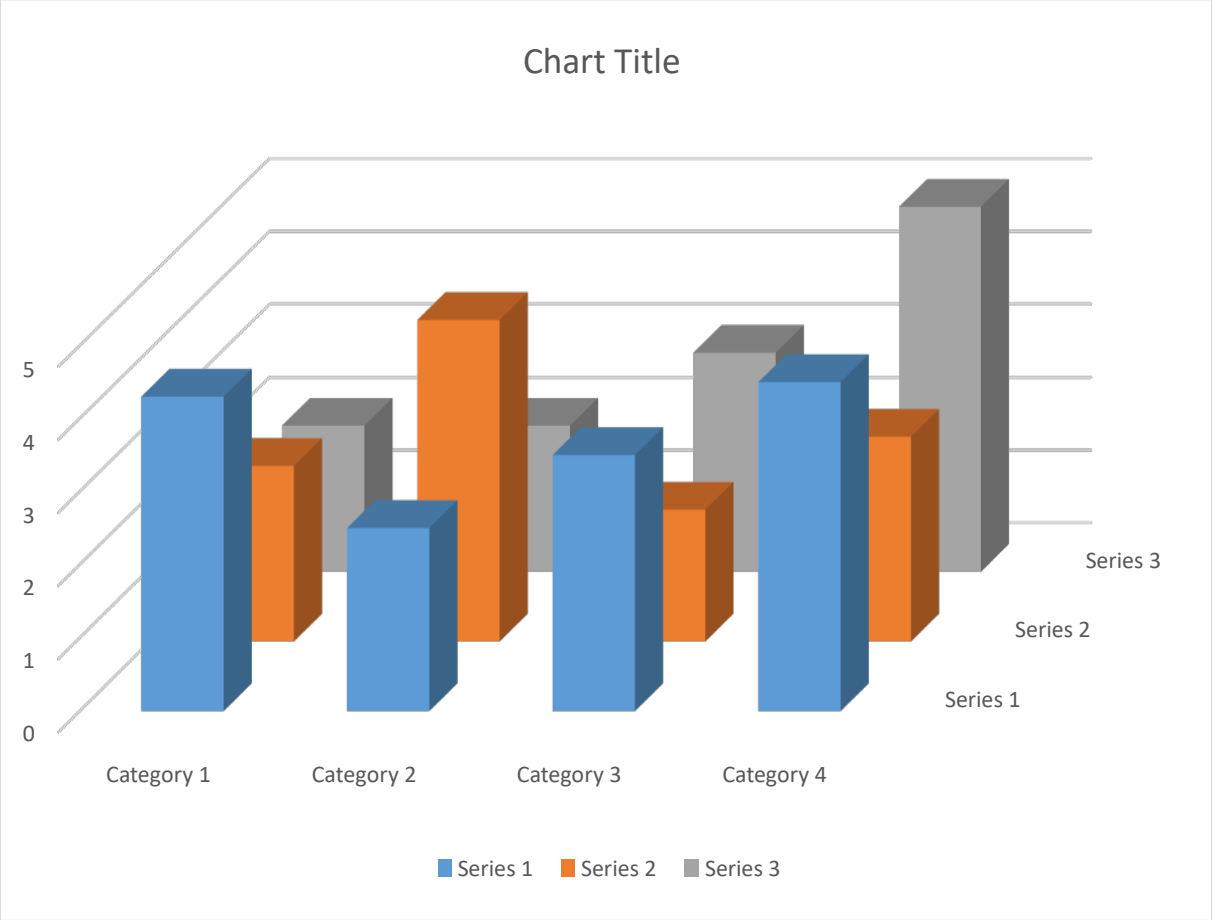
- In herbal combination products, the most hazardous component (e.g., neem for reproductive toxicity) determines the overall reproductive safety classification.
- Marketing claims such as "safe during pregnancy" or "supports fertility" require direct, well-documented evidence; otherwise, they may be deemed misleading and prohibited.

Herb	Concerns	Regulatory action	Pregnancy/ lactation label
Neem	Antifertility, helps in abortion, hormonal modulation.	Full reproductivity toxicity testing, pregnancy contraindication.	Avoided during pregnancy & lactation.
Giloy	No strong reproductive hazards, but limited human data.	Basic reproductive toxicity screening, precautionary warning.	Use with caution, consult with physician.

5. Conclusion:

TABLE11.2

Parameter	Neem	Giloy
Fertility (male/female)	Antifertility at high doses but is reversible.	No impairment at therapeutic doses; antioxidant support possible
Embryo foetal development	Embrotoxocity with high doses but leaf extracts aer safer at therapeutic range.	No teratogenicity in animals but data for humans is limited.
Pregnancy	Contraindicated specially during first trimester.	Insufficient data; medical supervision advised.
Lactation	Avoided systemic use due to lack of data.	Limited data.
Label expectation	Avoided in pregnancy and lactation.	Use with caution; consult physician.
Regulatory trigger	Full OECD/ICH package mandatory for chronic use.	Screening battery adequate; limited data.

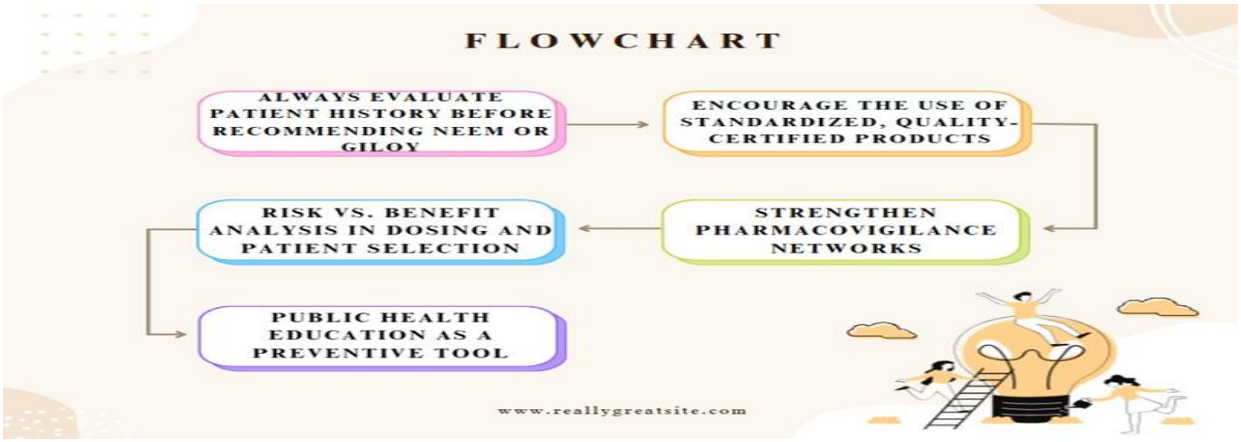


Module 12 Clinical Toxicity & Pharmacovigilance Signals

12.1Introduction

While preclinical studies in animals provide valuable baseline safety information, the true picture of toxicological risk emerges from human clinical data. This includes both controlled trials and real-world post-marketing surveillance. In the case of Neem (Azadirachta indica) and Giloy (Tinospora cordifolia), human data are scattered — often derived from case reports, small-scale studies, or spontaneous adverse event reporting systems. This makes pharmacovigilance (the science of detecting, assessing, and preventing adverse drug reactions) crucial for these botanicals.

12.2 Key takeaways for safety practice:



12.3 Always evaluate patient history before recommending Neem or Giloy

- Why it matters: Many adverse reactions happen not because the herb is inherently toxic, but because it is taken by someone with a hidden vulnerability — for example, liver disease, autoimmune conditions, or a history of allergic reactions.
- Practical approach:
  - Before recommending, ask about existing illnesses, medications, and previous reactions to herbal products.
  - For Neem, avoid use in infants and children, pregnant women, and people with neurological conditions.
  - For Giloy, be cautious in patients with autoimmune diseases (e.g., lupus, rheumatoid arthritis) or unexplained liver enzyme elevations.
- Clinical example: A patient with preexisting hepatitis may experience worsening symptoms if given highdose Giloy.

12.4 Encourage the use of standardized, quality-certified products

- Why it matters: The safety and efficacy of botanicals depend heavily on consistent phytochemical composition. Poorly processed products may contain excess active compounds, contaminants, or adulterants.
- Practical approach:
  - Recommend products tested for purity, potency, and absence of heavy metals, pesticides, or microbial contamination. o Avoid raw, unprocessed neem oil for oral use — as azadirachtin concentration can vary greatly and cause neurotoxicity.
  - Choose Giloy from reputable sources with botanical verification to avoid substitution with *Tinospora crispa*, which has higher toxicity potential.
- Evidence: Several Giloy-related hepatitis cases were traced back to misidentified plant material in noncertified formulations.

12.5Strengthen pharmacovigilance networks

- Why it matters: Herbal toxicity cases are often underreported — meaning safety issues go unnoticed until multiple severe events occur. A robust pharmacovigilance system can detect these problems early.
- Practical approach:
  - Healthcare professionals and AYUSH practitioners should be encouraged to report all suspected herb-related adverse events, even mild ones. o Encourage public awareness campaigns on reporting herbal side effects to national drug monitoring centers.
  - Integrate herbal monitoring into existing WHO VigiBase and AYUSH AEMCs for realtime trend analysis.
- Impact: This leads to updated safety guidelines, better regulatory actions, and product recalls if needed.

12.6Risk vs. Benefit analysis in dosing and patient selection

- Why it matters: Even safe herbs can become harmful if overdosed, used long-term without monitoring, or taken by high-risk individuals.

- Practical approach:
  - Limit Neem use to short-term courses unless under medical supervision.
  - Use Giloy intermittently for immune support rather than as a daily long-term tonic, unless prescribed for chronic illness. o Adjust dose according to body weight, age, and health status — avoid one-size-fits-all dosing.
- Example: Neem paste applied topically for skin infection carries minimal risk, but oral ingestion of neem oil can cause life-threatening toxicity.

12.7Public health education as a preventive tool

- Why it matters: Many adverse outcomes occur because the public assumes "natural means safe" and selfmedicates without considering risks.
- Practical approach:
  - Disseminate simple, culturally adapted safety guidelines via social media, community health workers, and pharmacies.
  - Educate consumers about correct dosing, contraindications, and possible drug-herb interactions (e.g., neem may potentiate hypoglycemic drugs; Giloy may interact with immunosuppressants).
  - Promote the idea that herbs require the same caution as pharmaceutical drugs.
- Benefit: Reduces hospital visits due to avoidable toxicity cases and builds trust in safe herbal use.

Module 13 Benefit-Risk Assessment and Comparative Toxicity Profile

13.1 Introduction

The use of medicinal plants such as Neem (Azadirachta indica) and Giloy (Tinospora cordifolia) has gained wide popularity due to their therapeutic potential in conditions like fever, infections, metabolic disorders, and immune-related illnesses. However, while these herbs are traditionally considered beneficial, modern toxicological research highlights the importance of carefully weighing their benefits against potential risks.

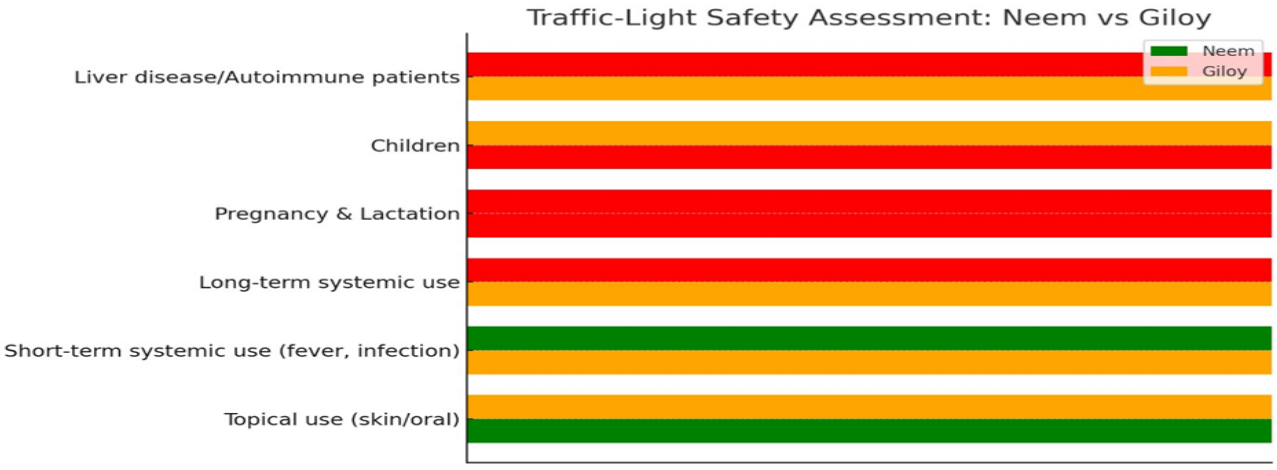
A benefit–risk assessment helps in understanding both the therapeutic potency (how effective the herb is) and the toxicity profile (possible harmful effects and safety concerns). This comparative approach allows us to identify the relative safety margins, i.e., how much safer one herb is under specific conditions compared to the other.

Neem is widely known for its antimicrobial, anti-inflammatory, and dermatological benefits, particularly in topical and oral health applications. It generally has a wider safety margin, but caution is needed in pregnancy, children, and when consumed orally in high doses. Giloy, on the other hand, is valued for its immunomodulatory and antipyretic (feverreducing) properties, making it useful for systemic illnesses and fever management.

However, it carries a higher risk of liver toxicity and immune-related complications, especially in long-term use or in susceptible individuals.

To clearly visualize these differences, a trafficlight safety chart is used. This approach categorizes the safety of each herb into:

- Green: Generally safe use
- Orange: Use with caution under medical supervision
- Red: Avoid due to high risk



13.2Benefits (Therapeutic Potency):

Neem (Azadirachta indica)

- Neem has been used traditionally for skin diseases, oral health, and as a blood purifier.
- Scientific studies show that it has antibacterial, antifungal, antiviral, and anti-inflammatory actions. •
- It may help in reducing blood sugar and cholesterol, but evidence is not very strong compared to its topical uses.

Most reliable area of benefit: treating skin infections (eczema, acne, wounds) and oral conditions (gum disease, tooth decay).

Giloy (*Tinospora cordifolia*)

- Giloy is widely known in Ayurveda as an "Amrit" (elixir) for boosting immunity.
- Research suggests it can reduce fever, inflammation, and oxidative stress, making it useful in infections and chronic fatigue.
- It has some effect in diabetes management by lowering blood sugar and improving metabolism.
- Most reliable area of benefit: managing acute fever (like malaria, dengue, viral fevers) and improving immune strength during illness.

Comparison: Neem is better for local issues (skin, oral health), while Giloy is better for systemic conditions (fever, immunity).

13.3Risks (Toxicity Profile):

Neem

- Oral/systemic toxicity: High doses or concentrated extracts can damage the liver and kidneys**.**
- Pregnancy/Lactation: Animal studies show antifertility and possible abortifacient effects → unsafe for pregnant or breastfeeding women**.**

Comparative Benefit–Risk Profile:

TABLE13.1

Aspect	Neem	Giloy
Efficacy	o Moderate to good. o Best for skin & oral infections.	o Strong o Best for fever & immunity.
Toxicity risk	Moderate(serious only with high-dose oral or seed oil ingestion)	Higher(liver damage & autoimmune flare)
Best safe use	Topical & controlled oral doses.	Short-term systemic use in fever & inflammation.
High-risk groups	Children, pregnancy, liver & kidney patients.	Liver disease, autoimmune disease, pregnancy.

- Children: Neem seed oil ingestion has caused severe poisoning and deaths in infants**.** Strictly avoid in children.
- Topical safety: Creams, pastes, and oils applied on skin are usually safe, except for rare cases of skin irritation or allergy**.**

Giloy

- Liver toxicity: Several human case reports link Giloy with serious liver injury, especially when taken in large amounts or long-term.
- Immune system risk: Because it boosts immunity, it may worsen autoimmune diseases like rheumatoid arthritis or lupus.
- Pregnancy/Lactation: No reliable studies proving safety → best to avoid.
- Drug interactions: May enhance the effects of diabetes medicines (causing low blood sugar) or interfere with livermetabolized drugs.

Comparison: Neem toxicity is more dangerous in children and pregnancy, while Giloy toxicity is more dangerous for the liver and autoimmune patients**.**

13.4 Relative Safety Margins:

This refers to how much "safe space" exists between the effective dose (where it works) and the toxic dose (where harm starts).

- Neem:
  - Large safety margin for topical use (skin creams, dental rinses). o Narrower margin when used orally at high doses → can stress liver/kidneys.
  - Unsafe in children with seed oil ingestion (almost no margin of safety).
- Giloy: o Works well at moderate doses for fever and immunity.

o Safety margin is narrower because toxicity (liver injury) has been reported even at therapeutic doses in sensitive people. o Safe mainly for short-term use, not for long continuous use.

Conclusion: Neem has a wider safety margin, while Giloy has a narrower margin and needs careful monitoring.

13.5 Overall Assessment:

- Neem
  - Good option for long-term and daily use, especially in topical products.
  - Moderate systemic benefits, but oral use should be cautious and controlled.
  - Safer in general compared to Giloy.
- Giloy o More powerful in short-term conditions like fever and acute infections.
  - Risk of serious liver problems limits its use for long-term or casual daily use.
  - Needs supervision and liver monitoring when used systemically.

13.6 Simple Conclusion:

- Neem**:** o Safer herb overall. o Best for topical applications (skin creams, dental care) and can be used longer.

o Should not be used in children, pregnancy, or in high oral doses.

- Giloy: o More effective in short-term systemic illnesses (fever, inflammation, immunity).
  - Higher risk, especially for liver damage and in people with autoimmune diseases.
  - Should be used short-term and under medical supervision.

Module 14 Regulatory Perspective and Safety Standards

14.1Introduction

The increasing global use of herbal medicines such as Neem (Azadirachta indica) and Giloy (Tinospora cordifolia) highlights the urgent need for robust safety regulations and quality standards. Unlike synthetic drugs, herbal preparations are highly variable due to differences in plant species, growing conditions, harvesting, processing, and formulation. This variability can lead to inconsistent therapeutic effects and potential safety risks if not regulated properly.

International and national agencies, including the World Health Organization (WHO), Ministry of AYUSH (Government of India), and Organisation for Economic Co-operation and Development (OECD), have developed guidelines to ensure the safe use, standardization, and quality control of herbal medicines. These frameworks set out rules for toxicological testing, permissible limits of contaminants, good manufacturing practices, and pharmacovigilance systems.

For herbs like Neem and Giloy, which are widely used in traditional medicine but also face concerns over toxicity (e.g., hepatotoxicity, reproductive toxicity), these regulatory perspectives provide a scientific basis for evaluating their risks and ensuring consumer safety.

14.2WHO Guidelines:

- WHO provides a global framework for the safe use of traditional and complementary medicines.
- Key focus areas include:
  - Quality assurance: Good Agricultural and Collection Practices (GACP) for medicinal plants.
  - Safety evaluation: Preclinical studies on toxicity (acute, subchronic, chronic, reproductive, mutagenicity).
  - Pharmacovigilance: Reporting and monitoring of adverse drug reactions (ADRs) related to herbal medicines.
  - Standardization: Use of monographs (e.g., WHO monographs on selected medicinal plants, including Neem and Giloy) to describe plant parts, dosage, preparation, and safety.
- Relevance: For Neem and Giloy, WHO stresses the importance of identity verification**,** dosage standardization**,** and adverse effect monitoring, especially due to reported toxicities.

14.3AYUSH Guidelines (India):

- The Ministry of AYUSH regulates Ayurveda, Yoga, Naturopathy, Unani, Siddha, and Homeopathy in India.
- Guidelines include: o Pharmacopoeial standards: Official Ayurvedic Pharmacopoeia of India (API) provides monographs for Neem and Giloy describing their botanical identity, active compounds, formulations, and therapeutic uses.

- o Safety standards: Limits for heavy metals (arsenic, lead, cadmium, mercury), pesticide residues, microbial contamination, and aflatoxins.
- o Good Manufacturing Practices (GMP): Compulsory for all licensed herbal product manufacturers to ensure consistency and safety. o Pharmacovigilance system: Mandatory reporting of adverse events linked to Ayurvedic formulations.
- Relevance: AYUSH emphasizes quality control in raw materials, preventing adulteration (e.g., misidentification of Giloy with *Tinospora crispa*), and batch-to-batch uniformity**.**

14.4OECD Guidelines:

- OECD provides internationally accepted toxicological testing protocols, especially for herbal and natural products.
- Commonly applied OECD guidelines for herbs:
- o Acute Oral Toxicity (OECD TG 420, 423, 425) o Repeated Dose 28-day/90-day Oral Toxicity (OECD TG 407, 408) o Reproductive and Developmental Toxicity (OECD TG 414, 421, 422) o Genotoxicity Tests (OECD TG 471, 473, 474)

- These ensure herbs are scientifically tested for safety before human use.
- Relevance: Neem and Giloy extracts should undergo standardized OECDbased toxicology studies to confirm safe dosage ranges and rule out longterm risks (e.g., hepatotoxicity, reproductive effects).

14.5Standard Permissible Limits:

To protect consumers, regulators set maximum permissible levels for contaminants in herbal products:

- Heavy metals (as per WHO/AYUSH):
- o Lead (Pb) ≤ 10 ppm o Arsenic (As) ≤ 3 ppm o Cadmium (Cd) ≤ 0.3 ppm o Mercury (Hg) ≤ 1 ppm
- Pesticide residues: Must not exceed WHO/Food Codex maximum residue limits.
- Microbial contamination**:**
- o Total bacterial count ≤ 10^5 CFU/g o Absence of pathogenic microbes

(E. coli, Salmonella, Shigella).

- Aflatoxins**:** ≤ 0.5 ppm for total aflatoxins.
- Relevance: These limits are critical for Neem and Giloy formulations, since poor harvesting or storage can lead to contamination that poses greater health risks than the herb itself.

14.6Need for Quality Control:

- Quality control ensures that herbal medicines are safe, effective, and consistent.
- Key measures include: o Botanical verification: Confirming correct species identity (e.g., Neem vs. similar Meliaceae plants; Giloy vs. Tinospora crispa). o Standardization: Ensuring consistent levels of bioactive compounds (e.g., nimbolide in Neem, tinosporaside in Giloy).

- o Good Manufacturing Practices (GMP): Maintaining hygiene, controlled processing, validated methods. o Phytochemical fingerprinting: Using HPLC, TLC, or spectroscopy for batch consistency.
- o Stability testing: Shelf-life studies to ensure product safety over time.
- Relevance: Without proper quality control, Giloy products risk adulteration, and Neem products risk toxicity from concentrated seed oil.

 Summary Table: Regulatory Perspective and Safety Standards:
TABLE 14.1

Regulatory body/ Standard	Key focus areas	Application to Neem & Giloy
WHO Guidelines	o GACP o Herbal pharmacovigilance o Monographs for standardization	o Stress on identity verification o Monitor adverse effects o Standard dosage and preparations
AYUSH(India)	o Pharmacopoeial standards o GMP for herbal products o Limits for heavy metals pesticicides, microbial load, aflatoxins	o Ensures formulation quality & safety o Prevents adulteration o Maintains batch consistency
OECD Guidelines	andard toxicology testing protocols: ➢ Acute oral toxicity ➢ Repeated dose toxicity ➢ Reproductivity & developmental ➢ Genotoxicity	o Ensures Neem & Giloy undergo scientific safety evaluation Confirms dose ranges & toxicity endpoints o Prevents long-term risks
Standard permissible limits	o Heavy metals: Pb<10ppm, As<3ppm, Hg<1ppm o Microbials: $<10^5$CFU/g, no pathogens o Aflatoxins <0.5 ppm o Pesticide residue within codex limits	Prevents contamination related health hazards o Critical for Neem seed oil & Giloy extracts
Quality control needs	o Botanical verification o Phytochemical standardization o GMP compliance o Stability & shelf-life testing	Ensures authentic Neem & Giloy products. o Avoids adulteration & toxicity
	o Analytical tools: HPLC, TLC, spectroscopy	o Guarantees safe, effective, consistent formulations.

Module 15 Risk Mitigation Strategies &
Future Research Needs

15.1Introduction: This module is the translation from modules 12-14 in an actionable safety step and detects the key research gaps for both Neem (*Azadirachta indica*) & Giloy (*Tinospora cordifolia*). The aim is to maximize therapeutic benefit, minimize harm, generates evidence where there is uncertainity.

Core Risk-Mitigation Strategy:
TABLE15.1

Step	Strategy	Purpose
1	Verify species, plant part & standardization markers	Ensures correct identity & consistent potency
2	Avoid high-risk forms (Neem seed oil orally, unlabelled mixes)	Eliminates formulations with highest risk of harm
3	Start low, reassess at 2 weeks	Reduce dose-related toxicity risk
4	Prefer short systemic courses	Minimizes cumulative exposure risk

5	Screen high-risk groups (pregnancy, liver, renal, autoimmune)	Identify users with greater susceptibility
6	Check for drug-herb interactions	Prevent harmful herb-drug interactions
7	Baseline & follow-up labs (LFTs, renal, glycemic if needed)	Detect toxicity early & guide safe use
8	Patient counseling (benefits, warning signs, storage)	Empower patients with knowledge & safe practices
9	Document everything (batch, dose, duration, labs, AEs)	Enable traceability & accountability
10	Stop rules: liver signals, jaundice, rash, autoimmune flare, child/pregnancy use	Immediate action on red-flag safety signals

15.2Detailed explanation of Core-risk Mitigation strategy:

1. Right product, right plant: Verify species (DNA/barcode or pharmacopeial ID), plant part, solvent, and standardization markers (e.g., nimbolide for Neem; tinosporaside/diterpenoids for Giloy).
2. Avoid high-risk forms: Do not use Neem seed oil orally. Avoid unlabelled poly-herb mixes and any Giloy products lacking species confirmation (risk of *T. crispa* mixups).
3. Start low, reassess early: Begin at the lowest effective dose; reassess at 2 weeks for benefit and AEs before any escalation.
4. Short courses for systemic use: Prefer short, indication-bound courses (e.g., febrile/inflammatory episodes for Giloy); avoid long, casual daily use.
5. Population screening: Flag pregnancy/lactation, pediatrics, liver/renal disease, and autoimmune disorders (higher risk with Giloy).
6. Interaction check: Review hypoglycemics/insulin, hepatotoxic drugs, anticoagulants/antiplatelets, immunosuppressants, and major CYP/P-gp substrates.
7. Baseline & follow-up labs: Obtain LFTs (ALT/AST/ALP/GGT/bilirubin) before systemic use in at-risk users; repeat at 2–4 weeks or sooner if symptoms.
8. Patient counseling: Explain expected benefits, warning symptoms (jaundice, dark urine, pruritus, severe rash, symptomatic hypoglycemia), and storage/expiry.
9. Document everything: Record brand, batch/lot, dose, duration, concurrent meds, labs, and AEs (use the templates above).
10. Stop rules: Stop immediately and evaluate if ALT/AST >3× ULN with symptoms or >5× ULN without symptoms, any jaundice, severe rash, anaphylaxis, autoimmune flare, or child/pregnancy exposure.

Herb-Specific Safety Recommendations: TABLE15.2

Category	Neem	Giloy
Prefer	Topical (skin/oral) forms; standardized oral extracts when clearly indicated	Short-term systemic use for acute fever/inflammation in adults with baseline normal LFTs.
Avoid	Oral Neem seed oil; use caution with high systemic doses or prolonged use.	Long-term unsupervised use; use in autoimmune disease or active hepatic disease.

Special cautions	Pregnancy/lactation, children, existing liver/renal disease.	Concomitant hepatotoxic drugs; immunosuppressants (mechanistic opposition); diabetics (hypoglycemia risk).
Operational tips	Patch-test for topicals; for oral courses beyond 2–4 weeks, check LFTs; coordinate with diabetes therapy	Baseline LFTs → recheck at 2– 4 weeks; stop on any hepatic signal; ensure species ID (*T. cordifolia*, not *T. crispa*).

15.3Monitoring Parameters & Schedule:

1. Baseline (before systemic use or if highrisk)

- History & risk flags: pregnancy/lactation, child/elderly, autoimmune, liver/renal disease, alcohol use.
- Concomitant meds: hypoglycemics, hepatotoxins, immunosuppressants, anticoagulants/antiplatelets.
- Vitals: BP, HR, temperature.
- Labs:

o LFTs: ALT, AST, ALP, GGT, total/direct bilirubin. o Renal: creatinine, eGFR.

o Glycemic (if diabetic or at risk): FPG, PPG, ± HbA1c.

o CBC: WBC, eosinophils, platelets (hypersensitivity clues).

2. Follow-up checkpoints

- Week 2: symptom benefit; AEs review; LFTs if symptomatic or any Giloy systemic use; adjust dose.
- Week 4: repeat LFTs for systemic users; reassess need to continue; deescalate/stop if no benefit.
- End of course (≤8 weeks typical): final clinical review; document outcomes and any labs.
- Anytime: immediate labs and stop if warning symptoms appear.

3. "Stop Now" red flags

- Jaundice, dark urine, RUQ pain, persistent nausea/vomiting.
- ALT/AST rise crossing thresholds above; bilirubin elevation.
- Severe rash, angioedema, anaphylaxis.
- Autoimmune symptom flare (new joint swelling, rashes, fatigue).
- Symptomatic hypoglycemia.

4.Patient & Caregiver Counselling:

- How it helps (indication-specific goal).
- How to take/apply (dose, route, timing with meals, patch-test for topicals).
- What to avoid: alcohol excess, duplicate hepatotoxins, unsupervised poly-herb mixes, Neem seed oil orally.
- Watch for: jaundice, itching, dark urine, severe rash, hypoglycemia symptoms.
- What to do if symptoms appear: stop product → contact clinician → keep the pack with batch/lot number.
- Storage: cool, dry, away from sunlight; do not use after expiry; don't share.

5.Quality & Supply-Chain Controls:

- Supplier qualification: GMP certification; traceable farm source; adherence to GACP.
- Identity/adulteration control: macroscopic/microscopic ID, HPTLC/HPLC fingerprint; DNA barcoding where feasible to avoid *Tinospora crispa* substitution.
- Standardization: declare solvent, plant part, and marker ranges on label/COA.
- Contaminant testing: heavy metals, pesticides, aflatoxins, microbial load per pharmacopoeia.
- Stability program: real-time/accelerated studies for shelf-life.
- Documentation: retain COA + batch records with distribution logs for traceability/recall.

6.Adverse Event (AE) Detection & Reporting:

- At each visit: ask open questions ("any new symptoms since starting?").
- Grade severity: use CTCAE if available; otherwise mild/moderate/severe.
- Causality assessment: WHO-UMC or Naranjo (temporal relation, dechallenge/rechallenge, alternatives).

- Essential fields to capture: product name, batch/lot, dose, start/stop dates, indication, time-to-onset, labs, seriousness, outcome.
- Where to report: institutional pharmacovigilance unit / national AYUSH PV program as applicable.
  - Template: use the AE Reporting Form CSV provided to standardize records.

15.4Future Research Needs:

A. Clinical efficacy & safety

1. Indication-specific RCTs with standardized extracts (topical Neem for dermatoses/oral health; systemic Giloy for acute febrile illness).
2. Dose-finding & duration studies to define minimum effective and upper safe bounds.
3. High-risk cohorts: controlled studies in diabetes, elderly, and those on polypharmacy.
4. Pregnancy/lactation & pediatric registries: prospective safety tracking (currently major gaps).

B. Mechanistic & toxicology

5. Hepatotoxicity mechanisms for Giloy: immune-mediated vs. intrinsic toxicity; explore HLA or genetic susceptibility.
6. Reproductive/developmental toxicity for both herbs with modern designs (OECD TG 414/421/422).
7. Immunotoxicology: quantify autoimmune activation risk (Th1/Th17 pathways, cytokine signatures).
8. Chronic & sub-chronic toxicity with histopathology and NOAEL derivation for specific extracts.

C. Pharmacology & interactions

9. PK/PD profiling: absorption, key metabolites, and CYP/P-gp modulation (in vitro + clinical DDI studies).
10. Biomarker development: early liverinjury markers (GLDH, miR-122), and benefit markers (glycemic/CRP changes).

D. Quality science

Herb Safety Recommendations Table15.1:

Aspect	Neem (Azadirachta indica)	Giloy (Tinospora cordifolia)
Safe Forms	Leaf extracts, bark decoctions, topical pastes, standardized capsules/tablets	Aqueous stem extract, standardized capsules/tablets, decoctions
Avoid Forms	Neem seed oil (oral use – hepatotoxic & neurotoxic risk), unstandardized crude mixtures	Unverified polyherbal mixtures, excess dosing of raw stem
Recommended Dose	250–500 mg/day standardized extract (short-term use preferred)	300–500 mg/day standardized extract (short to medium-term use)
High-Risk Groups	Pregnant & lactating women, children, patients with liver/kidney disease	Autoimmune disorders (may worsen), transplant patients (immunostimulation), pregnancy
Drug–Herb Interactions	May potentiate hypoglycemic drugs, hepatotoxic drugs, and immunosuppressants	May interact with antidiabetics, immunosuppressants, corticosteroids
Monitoring Needs	Liver function tests (LFTs), renal function if prolonged use	Glycemic control, autoimmune flare markers, LFTs if combined with other hepatotoxic agents
Counseling Points	Avoid self-medication; stop if jaundice, nausea, or neurological symptoms occur	Use standardized products; discontinue if rash, autoimmune symptoms, or unexplained fatigue
Duration of Use	Prefer short-term systemic use (≤3 months); topical use safer long-term	Generally safe for medium-term use (3–6 months) if monitored

11. Adulteration detection pipelines: DNA barcoding + untargeted metabolomics to catch *Tinospora crispa* or non-Neem substitutions.
12. Contaminant risk modeling: heavy metals/aflatoxins vs. geography, season, processing; link to health outcomes.

15.5Suggested study designs:

- Randomized, double-blind, placebocontrolled trials with pre-registered protocols and centralized lab reading of LFTs.
- Active safety monitoring with predefined stop rules and independent DSMB for systemic studies.
- Core outcomes set: indication-specific primary endpoints + harmonized AE/LFT panels to allow meta-analysis.

Module 15 Risk Mitigation Strategies & Future Research Needs

15.1Introduction

This module serves as the action-oriented conclusion of the Neem (*Azadirachta indica*) and Giloy (*Tinospora cordifolia*) toxicology and safety dossier. While previous modules established benefit–risk balance and regulatory perspectives, it focuses on practical strategies to minimize harm, monitoring approaches for safe use, and identification of research gaps that must be addressed to strengthen evidence-based herbal medicine.

The goal is to translate scientific findings into clear safety guidelines, define monitoring tools for clinicians, and highlight areas needing further toxicological research.

15.2Risk Mitigation Strategies:

A. General Strategies

1. Standardization & Authentication o Only use products that are GMPcertified, with batch-specific Certificates of Analysis (COA).

o Confirm correct botanical identity (avoid misidentified Giloy species such as *Tinospora crispa*).
o Ensure standardization to marker compounds (e.g., nimbolide for Neem, tinosporaside for Giloy).

2. Formulation Selection o Prefer extracts, tablets, decoctions, or topical preparations from trusted sources.
   - o Avoid Neem seed oil orally due to hepatotoxic and neurotoxic risks.
   - o Use single-herb standardized products to minimize unpredictable polyherbal interactions.
3. Dose & Duration o Start with the lowest effective dose.
   - o Prefer short to medium courses (≤3 months) for systemic use.

Stop Rules	Discontinue if jaundice, hepatic enzyme rise, rash, seizures, or use in children/pregnancy	Stop if autoimmune flare, unexplained fever, severe rash, or pregnancy exposure

o Reassess periodically; avoid long-term, unsupervised use.

4. Target Population Caution o Avoid in pregnancy, lactation, children, and patients with preexisting hepatic, renal, or autoimmune disorders.

o Careful use in diabetics (risk of hypoglycemia when combined with anti-diabetic drugs).

5. Drug–Herb Interaction Precaution o Avoid combination with hepatotoxic drugs, strong immunosuppressants, corticosteroids, or multiple antidiabetics without medical supervision.
6. Stop Rules o Discontinue immediately if:
   - Jaundice, dark urine, unexplained fatigue
   - ALT/AST >3× ULN with symptoms or >5× ULN without symptoms
   - Autoimmune flare, severe rash, seizure, or allergic reaction

15.3Safety Recommendations:

Neem

- Prefer topical and oral-care formulations; systemic extracts only under supervision.
- Avoid oral seed oil entirely.
- Monitor LFTs during prolonged systemic use.
- Contraindicated in pregnancy, children, liver/renal patients.

Giloy

- Prefer short-term systemic use for fever/inflammation.
- Avoid in autoimmune disorders and liver disease.
- Ensure correct botanical species (*T. cordifolia*).
- Monitor LFTs and glycemic parameters during systemic use.

15.4Monitoring Parameters:

Stage	What to Check	Parameters
Baseline	Before starting systemic Neem/Giloy	LFTs (ALT, AST, ALP, GGT, bilirubin), renal function (creatinine, eGFR), glycemic control (FPG, PPG, HbA1c if diabetic), CBC, risk flags (pregnancy, autoimmune, alcohol use)
Follow-up (2 weeks)	Early response & safety check	Symptom relief, adverse effects, repeat LFTs if systemic Giloy or symptomatic
Follow-up (4 weeks)	Ongoing safety	Repeat LFTs, glycemic profile, dose adjustment
End of Course (≤8 weeks)	Discontinuation or extension decision	Clinical status, labs, record outcomes
Anytime	Onset of warning symptoms	Stop immediately, perform labs, report AE

Adverse Event Reporting:

- All moderate-to-severe AEs must be documented with:

o Product name, manufacturer, batch number, dose, duration. o Symptom description, lab values, seriousness, outcome.

- Use standardized pharmacovigilance forms (WHO-UMC or AYUSH PV program).
- Encourage patient reporting for early detection of safety signals.

15.5Future Research Needs:

A. Clinical Studies

- Dose-ranging studies to define minimum effective and maximum safe limits.
- Randomized Controlled Trials

(RCTs) for specific indications (e.g., Neem for skin disorders, Giloy for febrile illnesses).

- Long-term safety studies in diabetics, elderly, and polypharmacy populations.
- Pregnancy & pediatric registries to fill current evidence gaps.

B. Mechanistic & Toxicological Studies

- Hepatotoxicity in Giloy: establish whether intrinsic or immune-mediated.
- Reproductive & developmental toxicity (OECD 414/421/422 guidelines).
- Immunotoxicology: quantify autoimmune activation potential.
- Chronic/sub-chronic toxicity with histopathology and NOAEL determination.

C. Pharmacology & Interaction Studies

- PK/PD profiling of marker compounds (absorption, metabolism, elimination).
- Herb–drug interaction studies, particularly with antidiabetics, immunosuppressants, and hepatotoxic drugs**.**
- Development of early biomarkers (e.g., miR-122 for liver injury).

D. Quality & Supply Chain

- DNA barcoding & metabolomics to prevent adulteration (esp. Giloy species confusion).
- Contaminant risk mapping (heavy metals, pesticides, aflatoxins).
- Shelf-life and stability testing for standardized extracts.

16. CONCLUSION

Neem (*Azadirachta indica*) and Giloy (*Tinospora cordifolia*) represent two of the most widely used medicinal plants in traditional and contemporary healthcare systems, valued for their antimicrobial, anti-inflammatory, immunomodulatory, and metabolic benefits. However, growing evidence highlights that their safety cannot be assumed to be absolute. Reports of hepatotoxicity, neurotoxicity, autoimmune exacerbations, and herb–drug interactions underscore the need for a cautious, evidence-based approach to their use.

This dossier demonstrates that both Neem and Giloy possess significant therapeutic potential, but their benefit–risk balance depends heavily on the form

used, dosage, treatment duration, and patient population. Neem leaf extracts and topical preparations generally carry a favorable safety margin, whereas Neem seed oil is associated with serious toxicity and should be strictly avoided orally. Giloy stem extracts show promise in managing febrile and inflammatory conditions, but misidentification with toxic *Tinospora* species, as well as potential risks in autoimmune and hepatic disorders, raise important concerns.

The regulatory perspectives from WHO, AYUSH, and OECD emphasize the need for standardization, permissible safety limits, and rigorous quality control in herbal formulations. Risk mitigation strategies proposed in this report—including product authentication, dosing limits, monitoring parameters, adverse event reporting, and clear stop rules—offer practical pathways for safer integration of these herbs into therapeutic practice.

At the same time, significant research gaps remain, particularly in defining dose thresholds, long-term safety in vulnerable groups, herb–drug interaction mechanisms, and molecular pathways of toxicity. Addressing these gaps through controlled clinical trials, toxicological studies, and advanced quality assurance methods will be critical for ensuring global acceptance and safe utilization of Neem and Giloy.

In conclusion, Neem and Giloy are valuable herbal medicines with proven benefits, but they must be used with scientific caution, standardized formulations, and active safety monitoring. By combining traditional wisdom with modern pharmacovigilance and research, these herbs can continue to play a responsible role in integrative medicine while minimizing the risk of adverse outcomes.

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