A Review Study on Medicinal Importance of Ayurvedic Extracts of Azadirachta Indica

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***Abstract*—Azadirachta indica (commonly named Neem) has a variety of beneficial chemicals that may be applied to protect stored seeds from insects and are used as insect repellents. But in addition to their potential health benefits, they are widely employed in popular medicine for their antifungal, antibacterial, antimalarial, antiparasitic, anti-inflammatory and immune-modulatory qualities in many animal species. Neem leaves are used to treat snake and insect bites and are frequently given to ruminants in arid regions. Alkaloids, resins, gum, fluoride, Sulphur, tannins, oils, saponins, flavonoids, sterols, and calcium are all abundant in its twigs. Despite being a rich source of proteins and fatty acids, its seeds are mainly unutilized due to the presence of bitter, poisonous characteristics. This study aims to create a thorough safety dossier for several Azadirachta indica extracts. The study combines results from scientifically validated in-vitro and in-vivo studies with an assessment of human exposure derived from pharmacovigilance data and case reports. This thorough approach offers a solid foundation for evaluating the toxicological safety of neem extracts, hence bolstering their potential for usage in upcoming medicinal and nutraceutical applications.**

***Index Terms*—Azadirachta indica, in-vitro and in-vivo studies, toxicology study, therapeutical.**

I. INTRODUCTION:

Plants have been used for centuries to heal a variety of human ailments. Many plant species have compounds with potential medical uses in one or more of their organs. These are recognised by medicinal plants by the World Health Organisation (WHO)[1]. Azadirachta indica is regarded as a tree with many therapeutic uses. Neem is a natural source of raw materials for cosmetics that may be produced on a large scale because of its low toxicity and extensive dispersion in nature [2]. The Azadirachta indica tree, which is native to India and belongs to the Meliaceous family, has been utilized by traditional medical systems such as Ayurveda for thousands of years. The different parts of the neem tree, such as the leaves, flowers, fruits, and roots, are capable of delivering a wide range of phytochemicals that possess a variety of biological and pharmacological activities [3]. Antidiabetic, anti-inflammatory, anti-cancer, anti-bacterial, antifungal, antiviral, antileishmanial, anthelmintic, antimalarial, antipyretic, analgesic, anti-inflammatory, anti-allergenic, neuroprotective, cardioprotective, and pesticidal properties are some of the pharmacological activities that are included in this category. The neem tree is frequently referred to as a "village pharmacy," especially in India, due to its therapeutic qualities [4]. Other pharmacological characteristics were also found, including antileishmaniasis, microbicidal, nematicidal, antidiabetic, hypolipidemic, hepatoprotective, antipyretic, antifertility, hypoglycaemic, cardioprotective, antiulcer, neuroprotective, antioxidant, and microbicidal and nematicidal activities. Added significant concern is the safety of neem and its derivatives as pesticides; Boeke et al. [5,6] have already studied this topic. With an emphasis on the safety assessment in aquatic species and mammals, the current review looked to offer an ultramodern analysis of A. Indica's toxicity and safety investigation. To the best of our knowledge, this text provides an updated review of the subject.

1.1 Therapeutic Implications of Neem and Its Various Ingredients in Health Management

India's fabled medical tree, neem, has flourished with human settlement across the nation and has long been

an essential component of Indian culture. The history of Indian culture is closely intertwined with the history of the Neem tree. The Indian peasants have long regarded the neem tree as a friend and protector. Indians have long relied on this tree to improve their health and treat a wide range of illnesses. It has also been utilized as a natural insecticide and fertilizer for fields, as well as for the preservation of food and grains. Active ingredients break down bacterial cell walls, activate antioxidant enzymes to help treat diseases, and regulate cellular pathways to act as chemo preventives. Neem's pharmacological properties are thoroughly examined. The following table lists the various neem components and their applications. Nimba, which means "bestower of good health" in Sanskrit, is the root of the word NEEM. Ravisambha, which offers sun-ray-like health benefits, is another name for it. In rural India, the neem tree has long been revered because it offers hope in all circumstances. Patients with terminal illnesses have adopted neem as a way of life because they believe in the tree's magical healing abilities. [7]

Azadirachta indica

Taxonomy	Azedarach indica.
Kingdom	Plantae
Class	Magnoliopsida
Order	Sapindales
Family	Meliaceous
Genus	Azedarach
Species	A. indica

Neem parts	Key compounds	Common uses
Leaves	Flavonoids, quercetin, nimbin	Antiseptic creams, soaps, dental care, herbal teas, anti-inflammatory & antifungal uses.
Seeds	Azadirachtin, nimbin	Biopesticides, fertilizer, antiparasitic treatments |
Neem oil	Azadirachtin, fatty acids	Skin care (eczema, acne), hair care, biopesticide soap making
Bark	Tannins, nimbidin	Traditional medicine, mouthwash, astringent
Flowers	Terpenoids, flavonoids	Culinary uses (e.g., in south india), antifungal & anti-inflammatory remedies
Fruit/ pulp	Polysaccharides	Fertilizer, antibacterial and antifungal uses, animal feed (processed).
Twigs	Antibacterial compounds	Natural toothbrushes, gum, and oral health care |
Roots	Alkaloids	Traditional remedies for fever, inflammation, and pain (less commonly used)

In accordance with the Ayurvedic tradition, they lived in the shade of the tree and consumed an infusion made from various components of the tree, such as the bark and the leaves. To perform their dental hygiene routine first thing in the morning, they utilized young twigs. Additionally, they consumed tender leaves as salad or cooked leaves with vegetables as a source of nourishment. The lozenge form of neem gum was utilized for the purpose of alleviating thirst and dryness of the throat. During the summer months, the pulp of ripe, sweet fruit was extracted by sucking it. It is estimated that neem is present, in some form or another, in seventy-five percent of Ayurvedic formulations. Its therapeutic effects are mentioned in the ancient Sanskrit books known as the Puranas. In contrast to neem seed oil, natural neem leaves have a pleasant aroma. A tincture made from neem leaves can be made into an alcoholic beverage, and it can also be made into a tea. There is a dark green color to the alcohol extract, and it has a duration of effectiveness that is several weeks. It is possible to incorporate it into anti-aging formulations that are nutritious, as well as mouthwashes, face washes, shower gels, soothing gels, face masks, skin tones, and other products

Parts of neem in sanskrit	Uses
Sheeta	Nimba confers cooling properties to the body.
Laghu	Undergoes digestion and absorption with relative ease and rapidity.
Grahi	Facilitates moisture absorption from the gut. Absorbs and eliminates moisture in wounds and ulcers.
Katu	Has a piquant flavour. It also experiences a transformation of its spicy taste post-digestion.
Agnikurt	Enhances the digestive process
Truthara	Alleviates excessive thirst. It mitigates Pitta, making it beneficial for fever and concomitant thirst.
Kasahara	Facilitates the alleviation of cough. It is highly beneficial in addressing infectious respiratory conditions. It possesses antibacterial properties.

Jwarahara	Beneficial in fever, owing to its powerful antibacterial phytochemicals.
Aruchihara	Facilitates the alleviation of anorexia. Bitter-tasting herbs, despite their challenging palatability, have the distinctive ability to alleviate anorexia.
Krumihara	The accurate translation is - alleviates worms. Nimba is effective against intestinal worms, infected wounds and serves as an antimicrobial agent.
Mehanut	Beneficial in diabetes and urinary tract diseases.
Kushtahara	Beneficial in several dermatological conditions
Chardi	Helps to relieve nausea and vomiting

Table: - Parts Of Neem Have the Following Health Benefits

Extraction And Formulation

Herbal drug development is based on extraction, which isolates the medicinally active plant constituents from inert or unwanted plant components. Yield, purity, safety, chemical stability, and pharmacological potential are all influenced by the extraction process. To prepare effective preparations, ancient Ayurvedic physicians used fermentation of plant pastes, steeping (phanta or infusions), and boiling (kashaya or decoctions). These processes suffered issues of stability and reproducibility, but they were sufficient for routine therapeutic applications. Contemporary drug sciences allow precise, selective, and reproducible extraction of phytochemicals by combining traditional knowledge with contemporary standards of safety and efficacy.

We enumerate below the major extraction methods.

The Cold Press

Oil is extracted mechanically from seeds, nuts, and fruits at low temperatures by cold pressing. Without the heat, the fragile balance of fatty acids, volatile oils, and sensitive phytochemicals remains intact. For extracts with high flavour, odour, and nutritional content, it makes the process advantageous [8]. Scientific Basis: Oil cells are mechanically ruptured by cold pressing without the addition of chemical solvents. Temperatures are kept below 50 °C to retain thermolabile compounds such as limonoids, sterols, and unsaturated fatty acids.

Advantages:

- Environmentally friendly and chemical solvent-free.
- Fragrance molecules, antioxidants, and vitamin E remain intact.
- Nutraceuticals and cosmetics are safe for use.

Limitations:

- Most yield less than supercritical or solvent extraction.
- Not leaves or bark, but oily seeds and fruits are most effective.

For example:

- Cold-pressing neem oil preserves azadirachtin, a powerful triterpenoid with antibacterial and insecticidal activity.
- Ayurvedic application of cold-extracted coconut oil for hair, skin, and internal purification is widespread due to the preservation of lauric acid and polyphenols.
- Sesame oil's antioxidant lignans, applied in Ayurvedic abhyanga (oil massage), are preserved through mechanical pressing.

Solvent Extraction:

The most widely used contemporary method is solvent extraction, which isolates a range of phytochemicals. With the right polarity solvent, scientists are able to dissolve and isolate fatty acids, alkaloids, flavonoids, and terpenes. Scientific basis: The principle of polarity, which is based on the belief that "like dissolves like," forms the scientific basis for the efficiency of solvent extraction. Although terpenoids and lipophilic compounds are best soluble in hexane and chloroform, phenols and glycosides are best soluble in water, ethanol, and methanol [9].

Advantages:

- Capable of extracting both polar and non-polar compounds.
- Gives higher yields than mechanical methods.
- Suitable for large-scale industrial processing.

Limitations:

- Solvent residues can leave safety hazards behind in the extract.
- Proper downstream processing is needed for the removal of solvents.

- Some solvents, like methanol and chloroform, are toxic and unsuitable for human use.

For example:

- Quercetin and nimbin, which are antioxidant and immunomodulatory agents, are isolated from the leaves of neem using ethanol.
- Ethanol is employed to isolate the liver-protective tinosporin and berberine-like alkaloids from giloy stems.
- Standardised curcumin nutraceuticals are facilitated by the purification of curcuminoids after turmeric extraction with ethanol or acetone.

Steam Distillation

Steam distillation is an ancient but useful method for the separation of aromatic plant oils. Steaming plant material to vaporize essential oils is done by this process. These vapors split pure oils from water upon cooling and condensation. Scientific Foundation: The primary components of essential oils are the aromatic esters, phenylpropanoids, and volatile terpenes. Steam is used for heat-free collection, which reduces the boiling points of these compounds.

Benefits:

- ❖ Pure essential oils are created for medicinal, cosmetic, and aromatherapy purposes.
- ❖ Solvent-free.
- ❖ Standardized and tested method.

Disadvantages:

- Only volatile compounds: non-aromatic phytochemicals excluded.
- Heat can kill heat-sensitive compounds.
- Hydrosol distillation can extract water-soluble aromatic chemicals.

Examples:

- Eugenol and methyl eugenol-containing tulsi oil (Ocimum sanctum) is known for its antibacterial and adaptogenic properties and is steam-distilled.
- It produces eugenol-containing clove oil, which is employed in dentistry and the management of pain.
- Similarly extracted eucalyptus oil has been used for centuries for respiratory infections and chest congestion.

Soxhlet Extract

The Soxhlet apparatus from the late 19th century continues to be employed in lab work for thorough extraction. This method solubilizes phytochemicals by continuously passing the solvent over plant material until saturation is achieved [10]. Scientific Basis: Ongoing solvent percolation ensures the gradual extraction of poorly soluble substances. This method is effective for dried plant material with rigid cell walls that harbor particular chemicals.

Advantages:

- ❖ Assures maximum extraction.
- ❖ Ideal for semi-volatile and non-volatile chemicals.
- ❖ Reproducible results make it a research gold standard.

Limitations:

- ❖ A long heat exposure degrades thermolabile compounds.
- ❖ Time- and solvent-costly.
- ❖ Less environmentally friendly than today's practices.

Examples:

- In Soxhlet conditions, ethanol or acetone extracts curcumin from turmeric rhizomes for pharmacological evaluation.
- Brahmi (Bacopa monnieri) glycosides (bacosides) have been separated by Soxhlet for use in improving cognitive functions.

CO_2 Supercritical Extraction

The pharmaceutical and nutraceutical industries are increasingly embracing supercritical CO_2 extraction, the newest technology available in phytochemistry [11]. The method considers carbon dioxide a gas and liquid at temperatures and pressures above its critical point (31° C and 73 atm). CO_2 in the "supercritical state" can penetrate plant tissues as a gas but extract bioactive compounds as a liquid. Scientific Principle: Temperature and pressure can be altered to change the supercritical CO_2's solvating ability. This allows selective extraction of lighter volatile oils under low

pressure and heavier carotenoids and flavonoids at high pressure [12].

Benefits:

- Solvent- and toxin-free.
- Operates at low temperature to retain heat-sensitive chemicals.
- Environmentally friendly, since CO_2 is recyclable.
- Adjust settings for selective separation.

Disadvantages:

- Capital-intensive startup and operational costs.
- Requires skilled labor and equipment.
- Not always accessible to small herbal enterprises.

Examples:

Supercritical CO_2 use in extracting lycopene from tomatoes and curcuminoids from turmeric has gained international interest. This method generates azadirachtin-barc neem seed extracts for environmentally friendly insecticides and pharmaceuticals. Green tea catechins are included in anti-obesity tablets, isolated by this new process.

Toxicological Study of Neem Extract

The evergreen tree Azadirachta indica, more often referred to as neem, is a member of the Meliaceae family and is indigenous to India. Since the beginning of time, Ayurvedic medicine and other traditional medicinal practices have used it. The many parts of the neem tree, including its leaves, bark, seeds, blooms, fruits, and roots, are responsible for the production of a number of phytochemicals that possess a wide variety of biological and pharmacological characteristics. An assortment of compounds, such as Azadirachtin, Nimbolide, Gedunin, Azadirone, and Salannin, are included in this category. A number of pharmacological actions have been observed, including but not limited to the following: anti-inflammatory, anti-diabetic, anti-cancer, antibacterial, antifungal, antiviral, antileishmanial, anthelmintic, antimalarial, antipyretic, analgesic, neuroprotective, cardioprotective, and pesticidal properties. Due to the pharmacological properties of A. indica extracts and isolated compounds, it is necessary to conduct an evaluation of their safety, particularly in mammalian models. Medicinal plants can be swiftly assessed for acute and subacute toxicity, yielding insights on the safety and possible hazards of the associated chemicals. The animals exhibited severe toxicity when administered injections intramuscularly or intraperitoneally. The toxicity of neem extracts and byproducts is contingent upon dosage in relation to subacute and subchronic toxicity. Irrespective of the mode of administration, elevated levels were shown to be more hazardous. Most research investigated changes in the male and female reproductive systems; however, several reported no effects on teratogenicity or reproductive outcomes. [13]

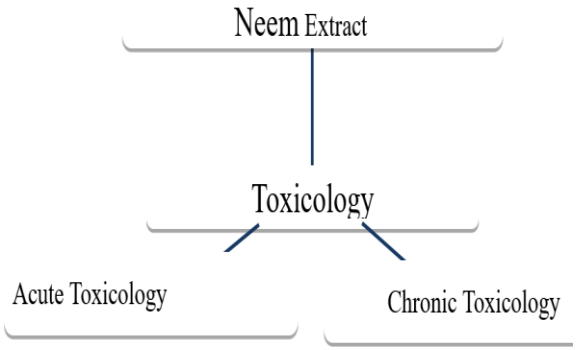

Fig 1: Toxicology study of Neem Extract.

Acute Toxicology

Within the context of the traditional Indian medical system, the therapeutic effects of neem oil, which is also referred to as the seed oil of Azadirachta indica, are usually well recognized. After the drug was given to rats and rabbits by oral administration, acute toxicity was reported in both species. A number of biochemical and histological signs of toxicity were identified, in addition to the dose-related pharmacotoxic symptoms that were reported. In rats, the lethal dose (LD50) was found to be 14 milliliters per kilogram of body weight, however in rabbits, it was found to be 24 milliliters per kilogram of body weight. The lungs and the central nervous system were the organs that were responsible for the poisoning in the animals of both species, and prior to their deaths, they had symptoms that were comparable regarding pharmacotoxicity. In order to assess the extent to which the physical characteristics of an oil may contribute to the oral toxicity of neem oil, a similar test was carried out on edible mustard seed oil at a concentration of 80 milliliters per kilogram. 14 in total Within the framework of the traditional Indian medical

system, neem oil, which is also referred to as the seed oil of Azadirachta indica, is commonly recognized for the healing characteristics that it has. The acute toxicity of the chemical was proven in rats and rabbits when it was given to them in the form of an oral administration. A number of biochemical and histological signs of toxicity were identified, in addition to the dose-related pharmacotoxic symptoms that were reported [15]. According to the findings of the study, the fatal dosage (LD50) after twenty-four hours was found to be fourteen milliliters per kilogram of rat and rabbit body weight, respectively. It was determined that the organs responsible for the poisoning in the animals of both species were the lungs and the central nervous system. Prior to their deaths, the animals exhibited symptoms that were equivalent in terms of pharmacotoxicity.

Additionally, studies were carried out using edible mustard seed oil at a dosage of 80 milliliters per kilogram in order to determine the extent to which the physical characteristics of an oil might impact the oral toxicity of neem oil. These experiments were carried out in the same way as earlier ones. Intramuscular injections of the extracts at a concentration of 0.07 milliliters per gram of body weight were administered to the animals throughout the length of the experiment. Two days were spent administering the shots to the patients. The injection of aqueous extracts of leaves and seeds into rats resulted in the observation of acute toxicity in the rats. Both of the extracts had LD50 values of 9.4 mL/kg, with the former being 6.2 grams per kilogram. For a period of seven days, mice were administered an oral dosage of an ethanolic extract of A. indica leaves at a rate of 5 grams per kilogram of body weight. [Numeric] the LD50 value was determined to be greater than 5 g/kg due to the absence of any indications of acute toxicity or deaths.

The blossoms of the indica plant, which are another component of the plant, have also been investigated for their acute toxicity towards rats. Oral administration of methanolic extracts of A. indica flowers at various dosages (six, nine, and twelve grammes per kilogramme of body weight) over a period of fourteen days resulted in the absence of any harmful effects. Consequently, it was determined that the LD50 was greater than 12 grammes per kilogramme. (17) [19] This study studied the acute toxicity of bioneem oil against D. magna and D. rerio after 48 and 96 hours of exposure, respectively. Bioneem oil is composed of 90 percent neem oil and 10 percent emulsifiers and synergistic chemicals. The median effective concentration (EC50) for immobility in the instance of D. magna was found to be 0.17 mL/L. This was determined by exposing the organisms to a number of different dosages of Bioneem oil (0.15, 0.031, 0.065, 0.125, 0.250, 0.50, 1.0, and 2.0 mL/L). Concentrations of 0.16, 0.2, 0.32, 0.4, and 0.8 mL/L were used in the experiment, and the LC50 value for D. rerio was found to be 0.22 mL/L. [18]

Chronic toxicology

C. dubia was used to investigate the long-term toxicity of bioneem. The amounts of the drug that were administered to these animals were as follows: 0.001, 0.002, 0.004, 0.008, and 0.016 millilitres per litre over the course of seven days. After this period of time, there were increased concentrations of newborns, but there were fewer neonates per female. On the basis of this, Bioneem TM demonstrated a cytotoxic effect on the reproduction of C. dubia. The toxicity of bioneem oil over a prolonged period of time was evaluated. At the end of the experiment that lasted for twenty-one days and used five different concentrations—0.0106, 0.0212, 0.0425, 0.0850, and 0.17 mL/L—a comparison was made between the control versus the treatments in terms of the average number of neonates produced and the sizes of adult organisms. By doing this long-term test, it was observed that Bioneem oil has a variety of impacts, including a reduction in the number of neonates and an inhibition of size. The conclusion that Bioneem oil may have negative effects on aquatic creatures even at low concentrations was reached after taking into account these findings as well as the results from the acute toxicity testing. Furthermore, it was possible to realise that the means of both parameters were significantly lower than those of the control, which indicated a toxic impact. It is [19].

Organ-specific toxicity

Although neem has long been used for its many and trustworthy medicinal advantages, toxicological research is required as the regulated dose of the plant has not been determined. In addition, it is common practice to conduct toxicity experiments on animals in order to assess the potential dangers that chemical compounds or plant extracts pose to human health. These unfavourable outcomes may lead to substantial

alterations in the normal histomorphology, concentrations of biomolecules including metabolic products and enzymes, and the organ's normal functioning. The kidneys of the control group of Wistar rats showed no abnormalities in microarchitectural features of the cortex and medulla when compared to the experimental groups. Nephrotoxic effects are observed at doses of 500, 1000, and 2000 mg/kg body weight when a methanolic extract of neem root bark is administered orally. To that end, it is unclear if these doses of the methanolic Azadirachta indica root bark extract are completely safe for therapeutic usage when taken orally. Use it with caution, then. In [20],

The neem plant, also known as Azadiracta indica Juss, is the source of neem oil, which is also often referred to as margosa oil. In order to extract the oil from neem seeds, the kernels must first be broken open. The neutral oils that may be found in it include stearic and palmitic acids, among others. Terpenoids, which include sialin, nimbin, picrin, and azadirachtin, are the components that are responsible for the therapeutic effects. In addition, there is a very minute amount of aflatoxin present. Neem oil contains azadirachtin, which is responsible for its insecticidal properties.

Neem oil has several uses, including in cosmetics and as a base for natural therapies. Supposedly, it is also effective in preventing pregnancy in men. Neurodeficits were the outcome of hypoxic brain damage in our case, following cardiac arrest. However, cases without cardiac arrest but with neurological consequences have occurred. The forensic lab's mice exhibited symptoms such as salivation, diarrhoea, tremors, convulsions, and death throughout its animal studies.

Dosage determined the severity of symptoms. The exact dose levels at which persons become toxic are unclear. It is not recommended to use gastric lavage, and no specific antidote is currently recognised. Symptomatic therapy accounts for the bulk of the treatment. Patients with mild gastrointestinal and vomiting symptoms are far better off than those with symptoms affecting the central nervous system. [21]

According to the findings of the research, the group that was given OTA appeared to have a greater level of oxidative stress. An increase in the amounts of reactive oxygen species and reactive nitrogen species, as well as a decrease in the activity of antioxidant enzymes, presented evidence of this phenomenon. As a consequence, proteins were broken down, and the levels of cytokines that cause inflammation rose as a result. As a result of an increase in inflammatory cytokines, oxidized biomolecules accumulated in mycotoxicosis, which led to a breakdown in the homeostasis of oxidative stress in the quality control systems of the cells. This was the cause of mycotoxicosis. Damaged proteins and lipids have a propensity to accumulate in cells that are under stress, which can result in changes to the structure of protein adducts as well as changes in the function that they are likely to perform.

These modifications can lead to changes in cellular metabolism and mycotoxicosis. Both NADPH-dependent and ascorbate-dependent mechanisms are responsible for the induction of lipid peroxidation by OTA via the cofactor ferrous ions (Fe3+). The administration of A. indica oil at a concentration of 170 mg/mL resulted in the proliferation of normal hepatocytes and most likely had an anti-inflammatory therapeutic effect. Based on the facts presented above, it can be inferred that A. indica oil can limit inflammation caused by OTA and reduce the influx of reactive oxygen species (ROS) by regulating the equilibrium of interleukin-1β, interleukin-6, interleukin-10, interferon-gamma (IFN-γ), and tumour necrosis factor-alpha (TNF-α) over a period of 28 days. The toxicity caused statistically significant increases in cytokine levels, abnormal hepatic and antioxidant enzymes, and markers of oxidative stress. This is because OTA is a mycotoxin that induces hepatotoxicity. It is conceivable that the restorative and protective actions of A. indica oil are responsible for the restoration of hepatic enzymes as well as antioxidant defences. [22] [22]

Lethal and chronic doses of the neem leaf extract

More than 5,000 milligrams per kilogram of body weight is the median fatal dosage (LD50) of each component, according to research. Both the hexane and ethyl acetate fractionated extracts of Azadirachta indica leaf did not cause any toxicity or death in mice when they were given as an acute oral dosage of 5,000 mg/kg body weight respectively. However, the subchronic oral doses of the fractionated extracts caused a minor change in the biochemical markers of the hepatic and renal functions, as well as the haematological and histopathological parameters. This was the case even if the changes were not significant.

Even when hexane or ethyl acetate was utilized as the extraction solvent, our findings showed that A. indica leaf extracts would not be safe for sub-chronic use. Both of these solvents were used [23].

II. MEDICINAL AND NUTRACEUTICAL APPLICATION OF AZADIRACHTA INDICA

Neem's use in topical medications, cosmetics, and dermatology:

As a result of its antibacterial, anti-inflammatory, and skin-soothing qualities, neem (A. indica) is extensively utilised in the formulation of topical pharmaceuticals, cosmetics, and dermatological care [24]. Neem is a component that is frequently found in natural and Ayurvedic cosmetics due to the numerous applications it has in the care of the skin and hair. Because of their antimicrobial, antifungal, and antibacterial properties, neem oil and extracts are utilised in face creams and lotions. These properties aid to calm skin that is irritated caused by inflammation. Neem's antioxidants, including as carotenoids, protect the skin from the damage caused by free radicals, which helps to prevent the appearance of signs of ageing. These antioxidants are found in anti-aging products. Additionally, it is widely used in face cleansers and toners, where its antibacterial characteristics support the reduction of acne and blemishes [25,26,27,28]. This is because of its ability to kill germs. Neem oil is used topically to the skin in order to cure psoriasis, acne, and eczema. This is done because of the anti-inflammatory and antibacterial qualities that it possesses. As a result of its ability to facilitate the healing process and prevent the development of secondary infections, it is also contained in ointments and creams that are utilised for the treatment of fungal infections, wounds, and ulcers. The use of neem extract in lip balms is common since it is effective in treating dry and cracked lips [29, 30]. It is also common knowledge that neem has beneficial effects on the skin. This treatment effectively treats acne by reducing the production of sebum and eliminating the microorganisms that cause acne, so preventing further outbreaks. Its moisturising, anti-inflammatory, and antifungal properties make it useful in the treatment of chronic skin illnesses such as psoriasis and eczema [31, 32]. It keeps the skin moist, reduces inflammation, and calms itching, all of which contribute to its effectiveness. Additionally, neem encourages the synthesis of collagen and lowers the risk of infection, both of which contribute to the acceleration of wound healing [33]. Athlete's foot, ringworm, and candidiasis are only few of the illnesses that can be effectively treated with its antifungal characteristics. As an additional benefit, the anti-inflammatory properties of neem help reduce the redness, swelling, and irritation that are brought on by allergic reactions, dermatitis, or skin injuries [34,35].

Use of Neem in Pharmacology:

1. Antimicrobial Effect:

Neem and its components suppress the growth of several microorganisms, including viruses, bacteria, and dangerous fungi. The role of neem in the prevention of microbial growth is explained individually below.

1.1. Antibacterial Activity

An investigation into the antibacterial efficiency of herbal alternatives as endodontic irritants was carried out in order to evaluate their effectiveness in comparison to sodium hypochlorite, which is a traditional irritant. The findings demonstrated that grape seed and leaf extracts displayed zones of inhibition, which is an indication that they had antibacterial capabilities. Additionally, as compared to sodium hypochlorite at a concentration of 3%, leaf extracts exhibited considerably broader boundaries of inhibition [36]. To evaluate the antibacterial properties of Azadirachta indica (neem) bark, leaf, seed, and fruit extracts, an additional test was carried out on bacteria that were isolated from adult mouths. The results of the study demonstrated that the bark and leaf extracts have antibacterial activity against each and every germ that was tested. Furthermore, the antibacterial activity of seed and fruit extracts was only observed at higher dosages [37].

1.2 Antiviral Activity:

In terms of antiviral activity, the findings indicated that the neem bark (NBE) extract, when administered at concentrations ranging from 50 to 100 μg/mL, significantly blocked the entry of HSV-1 into cells. Additionally, the inhibitory action of NBE was shown when the extract was preincubated with the virus but not with the target cells, which indicates that the neem bark possesses a direct anti-HSV-1 capability [38].

Neem (Azadirachta indica A. Juss.) leaf extract (NCL-11) has shown virucidal efficiency against coxsackievirus B-4, as revealed by virus inactivation and yield reduction experiments [39]. In addition to interfering at an early stage of the replication cycle, neem leaf extract has also been shown to be effective against the virus.

1.3 Antimalarial Activity:

Extraction of neem leaf and stem bark was found to reduce the number of parasitemia in infected mice by approximately 51–80% and 56–87%, respectively, according to the findings of an experiment that was conducted to evaluate the effectiveness of extracts against malaria using albino mice that were infected with Plasmodium berghei [40]. The experiment was carried out in order to determine whether or not extracts were effective against malaria.

The in vitro testing of crude acetone/water (50/50) extract of leaves (IRAB) against the malaria parasite, Plasmodium falciparum, revealed that the number of parasites in separate 72-hour cultures of both asexual parasites and mature gametocytes treated with IRAB (0.5 microg/mL) was less than fifty percent of those in control cultures, which had eight percent and eight five percent parasitemia, respectively. This revealed that the IRAB treatment was effective against the malaria parasite.

Effect of Neem as Anti-Inflammatory:

It is common practice to use plants or their separated compounds as anti-inflammatory medicines. According to a study's findings, rats' cotton pellet granuloma assay revealed a notable anti-inflammatory effect when given an extract of A. indica leaves at a dose of 200 mg/kg, p.o. [42]. Other research findings indicated that neem leaf extract had a notable anti-inflammatory impact, although it was not as effective as dexamethasone [43], and research findings indicate that nimbidin inhibits the inflammation-related functions of neutrophils and macrophages [44].

Neem as an organic pesticide:

Due to new laws like the Food Quality Protection Act and the Worker Protection Standard, which limited the use of conventional chemical pesticides, the agricultural industry encountered difficulties controlling diseases and pests in the 1990s. As a result, the use of natural themes and integrated pest control (IPM) became more popular. Insecticides derived from neem, particularly those that include azadirachtin, became the answer. Azadirachtin is harmless to beneficial insects and has a minimal influence on the environment. Pests such as aphids, whiteflies, psyllids, leafminers, and more than 300 kinds of insects are effectively gone. Even though there were some problems with manufacturing and regulations when azadirachtin-based pesticides first came out in the US in 1993, they have now become an important part of crop protection.

They still only make up a minor portion of the market for insecticides overall, but [45]. Due to its extensive history of usage as a biopesticide, pests have evolved some resistance to neem oil, making it less effective in protecting crops. The latest advancements in nanotechnology hold the key to overcoming this obstacle.

Nanoencapsulation of neem oil has the potential to provide more stable formulations for the control of crop-damaging insects, particularly those that are needed for human nutrition. Another issue that often develops as a result of the accidental and widespread usage of neem oil is insect resistance; nanotechnology may help with this [46].

Use of Neem in ASU&H formulations:

An assortment of botanical, mineral, metallic, animal, and marine constituents make up ASU&H formulations, which are used in Ayurveda, Siddha, Unani, and Homeopathy. Given its wide range of medicinal uses, the neem tree is revered as "the village pharmacy" in India. The tree's bark, leaves, blossoms, fruits, seeds, and oil are all used for various medicinal purposes. Traditional Indian medicine (Ayurveda, Siddha, Unani, and Homeopathy) extensively makes use of neem, which is an important ingredient in many polyherbal medicines.We have documented various ASU&H formulations that include Neem as an ingredient, along with their therapeutic effects. The Neem tree is known as 'Arista' in Sanskrit, which means 'reliever of disease'; so, in our Indian heritage, it is frequently referred to as 'sarba roganibarini', which means the one who or which can heal all types of maladies.

Antioxidant Properties:

It is a rich source of compounds that scavenge free radicals and help reduce oxidative stress, which is

beneficial in managing metabolic syndrome and related complications.

Neem in the dental care industry:
Several parts of the Neem tree have astringent and antibacterial properties. Leaf extracts have long been used in dentistry to make toothpaste and mouthwash. Its antibacterial qualities are attributable to the presence of nimbidin, azadirachtin, and nimbinin, which assist in eradicating numerous oral aerobic and anaerobic microorganisms from the oral cavity. Neem bark and leaf extract are most efficient at preventing cavities and gum disease. Neem mouthwash reduces bleeding and painful gums, as well as tooth decay and other oral illnesses. People throughout India utilize twigs from the Neem tree as chewing sticks.

Safety and Threshold Capacity of Azadirachta indica Extracts
Safety assessment is a significant component of toxicological evaluation, which is a crucial aspect of herbal dossier preparation. Azadirachta indica, sometimes known as neem, has long been used in Ayurvedic medicine due to its antibacterial, anti-inflammatory, hepatoprotective, and immunomodulatory properties. Although it has been used for millennia, rigorous toxicology study is required to identify safe dosages, threshold levels, and potential side effects. The next segment evaluates evidence on the acute, sub-chronic, reproductive, and clinical toxicity of neem extracts to determine their safety and threshold levels. Neem leaf and seed extracts have high LD_{50} values, indicating little acute toxicity. Mice given an oral dosage of 5000 mg/kg of ethanolic neem leaf extract showed no toxicity or mortality, indicating a large margin of safety [48]. The principal bioactive constituent, azadirachtin, has an LD_{50} of around 3550 mg/kg in rats [49].

Adverse Effects (Acute):
Neem oil is, however, extremely toxic even in small quantities. Indian case reports have indicated that children can develop seizures, metabolic acidosis, and encephalopathy upon consuming a mere 5 milliliters of neem oil [50]. Leaf extracts are generally safe, but the above cases indicate that consumption of oil can be extremely hazardous, particularly for children.

Immediate Side Effects

- The use of neem oil is strongly linked to acute neurotoxicity. Clinical case reports [51] indicate that even 5 ml of neem oil can lead to convulsions, vomiting, metabolic acidosis, and coma in children.
- High doses of neem bark preparations have nauseated and caused stomach upset and diarrhea. These examples illustrate that neem leaves can be safely consumed, but neem oil is not very safe.

Sub-Chronic and Chronic Toxicity
Evidence of safe limits is better provided by long-term animal experiments. Neem oil did not cause any adverse effects in a 90-day sub-chronic toxicity study on mice when administered levels as high as 177 mg/kg/day. Hormonal disruption, renal failure, and liver toxicity were dose-dependent adverse effects at higher dosages (533-1600 mg/kg/day) [52].
A supporting investigation [53] found that rats given 100-300 mg/kg/day of neem leaf extract for 28 days had no discernible alterations in kidney and liver histology, confirming a NOAEL of 300 mg/kg/day.
Yet, there was no discernible rise in liver enzyme levels at 300 mg/kg/day for 21 days of bark extract, suggesting a narrowing margin of safety with increasing dosages [54].

Adverse Effects (Chronic):
Topical neem preparations hardly produce hypersensitivity reactions such as contact dermatitis, and extensive use of neem oil over a long period can harm the liver and kidneys. Bark preparations can also irritate the stomach and cause mild damage to the liver if taken in excess.

Impact on Fertility and Infant Growth
Neem seed and oil extracts are toxic to the reproductive system even in low doses. Fetal resorption, degeneration of follicles, and post-implantation survival were negatively impacted in pregnant rats given neem extract at doses of 100-200 mg/kg/day [55]. Researchers have also examined the spermicidal activity of neem oil and have determined that when administered to rabbits via the uterus, it rendered them temporarily sterile [56].
Neem seed and oil extracts appear to have a greater impact on reproductive well-being than neem leaf

extracts. Earlier studies in pregnant rats indicated that doses up to 1200 mg/kg were not responsible for fetal abnormalities [57].

Adverse Effects (Reproductive):
Neem seed or oil induces infertility, defects in sperm production, and embryo damage. Pregnant or lactating females should avoid using this product since it may be hazardous.

Genotoxicity and Carcinogenicity
Genotoxicity assays such as the Ames test [58] indicate that neem leaf extracts are not carcinogenic. Indeed, neem chemicals have been indicated to possess chemoprotective actions to reduce DNA damage and tumor number in in vitro and in vivo models of cancer [59]. Thus, neem can be more likely to be protective than causative against cancer when it is applied in a controlled environment.

Adverse Effects (Genotoxic/Carcinogenic):
There have been no mutagenic or carcinogenic effects associated with therapeutic doses; however, high doses can cause indirect liver stress instead of DNA damage.

Human Safety and Clinical Evidence
Ensuring that People and Clinical Information Are Safe. Clinical experience is essential in helping us understand threshold safety better. Neem leaf powder has been safely used in conventional Ayurvedic practice for centuries at doses of 500 to 1000 mg daily. There were no significant effects on the liver or kidneys in a 12-week controlled clinical trial with 500 mg of neem leaf capsules every day.
There is, however, repeated evidence that neem oil is extremely poisonous to humans. For example, a 5-year-old was hospitalized after ingesting 5 ml of neem oil, which resulted in acidosis and intractable seizures. This clinical illustration demonstrates that neem oil is unsafe for ingestion, particularly by young infants.

Adverse Effects in Humans:

- Neem oil poisoning: Several case reports (mostly in children) describe seizures, encephalopathy, and death after 5–30 ml ingestion.
- Gastrointestinal effects: Nausea, vomiting, and diarrhea with neem bark decoctions at high doses.
- Dermatological reactions: Rare allergic dermatitis when applied topically in sensitive individuals.
- Metabolic effects: Prolonged high doses may cause hypoglycemia in non-diabetic individuals.

Safety Thresholds and Acceptable Daily Intake (ADI):
Safety Limits and the Correct Amount to Use (ADI)
There exists a wide therapeutic margin when administering Azadirachta indica leaf and bark extracts, and the plant is generally safe for use, provided one does so properly. However, neem oil and seed extracts have significant toxicities, ranging from reproductive effects to acute toxicity in humans. There should be established dosing recommendations and caution in at-risk groups (children, pregnant females, elderly) because of reported harmful effects that include gastrointestinal irritation and neurotoxicity. Establishment of the threshold capacities (NOAEL, LOAEL, ADI) ensures that neem is safe to be employed both in Ayurvedic medicine and conventional medicine.

Table: Safety Thresholds, ADI, and Adverse Effects of Azadirachta indica Extracts

Extract Type	NOAEL (Animal Studies)	Human Safe Dose	Proposed ADI (mg/kg/day)	Reported Adverse Effects
Leaf Extract	~300 mg/kg/day (28-day oral study in rats)	500–1000 mg/day (capsules, decoction in clinical trials)	5–7 mg/kg/day	Mild GI upset at very high doses; rare allergic dermatitis; hypoglycemia in non-diabetics with prolonged use.
Bark Extract	~250–300 mg/kg/day (21-day study in rats)	250–500 mg/day (traditional decoctions)	3–5 mg/kg/day	Gastric irritation; mild elevation of liver enzymes at >1.5 g/day

Seed Extract	Reproductive toxicity at 100–200 mg/kg/day (rats)	50–100 mg/day (ethnobotanical use)	<2 mg/kg/day	Infertility, embryotoxicity, altered spermatogenesis; contraindicated in pregnancy/lactation
Neem Oil	177 mg/kg/day (90-day oral study in mice)	Not recommended for oral use	None established (unsafe)	Acute neurotoxicity, seizures, vomiting, and encephalopathy.

III. CONCLUSION

Neem (Azadirachta indica) has long been valued for its healing properties and is still widely used in traditional medicine. It provides many benefits, such as fighting infections, reducing inflammation, and supporting overall health. At the same time, research shows that its safety depends greatly on which part of the plant is used, how it is prepared, the amount taken, and how long it is used. Neem oil and seed extracts can be harmful when consumed in excess, with toxic effects reported on the liver, kidneys, nervous system, reproductive organs, and blood.

This makes it clear that while neem is a powerful natural remedy, it must be used with care. To make the best use of its medicinal value, proper guidelines for dosage and preparation should be established, and more clinical research is needed. By combining traditional knowledge with modern scientific validation, neem can be used safely and effectively, allowing us to receive help from its strengths without exposing people to unnecessary risks.

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