***Review Article***

**A REVIEW ON ACUTE TOXICITY STUDIES OF *AZADIRACHTA INDICA***

**ABSTRACT**

*Azardirachta indica,* commonly referred to as neem, is a traditional Indian plant recognized for its therapeutic properties. It has been utilized in the treatment of numerous health issues due to its diverse activities, including anti-diabetic, anti-cancer, anti-bacterial, anti-fungal, and anti-inflammatory effects, among others. Neem oil, which has anti-inflammatory and wound-healing qualities, is its most popular version. In the pharmaceutical and pharmaceutical sectors, it is also utilized as a solvent and as a field fertilizer. The Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) state that acute oral toxicity of ethanol neem containing 3000 ppm Azardirachtin is registered with the Environmental Protection Agency (EPA) in the United States. These values are based on experimental data. According to a study, neem oil caused acute cutaneous toxicity that caused primary skin irritation in rats and rabbits, with rat 24-hour LD50s of 14 and 24 ml/kg, respectively. The toxicity of aqueous leaf extract in chickens (Gallus domesticus) was found to be dosage dependent. Conclusion: Numerous research' conclusions advise using them cautiously and checking for dose dependency. This page provides a thorough analysis of the findings of several studies on Azardirachta Indica’s acute toxicity.

Keywords: Anti-diabetic, Anti-cancer, Anti-bacterial, Anti-fungal and Anti-inflammatory activities

**INTRODUCTION:**

Native to the Indian subcontinent, *Azardirachta indica* is a plant that grows in tropical and subtropical climes and goes by several names, such as neem, margosa, or Indian lilac. *Azardirachta indica*, a member of the Meliaceae family, is commonly grown in Bangladesh, India, and Pakistan1. Due to its claimed effectiveness as a herbal treatment called spermicidal, it is considered a perfect, all-encompassing, and indestructible gift from nature (Jhariya et al., 2013)2.

With an annual production of over 4,42,300 tons of neem seeds, which are used to generate 3,53,800 tons of neem cake and 88,400 tons of neem oil, India is the world's largest producer of neem seeds3.

*Azardirachta indica* contains a wide variety of phytochemicals in its fruit, seeds, leaves, stems, and bark, some of which were first identified in its seed extracts4.

*Azadirachtin*, for instance, was developed in the 1960s as an antifeedant, growth disruptor, and insecticide5. Furthermore, glycerides, various polyphenols, nimbolides, triterpenes, and beta-sitosterol are present in *Azardirachta* seed oil. The oil is yellow, bitter, and contains 2% limonoid chemicals. It smells like garlic. Vitamin C, carotenes, catechins, and quercetin are all present in the leaves6.

**Neem seed oil**

The fruit, seeds, and blossoms of the neem tree7 yield the oil that is produced from the seeds of *Azardirachta*, often known as neem. This oil is a golden to dark-brown liquid with a very disagreeable and foul odor7. It is made up of calcium, triglycerides, fatty acids, limonoids, vitamin E, and antioxidants. This plant is crucial to many fields, such as medicine and agriculture.

* A combination of neem oil and urea, pusa neem golden urea is an agrochemical used to prevent nitrification8.
* Because neem oil is environmentally benign and its residues break down rapidly in the environment, it can be utilized as a green solvent9.
* Neem seeds have a significant potential for producing biodiesel and can contain up to 40% oil10.

Neem oil can be extracted using a variety of techniques, however solvent extraction is the most often used approach since it produces a higher yield of clear oil than other techniques11.

**Composition of neem seed oil**

The composition of neem seed oil includes acidic substances such as

1. Oleic acid – 61.9%

2. Palmitic acid – 14.9%

3. Stearic acid – 14.4%

4. Linolic acid – 7.5%

5. Arachidic acid – 1.3%12

The neem seed oil also contains Glycerides likeFully saturated Glycerides – 0.6%

1. Tri-unsaturated Glycerides – 22%

2. Stearodiolein – 34%

3. Palmito-diolein – 26.0%13

4. Oleopalmitostearin – 12%

5. Oleodipalmitin- 5%

In addition, 1.2–1.6% Nimbidin, 0.1% Nimbin, 0.01% Nimbinin, and 0.2–1.0% acare present in neem oil. Nimbidin includes sulfur and makes oil taste harsh. Nimbidin is hydrolyzed to produce nimbidinic acid14.

**Neem leaf composition:**

1. Crude protein – 12.40- 18.27%

2. Crude fibre – 11.40- 23.08%

3. N free extract – 43.32- 66.60%

4. Ether extract – 2.27- 6.24%

5. Total ash – 7.75- 18.37

**TOXICITY STUDIES (Acute toxicity)**

A series of tests or procedures known as toxicity studies are used to ascertain the harmful effects of a medication on the body immediately following administration, typically within a 24- to 14-day period15.

**Regulatory values**

Short-term exposure limits, such STELs or CVs, are only established when a chemical has a specific acute toxicity. Based on experimental data, the Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) have established these limitations16. These organizations' values don't always align, and in the chemical sector, it's standard procedure to select the more conservative value to protect worker safety. Usually, a material safety data sheet contains the values. Additionally, the levels vary according on the compound's entry mode (oral, cutaneous, or inhalation)17.

* Value-time-weighted-average threshold limit: The highest level of concentration to which an employee can be exposed for eight hours each workday without suffering any negative health consequences.

The concentration to which no individual should be exposed for more than 15 minutes during an 8-hour workday is known as the Short-Term Exposure Limit, or STEL or Threshold Limit Value-Short-Term Exposure Limit, or TLV-STEL18.

* The concentration to which no one should ever be exposed is known as the ceiling value, CV, or threshold limit value-ceiling, or TLV-C.19.

**Experimental values**

* No-observed-adverse-effect level, NOAEL
* Lowest-observed-adverse-effect level, LOAEL
* Maximum tolerable concentration, MTC, LC0; Maximum tolerable dose, MTD, LD0
* Minimum lethal concentration, LCmin; Minimum lethal dose, LDmin
* Median lethal concentration, LC50; Median lethal dose, LD50; Median lethal time, LT50 (LT50)
* Absolute lethal concentration, LC100; Absolute lethal dose, LD100

The median lethal dosage, or LD50, is the most frequently cited figure in the chemical business. This is the amount of the chemical that killed half of the test subjects in the lab, usually mice or rats20.

I. Fatal Dose50: The acronym for fatal dose is LD50. It is a measurement of the acute toxicity of chemicals, either liquid or solid, that enter the body through any other route outside the respiratory system21. It is the quantity of a drug needed under specific circumstances to kill 50% of the experimental animals in a group after just one dose. It is measured in mg/kg. 2. fatal Concentration50: The acronym for fatal concentration is LC50. The toxicity of chemical compounds in gas form that enter the body by inhalation is measured by this method22. The amount of a chemical in the pulmonary air that, under specific circumstances, causes 50% of the experimental animals in a group to die from respiration. ppm or mg/m3 of air is its unit. III. Minimal Lethal dosage: The acronym for minimal lethal dosage is MLD for short. It is the smallest quantity of chemical that can cause an animal's death. Here, the LD50 assay will receive special attention. Acute toxicity assessment needs to meet specific requirements in order to get reliable results.

I. Traven method: If the dosages are processed in the x-axis and the corresponding percentages of deaths are processed in the y-axis, the typical sigmoidal curve is produced on a graph paper. This is the graph's LD50 value. The Reed-Muench technique It uses a graphical approach23. Finding the x-axis counterpart of the 50% mortality rate is how the percentage measurements are determined, which sets it apart from the other two methods discussed.

The most reliable method is the probit-analysis method (24). Statistical calculations must be performed on a specifically prepared logarithmic-Probit graph paper with a regulated Probit-Analysis chart in order to apply this method25. The yaxis shows the probit answers from the probit ruler of the percentage of deaths, while the x-axis is labeled with the logarithmic-Probit graph paper's doses. Consequently, a line that can be represented by the formula y = ax + b is produced, making operations easier26.

**Types of Acute Toxicity Studies**

Adverse drug reactions that occur in the body and impact various routes of administration—most commonly the oral route—as well as their organs are typically monitored, screened, analyzed, and assessed27. To ensure the safe use of a drug, many laboratory tests are conducted to forecast the potential adverse drug reactions (ADRs). The Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) state that acute toxicity estimates are based on experimental data and that the limits for various substances vary28.

* The threshold limit value-time-weighted-average is the highest concentration to which an employee can be exposed for eight hours each workday without suffering any negative health consequences.
* Short-Term Exposure Limit (STEL) or Threshold Limit Value-Short-Term Exposure Limit (TLV-STEL): The maximum concentration to which an individual should be exposed in an 8-hour workday for more than 15 minutes.
* The concentration to which no one should ever be exposed is known as the ceiling value, CV or threshold limit value-ceiling, or TLV-C.

**Toxicological Effect of *Azardirachta indica***

Despite extensive research on the pharmacological efficacy of neem extracts, little toxicological analysis has been conducted29. Neem leaves are said to have harmful effects on guinea pigs, goats, and sheep (Ali and Salih, 1982; Ali, 1987). a dosage more than what would cause guinea pigs to die.

Nevertheless, it was discovered that 200 mg/kg administered in the same manner was not harmful to rabbits (Thompson and Anderson, 1978)30.

The American Journal of Neuroradiology states that if margosa oil is ingested in amounts greater than 150 milliliters (5.07 US fluid ounces), it may result in certain types of toxic encephalopathy and ophthalmopathy.

**Toxicity in *Azadirachta indica***

1. Acute toxicity 2. Sub-acute toxicity
2. **Acute toxicity Studies**

It explains a substance's harmful effects that arise from either a single exposure ("The MSDS Hyper Glossary, 2006") or from several exposures in a short period of time (often less than 24 hours). The negative effects must manifest within 14 days of the substance's ingestion in order for it to be classified as acute toxicity.

• Oral acute toxicity The Environmental Protection Agency (EPA) in the United States has registered ethanol neem with 3000 ppm *Azadirachtin* (± 10%). Up to a dose of 5 milliliters per kilogram, the findings on acute oral toxicity in rats revealed no adverse effects (National Academy Press, Washington, D.C., 1992)31. In a different study, acute toxicity tests on mice using methanolic leaf and bark extracts revealed an oral LD50 (Lethal dosage, 50%) of roughly 13g/kg. The animal displayed symptoms of general illness and pain, including gastrointestinal spasms, apathy, coldness, and refusal of food and water. Terminal convulsions caused the mice to perish. The autopsy revealed no gross microscopic lesions (Okpanyi and Ezeukwv, 1981).

• Neem seed oil was used in a comprehensive acute toxicity investigation in rats and rabbits, yielding 24-hour LD50 values of 14 and 24 ml/kg for rats and rabbits, respectively. The lungs and central nervous system were the intended organs of toxic effects (Gandhi et al., 1988)32. However, when given orally to mice at a dose of 200 mg/kg, the methanol-soluble and insoluble fractions from an aqueous leaf extract were not harmful within 24 hours (Singh et al., 1987).

• Morgosan-O's acute cutaneous toxicity LC50 (lethal concentration, 50%) in albino rabbits is greater than 2 milliliters per kilogram (National Academy Press, Washington, D.C., 1992)33. When a seed ethanol extract was administered intradermally to guinea pigs' shaved skin, there was no discernible skin-sensitive reaction (Gupta and Bhaid, 1981). Margosan-O was applied in patches to the shaved and abraded parts of albino rabbits. According to the findings, the shaved region patch had low to moderate primary irritation, whereas the abraded area had high to moderate irritation (National Academy Press, Washington, D.C., 1992).

• Male albino New Zealand rabbits had no reaction when 0.1 mg of an ethanol extract of neem seed was injected into their eyes, causing ocular discomfort (Jeter, 1980). When injected into the eyes, a sodium nimbinate solution (1–5%) did not result in any eye discomfort or changes in the size of the papillaries (Gaitonde and Sheth, 1958). Additionally, the ethanol extract of the seed that was injected into the rabbit's eye did not irritate it in any way (Gupta and Bhaid, 1981). When given to one albino rabbit's cleaned eye and one that had not over the course of seven days, EPA-registered Morgoson-O caused very little eye irritation in either eye (National Academy Press, Washington, D.C., 1992).

In this investigation, five experimental groups of hens were used. Group five served as a control, while the other four groups received intraperitoneal dosages of the extract at 800 mg/kg, 1600 mg/kg, 3200 mg/kg, and 6400 mg/kg, respectively. They computed the LD50. Microscopic lesions, tissue post mortem, and clinical symptoms were documented. Findings: 4800 mg/kg was determined to be the median lethal dose (LD50). The degree of the microscopic and gross tissue abnormalities and clinical symptoms seen at post-mortem was dose-dependent.

*Azadirachta indica* (neem) leaf aqueous extract is hazardous, dose-dependent, and should be used cautiously in ethno-veterinary practice, according to this study.

**Levels of Heavy metals in *Azadirachta indica***

Finding the phytochemical components, median lethal dosage (LD50), and heavy metal concentrations in *Azadirachta indica* leaves sourced from Kano State, Nigeria, was the goal of the current investigation. While cardiac glycosides were not present in the methanol extracts, alkaloids, flavonoids, carbohydrates, tannins, saponins, anthraquinones, and steroids were detected in the initial phytochemical screening of *Azadirachta indica* leaves. The findings showed that the concentrations of heavy metals in *Azadirachta indica* leaves were 0.06± 0.01 mg/kg, 0.05± 0.01 mg/kg, and 0.07± 0.00 mg/kg for Zn, As, and Mn, respectively. The concentrations of Cd, Cu, Ni, Co, and Pb were not detectable, and the concentrations of Zn, As, Co, and Mn were 0.02± 0.01 mg/kg, 0.05±0.02 mg/kg, 0.02±0.00 mg/kg, and 0.15±0.00 mg/kg, respectively. Using Lorke's method, the LD50 of the methanol leaves extract of *Azadirachta indica* ranged from 500 to 5000 mg/kg body weight, indicating that the extracts were mildly hazardous. The World Health Organization's (WHO) allowable limits for metals in medicinal plant and herbal products were fulfilled by the quantities of heavy metals found.

The results of this study show that the extracts are somewhat toxic at LD50, which may imply that they should be used with caution and that more research is necessary to evaluate their short- and long-term cumulative risk in order to increase their safety.

**Genotoxicity**

Chandra and Khuda-Bukhsh evaluated the genotoxic effects of *Azadirachtin* in fish, Oreochromis mossambicus. following receiving an intramuscular injection of 0.005% *Azadirachtin* at a rate of 1 mL/100 g body weight (b.w.), the animals were housed apart and given a regular meal until they were killed (6, 24, 48, 72, and 96 hours following treatment). Break, terminal association, centric fusion, precocious centromeric separation, and C-mitosis were among the modifications found as a result of the treatment, which involved closely monitoring somatic metaphase complements for any structural or numerical abnormalities. The amount of protein bands (on the electrophoretic gel) was significantly decreased by *Azadirachtin* therapy in terms of proteotoxicity, especially in tissues like the kidney, dorsal muscle, and gills.

Analytically, *Azadirachtin* also caused modest alterations in the protein level of the kidney, liver, and spleen, as well as dramatic alterations in the gills and heart. Chandra and Khuda-Bukhsh therefore recommended that caution be exercised when using this natural product, *Azadirachtin*.

1. **Sub-acute Toxicity Studies**

Male but not female rats' growth was severely reduced by MENF in a subacute toxicity trial, whereas the relative liver weights of rats given 750 and 1,500 mg/kg bw of MENF were significantly higher. The majority of rats' blood chemical readings fell below normal limits.Male rats showed lower levels of AST and BUN but greater creatinine than the control groups, while the female group receiving MENF at 750 mg/kg bw had significantly higher ALP, creatinine, and potassium values. Histopathological study of visceral organs indicated no substantial alteration. To sum up, the LD50 value of MENF in rats was over 12 g/kg bw, which is almost 800 times the human dosage.

**CONCLUSION**

Among the golden medicinal plants of Indian botanical treasure, *Azardirachta indica* is used to treat a variety of ailments in different dosage forms. However, when neem oil is used in doses that exceed their consumption limits, it can have toxic effects on organ functioning. Therefore, its use must be restricted to avoid causing toxicity.

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