Review Article

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A REVIEW ON ACUTE AND SUBACUTE ORAL TOXICITY STUDIES OF SNUHI (*EUPHORBIA NERIIFOLIA* LINN.)

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ABSTRACT

Snuhi (*Euphorbia neriifolia* Linn.) is included in Upavisha as per Ayurvedic classics, and according to modern toxicology, it is classified as irritant organic vegetable poison. It is an active ingredient of many Ayurvedic formulations like Chitrakadi taila, Jatyadi varti, Snuhi kshara etc. These formulations are widely used to treat disease conditions like Shotha (Edema), Dushivisha, Aamdosha, etc. Before administering any potential drug to humans, it is essential to evaluate its safety in animals to define safe human doses through validated scientific toxicity studies. Hence, there is a need to conduct toxicity studies of Snuhi and its phytoconstituents in various extract forms to recognise its safety from the perspective of the Globally Harmonised Classification System. In this article, a special emphasis is done on gathering details on acute and subacute oral toxicity studies of Snuhi (*Euphorbia neriifolia* Linn.)

Keywords: Snuhi, *Euphorbia neriifolia* Linn, Toxicity study, Upavisha, Phytoconstituents.

INTRODUCTION

Snuhi (*Euphorbia neriifolia* Linn.) is included in upavisha (mild poison) in Rasatarangini[1]. Visha (poison), though having toxic properties, can be used as medicine if administered with the proper dose.[2] Upavisha Snuhi is included in irritant, organic vegetable poison.[3] Inspite of that, it is an active ingredient of many Ayurvedic formulations like Jalodarari ras, Vajrakshara, Snuhyadi taila, etc. and is effective in a variety of diseases conditions like Udar (Abdominal disorder), Gulma (Abdominal lump), Arsha (Piles) etc.

Snuhi has several pharmacological activities like anti-carcinogenic, antidiabetic, antimicrobial, etc. Hence, there is need to acknowledge the safety of Snuhi and its phytoconstituents in various extract forms using its toxicity studies.

Herbal Formulations like Snuhi may have been mistakenly regarded as safe due to their natural source. As the chemical compositions of these formulations are complex, some moderate to severe side effects may arise from their use. Prior to human administration, it is essential to investigate animal drug safety to define safe human doses through toxicity studies.[4]

A toxicity study provides safety, therapeutic indices, the safety of recommended doses, toxic doses and toxicity profile, mainly the evaluation of the toxic characteristics of the drug. These studies facilitate deciding whether a new drug is accepted for clinical use.

Limited oral toxicity studies are available on Snuhi (*Euphorbia neriifolia* Linn.) as per the scientific data reviewed. This article mainly focuses on a detailed review of acute and subacute oral toxicity studies of Snuhi (*Euphorbia neriifolia* Linn.) For this article, a methodical scientific documentary search was performed on PubMed, Research Gate, Nigerian Journal of Experimental and Clinical Biosciences, Academic Journal of Plant Sciences, Scholar Research Library, International Journal of Pharmaceutics & Drug Research, International Journal of Phytomedicine, Indian journal of pharmacology, Journal of Natural Remedies in March to May 2023 to probe the acute and subacute oral toxicity studies of Upvisha Snuhi (*Euphorbia neriifolia* Linn.) For this purpose, the search terms such as "toxicity study" OR "acute oral toxicity" OR "Sub acute oral toxicity" OR "Euphorbia neriifolia Linn." OR "Euphorbia neriifolia Linn. extract" OR "Snuhi" OR "Upavisha" and combinations were explored in the title, keywords and abstract of articles to find appropriate scientific data. The studies of Snuhi were assessed with clear case definitions of acute and subacute oral toxicity studies.

All appropriate documents concerned with acute and subacute studies of Snuhi were included in the review. For the selection of eligible articles, time limitation was not defined. There were no strict inclusion criteria for the data extraction. The following exclusion criteria are taken for easy retrieval of scientific findings and to avoid misinterpretation.[5]

1. Other than English language articles.
2. Duplicated data.
3. Review articles, conference papers and editorials.
4. Research articles on sub-chronic and chronic toxicity studies
5. Articles with inadequate data and inapplicable articles.

Literature Review

Table 1: Classical data of Upavisha Snuhi

Vernacular Names	Hindi - Thuhara, Sehunda Marathi - Nivadunga English - Common Milk Hedge, Indian spurge tree
Classification	Akrutrima (natural), sthavara (plant origin), upavisha (mild poison)
Synonyms	Vajradruma, Sudha, Samantadugdha, Snuk, Guda, Sehunda, Sinhatunda, Vajri
Gana	Charaka – Virechana, Shatashodhanavruksha Sushruta - Adhobhagahara, Shyamadi Vagbhata - Nikumbhadi (Virechana)
Rasa, Virya, Vipaka	Katu, ushna, katu
Guna	Laghu, tikshna
Doshaghnata	Kaphavatahara
Karma	Bahya - Vedanasthapana, lekhana Abhyantara - Tikshnavirechaka, raktashodhaka,shothahara, kaphanissaraka, twakdoshahara
Indications	Shoola (pain disorders), aamadosha, ashthilika, aadhamana, gulma (abdominal lumps), udara (abdominal disorders), arsha (piles), shotha (edema), vranshotha, jwara (fever), plihavikara (splenic-diseases), dushivisha
Parts Used	Kshira (latex), patra (leaves), kanda (stem), moola (roots)
Medicinal Dose	125-250 mg kshira (Latex)
Types	According to Charaka Samhita – 1. Alpakantaka 2. Bahukantaka
Formulations	Vajrakshara, Snuhyadi tail, Snuhi kshara [6, 7]

Table 2: Botanical record of *Euphorbia neriifolia* Linn.

Botanical Name	*Euphorbia neriifolia* Linn.
Family	Euphorbiaceae
Description	Xerophytic tree or shrub, It is glabrous erect branched succulent, 20 ft or 1.8-4.5 m high with jointed cylindrical or obscurely 5-angled branches.
Pharmacological Activities	Anti-carcinogenic, anti-inflammatory, antimicrobial, antioxidant, antianxiety, anti-arthritis, anti-thrombotic, antiulcer, cytotoxic, radioprotective, wound healing properties, anaesthetic, analgesic, immunomodulatory etc. [7]

Table 3: Toxicological profile of *Euphorbia neriifolia* Linn.

Classification	Irritant Organic Vegetable Poison
Toxic principle	Neriifolin, Euphol, Neriifoliene
Mechanism of Action	Local and systemic irritant.
Toxic Manifestations	**Local** Vesication, when applied to the skin Inflammation, when dropped into the eyes **Systemic** Vomiting Diarrhoea Convulsion Coma
Fatal Dose	Uncertain (25 -30ml of latex)
Fatal period	Uncertain (3 days)
Postmortem Appearances	Gangrenous patches may be found in the stomach; the spleen may be found in a rotten condition
Medicolegal Aspects	Used for procuring criminal abortion and rarely for homicidal purposes
Schedule (as per Drug and Cosmetic Act 1940)	E1 [8-10]

Table 4: Details of Phytoconstituents of *Euphorbia neriifolia* Linn.

Name of the plant part	Phytoconstituents
Latex (fresh)	Neriifolin-S, neriifolin, 24-diene-3β-ol(Neriifoliene), 5α-eupha-8, 24-diene-3β-ol(euphol)
Latex (dried)	9,19-cyclolanost-20(21)-en-24-ol-3-one(Neriifolione),cycloartenol, Neriifoliol, lectin
Leaves	Ephonerins A-G, Quercetin, Rutin, 3-O-acetyl-8-tigloylingol, (24R)-cycloartane-36,24,25-triol,5,4'-dihydroxy-3,7,3',5'-tetramethoxyflavone, pachypodol (5,4'-dihydroxy-3,7,3'-trimethoxyflavone), combretol (5-hydroxy-3,7,3',4',5'-pentamethoxyflavone) (Flavonols), Friedelan 3α- & 3ß-ols, taraxerol, Euphol (8,24-Euphadien-3 beta-ol)
Bark	12-Deoxy-4 beta -hydroxyphorbol-13-dodecanoate-20- acetate, euphol, euphorbol hexacozonate,n-hexacosanol and 24-methylene cycloartenol
Stem	Friedelan 3γ-ol, waxes, taraxerol Glut-5 (10)-en-1-one ent-3,4-seco-4.16b, 17-trihydroxykauran-3-oic acid, ent-16b-hydroxykauran-3,4-lactone,13b, 19-dihydroxy-3,15-dioxoatis-16-ene, 13b-hydroxy-3,15- dioxoatis-16-ene,16b,17,19-trihydroxy-3-oxo-atisane, 4,13b-dihydroxy-14- oxo-3,4-secoatis-16-en-3-oic acid,4,13b-dihydroxy-14-oxo-3,4-secoatis-16-en-3-oic acid methyl ester,
Root (fresh)	Antiquorin, neriifolene [11,12]

Acute oral toxicity refers to those adverse effects that occur following oral administration of a single dose of substance or multiple doses given within 24 hours.[13] These studies are performed per OECD guidelines for testing chemicals no. 420,423 and 425 in accordance with the guideline formulated by CCSEA.

Sub-acute oral toxicity: Subacute toxicity (repeat dose toxicity) focuses on adverse effects after administering a single dose or multiple doses of test samples daily from 14 to 28 days.[14] These studies are performed per OECD guidelines for testing chemicals no. 407, 410, and 412 in accordance with the guidelines formulated by CCSEA.

LD_{50} (Median Lethal Oral Dose): It is a statistically derived single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral route. The LD_{50} value is expressed in terms of the weight of the test substance per unit weight of the test animal (mg/kg).[13]

Globally Harmonised Classification System (GHS): It indicates whether chemical substances and mixtures are hazardous or safe based on their GHS class.[13]

It categorises the chemical substances and mixtures into five following hazard categories:

- Category 1 (0 mg/kg/b.w < $LD_{50} \leq$ 5 mg/kg/b.w)
- Category 2 (5 mg/kg/b.w < $LD_{50} \leq$ 50 mg/kg/b.w)
- Category 3 (50 mg/kg/b.w < $LD_{50} \leq$ 300 mg/kg/b.w)
- Category 4 (300 mg/kg/b.w < $LD_{50} \leq$ 2000 mg/kg/b.w)
- Category 5 (2000 mg/kg/b.w < $LD_{50} \leq$ 5000 mg/kg/b.w)
- Unclassified (LD_{50} > 5000 mg/kg/b.w)

Table 5: Acute Oral Toxicity Studies of Snuhi (*Euphorbia neriifolia* Linn.)

Plant Part Used	Extract Used	Phyto-constituent	OECD Guideline Used	Dose Ranges	Animal Model used	Study Duration	GHS Class	Findings
Leaves	*Euphorbia neriifolia* sapogenin fraction	Euphol	420, 425	50, 100, 150 mg/kg	Wistar albino rats	14 days	4	LD_{50} of ENSF is 979.24 mg/kg/p.o.
Leaves	Petroleum ether extract of *Euphorbia neriifolia*	-	423	500, 1000, 2000 mg/kg	Laca Mice	14 days	5	No signs of acute toxicity nor mortality up to a dose of 2000 mg/ kg/ p.o.
Leaves	Chloroform extract of *Euphorbia neriifolia*	-	423	500, 1000, 2000 mg/kg	Laca Mice	14 days	5	No signs of acute toxicity nor mortality up to a dose of 2000 mg/kg/p.o.
Leaves	Ethanol extract of *Euphorbia neriifolia*	-	423	500, 1000, 2000 mg/kg	Laca Mice	14 days	5	No signs of acute toxicity nor mortality up to a dose of 2000 mg/kg/p.o.
Leaves	Water extract of *Euphorbia neriifolia*	-	423	500, 1000, 2000 mg/kg	Laca Mice	14 days	5	No signs of acute toxicity nor mortality up to a dose of 2000 mg/kg/p.o.
Stem	Methanol extract of *Euphorbia neriifolia*	-	425	-	Swiss albino mice	14 days	5	LD_{50} of MEEN is 2000mg/ Kg/b.w
Leaves	Hydro-alcoholic extract of *Euphorbia neriifolia*	-	425, 420	100, 200, 400 mg/kg	Wistar Albino Rats	14 days	5	LD_{50} of extracts is 2779.71 mg/kg
Leaves	Ethanolic extract of *Euphorbia neriifolia*	-	425, 420	100, 200, 400 mg/kg	Wistar Albino Rats	14 days	5	LD_{50} of *Ephorbia neriifolia* leaf extracts is 2779.71 mg/kg
Leaves	Triterpenoid fraction of *Euphorbia neriifolia*	Triterpenes	425, 420	100, 250, 500 mg/kg/p.o.	Swiss albino mice	14 days	5	LD_{50} was found to be greater than 2000 mg/ kg. The effect of TFEN on general behaviour was only a slight decrease in locomotor activity at 250 and 500 mg kg doses, with all other normal responses
Leaves	Hydro-ethanolic extract of *Euphorbia neriifolia*	-	423	50,100, 150, 200, 300, 400, 800, 1600 mg/kg/bw	albino mice	14 days	4	Mortality was not noticed up to 400 mg/kg. LD_{50} of hydro-ethanolic extract of *Ephorbia neriifolia* is 1600 mg/kg/bw [15-21]

Table 6: Sub-acute oral Toxicity study of Snuhi (*Euphorbia neriifolia* Linn.)

Plant Part Used	Study Type	Animal Model used	Study Duration	Findings
Latex	Repeated dose sub-acute toxicity	Wistar Albino Rats	14 days	LD_{50}- 630 mg/100 gm. ED_{50}- 8.37 mg/100 gm. At a dose close to its ED_{50}, it produced mild gastric irritation, slight depression of blood glucose and elevation of biochemical markers of liver and kidney function without crossing the normal range and minimal histological changes in the kidney and liver. [22]

ABBREVIATION

LD_{50}- Median Lethal Dose
ENSF- *Euphorbia neriifolia* sapogenin fraction
MEEN- Methanol extract of *Euphorbia neriifolia*
TFEN- Triterpenoid fraction of *Euphorbia neriifolia*
ED_{50}- Median Effective Dose
Mg- Milligram
Kg- Kilogram
p.o- Per os
µg- Microgram

DISCUSSION

The systemic data obtained in this article shows toxicity studies of Snuhi (*Euphorbia neriifolia* Linn.). A total of 8 oral toxicity studies of Snuhi are presented. Among them, 7 are acute oral toxicity studies and a single repeated dose subacute oral toxicity study. After reviewing the scientific data comprehensively, the oral acute toxicity studies are discussed under the headings of - plant part used, type of extract used, phytoconstituents, OECD Guideline used, dose ranges, animal model used, study duration, GHS class and findings (Table 5). Sub-acute toxicity study is summarised as plant part, study type, animal model used, study duration and conclusions (Table 6).

In acute oral toxicity studies (single dose), plant parts used for analyses are leaves and stems. Leaves are commonly used plant parts. The extracts used are *Euphorbia neriifolia* sapogenin fraction, petroleum ether, chloroform, ethanol, water, methanol, hydro-alcoholic, ethanolic, triterpenoid fraction of *Euphorbia neriifolia*, and hydro-ethanolic. Ethanol extract is the most used for the studies. Phytoconstituents used are euphol and triterpenes. Most of the studies reveal the use of OECD guideline no. 420, 423 and 425. According to OECD guidelines, studies are 14 days in duration. The doses of extracts used are 50, 100, 150, 200, 250, 300, 400, 800, 1000, 1500, 1600, 2000 mg/kg/bw. The most commonly used study doses are 200 mg/kg/bw & 500 mg/kg/bw. Animal models used for studies are Wistar albino rats, Laca mice, Swiss albino mice, and albino mice.

Findings of acute oral toxicity studies reveal LD_{50} of *Euphorbia neriifolia* (leaves) sapogenin fraction, Methanol extract of *Euphorbia neriifolia* (stem) and hydro-ethanolic extract of *Euphorbia neriifolia* (leaves) as 979.24 mg/kg/p.o, 2000 mg/kg and 1600 mg/kg/bw respectively. LD_{50} of *Euphorbia neriifolia* (leaves) of hydro-alcoholic and ethanolic extract is 2779.71 mg/ kg.

LD_{50} of a triterpenoid fraction of *Euphorbia neriifolia* (leaves) is found to be greater than 2000 mg/ kg, whereas the effect of TFEN on general behaviour is noted as only a slight decrease in locomotor activity at 250 and 500 mg/kg dose with all other normal responses. No signs of acute toxicity nor mortality up to dose 2000 mg/kg/p.o. of pet ether, chloroform, ethanol and water extract of *Euphorbia neriifolia* (leaves) are seen. In most studies discussed, Snuhi (*Euphorbia neriifolia* Linn.) is included in GHS class 5.

In repeated dose subacute oral toxicity study, the plant part used for analysis is latex. LD_{50} and ED_{50} of *Euphorbia neriifolia* (latex) are found to be 630 mg/100 gm and 8.37 mg/100 gm, respectively. Mild gastric irritation, slight depression of blood glucose, elevation of biochemical markers of liver and kidney function without crossing the normal range and minimal histological changes in kidney and liver are found at a dose close to its ED_{50}.

CONCLUSION

The present review accomplishes acute and subacute oral toxicity studies of Snuhi (*Euphorbia neriifolia* Linn.). The acute oral toxicity studies mostly reveal no signs of toxicity nor mortality up to a dose of 2000 mg/kg/p.o. The LD_{50} is predominantly narrated as 2000 mg/kg/b.w < LD_{50} < 5000 mg/kg/b.w in various extract forms. Overall, Snuhi is included in GHS class 5. Repeated dose subacute oral toxicity study demonstrates LD_{50} as 630 mg/100 gm. Minimal toxicity is seen at a dose close to its ED_{50} (8.37 mg/100 gm). Further toxicity and clinical studies can be conducted on Snuhi (*Euphorbia neriifolia* Linn.) and its formulations to evaluate confirmed therapeutic doses of Snuhi (*Euphorbia neriifolia* Linn.).

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