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ACUTE AND SUBACUTE ORAL TOXICITY OF TINOSPORA CORDIFOLIA EXTRACT IN WISTAR RATS

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Abstract

Tinospora cordifolia (guduchi) is a widely used Ayurvedic climber, and safety questions follow its use in immune and metabolic preparations. This narrative review summarises published knowledge on acute and subacute oral toxicity testing of its extracts in Wistar rats. It covers the plant and its constituents, OECD guideline designs, the clinical signs, body weight, haematological, biochemical and histological end points that such studies report, and how the findings are interpreted. Published animal studies generally describe a wide margin of safety for standard extracts, but extract definitions, dosing and reporting vary and human liver injury reports call for care. This review reports no new experimental data.

1. INTRODUCTION

Tinospora cordifolia (Willd.) Miers, called guduchi or giloy in India, is a large deciduous climbing shrub that appears in many Ayurvedic formulations, usually under the Sanskrit name amrita. Stem preparations are taken as tonic, antipyretic and antidiabetic remedies, and as general immune supportive agents [1, 2, 3]. Commercial interest has grown in the last decade, and capsules, tablets, juices and powders of the stem are sold widely, often without any standardised content of active constituents. Wide use does not by itself establish safety, and the question of what a given dose of extract does to a healthy animal is the starting point of any serious safety dossier.

Toxicity testing of botanical extracts follows the pattern used for synthetic chemicals. An acute study looks for mortality and signs after a single oral dose, and a repeated-dose study over 28 days, usually called subacute, looks

for effects of continued exposure on body weight, blood, organ function and tissue structure [4, 5]. Rats are the usual species, with Wistar and Sprague-Dawley strains the most common, and studies are normally performed according to OECD test guidelines [5, 6, 7]. This review examines what such studies on T. cordifolia can and cannot tell us, how their results are generally reported, and the interpretive problems they raise.

The review is based on published guidelines, reviews of the plant and individual experimental reports from other laboratories. It does not report any original experiment, and the findings of individual papers are described qualitatively because extract preparation, dose units and strains differ so much that numerical comparison would be misleading. Representative findings are collected in table 1, and fig. 1 shows how they are distributed across the main themes. Readers who wish to design a study, rather than interpret one, should consult the guideline documents and reporting standards cited here directly [4, 5, 8, 9].

A point of terminology. Subacute is the term used in the older literature and in many Indian papers for repeated dosing over 14 to 28 days, whereas current OECD wording prefers repeated dose 28-day toxicity [5]. Subchronic refers to 90 days [7]. Sub-chronic and chronic designs, which run for three months and six to twelve months respectively, are needed for products intended for prolonged use, as guduchi preparations commonly are, and published reports of such designs on this plant are few compared with 28-day studies [7, 10]. Authors should state which design they followed.

2. PLANT, CHEMISTRY AND USE

Phytochemical work has identified several classes of compound in the stem. They include isoquinoline alkaloids such as berberine, palmatine and magnoflorine, diterpenoid lactones including columbin and tinosporaside, glycosides, steroids such as beta-sitosterol and ecdysterone, and polysaccharides [3, 10, 11]. The immunologically active polysaccharide fraction, the alpha-D-glucan G1-4A, has been shown to act on Toll-like receptor 4 in macrophages and B cells, and other fractions have been described as immune stimulants in cell models [12, 13, 14]. These compounds are not present in fixed proportions. Their content depends on the plant part, the age of the stem, the habitat and the extraction solvent, which is one reason why extract-based toxicity data cannot be generalised without care [11, 15].

Table 1: Published themes in toxicity assessment of Tinospora cordifolia and related guidance

Topic|feature	Reported finding or feature	Ref.
Acute toxic class OECD 423	Stepwise dosing in small groups, with a limit test at 2000 mg/kg for low-toxicity substances.	[4]
Up-and-down OECD 425	Sequential single-animal dosing that estimates LD^{50} with fewer animals.	[6, 16, 17]
28-day repeated dose OECD 407	Ten animals per group, daily gavage, haematology, chemistry and histopathology.	[5, 7]
Stem constituents phytochemistry	Alkaloids, diterpenoid lactones, glycosides and immunoactive polysaccharides vary with solvent and plant source.	[3, 10, 11]
Hepatoprotection toxin models	Reduced liver damage in rats given carbon tetrachloride or lead.	[18, 19, 20]
Glucose lowering diabetic rats	Hypoglycaemic actions seen in root and alkaloid preparations.	[21, 22]
Acute studies extracts	Reports of no mortality at limit doses and practically non-toxic classification.	[1, 10, 11]
Repeated-dose studies extracts	Generally unchanged haematology and organ markers with normal histology at tested doses.	[10, 11, 19]
Biomarker interpretation historical ranges	Changes judged against dose response, pathology and control ranges.	[23, 24, 25]
Herbal liver injury human reports	Idiosyncratic injury poorly predicted by rodent studies; pharmacovigilance advised.	[26, 27, 28]

The traditional preparation is usually an aqueous decoction, a fresh stem juice or a

powder, and the ethanolic or hydroalcoholic extracts used in many laboratories differ from these in composition. An aqueous extract is richer in polysaccharides and glycosides, while ethanol favours alkaloids and diterpenes. A study that finds an alcoholic extract to be safe says relatively little about the juice that a patient prepares at home, and vice versa [1, 10, 15]. Authors who state the extraction ratio, drying yield and chemical markers of their material provide far more useful information than those who describe a stem extract only by name [10, 11].

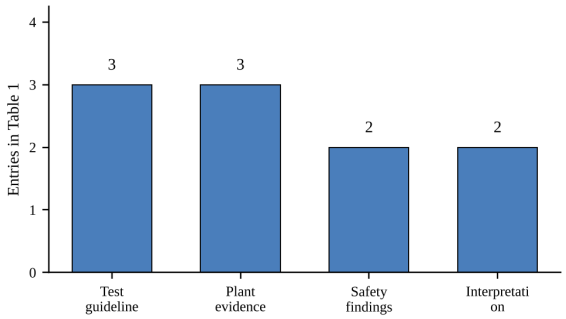


Fig. 1: Number of entries in table 1 by type of evidence

Pharmacological studies give the background against which safety is judged. Hepatoprotective effects against carbon tetrachloride and other toxins were reported in rats and attributed in part to modulation of Kupffer cell function [18, 19]. Immunomodulatory activity has been reported in cells, animals and in a clinical trial in allergic rhinitis [12, 13, 29]. Antihyperglycaemic effects of root and stem preparations in alloxan-diabetic rats have been described, and an alkaloid fraction has been reported to lower glucose in rodent models [21, 22]. Protective actions against lead and aflatoxin toxicity in rodents have also been reported [20, 30]. Such findings suggest bioactivity at doses of the same order as those used in toxicity studies, which is relevant when judging whether a given test dose is high enough to mean anything.

Quality of the plant material is a prerequisite for any toxicity statement. The stems are collected from wild and cultivated sources, and the botanical identity matters, because other species of Tinospora and other Tinospora species, as well as unrelated look-alike climbers, are traded under the same or similar local names. Contamination with heavy metals, pesticide residues and microbes is a recognised problem for herbal raw materials, and analyses of Ayurvedic products sold over the internet have found lead, mercury or arsenic in a notable share of samples, which is why botanical identification, voucher specimens and documented harvest, drying and storage matter as first controls on quality [11, 15, 31]. Many published toxicity studies describe the source of the plant only loosely, which makes it hard to relate their conclusions to a commercial product [10, 11]. Reporting these basics costs little.

Human use is mostly in the form of home preparation and over-the-counter products. Typical traditional doses of the dried stem powder are in the order of a few grams a day, and the equivalent for a 70 kg adult is a small fraction of the amounts given to rats in limit tests [1, 3]. This margin is usually presented as reassurance. It deserves a degree of caution, however, because the dose scaling between species, the conversion of dry stem to extract yield and the long duration of human use all affect the comparison [23, 27].

3. ACUTE TOXICITY STUDIES

Acute oral toxicity tests have changed a good deal since the classical LD_{50} determination. The Acute Toxic Class method (OECD 423) uses small groups of three animals of one sex, usually females, dosed stepwise at fixed levels of 5, 50, 300 and 2000 mg/kg, and assigns a hazard class from the mortality seen [4]. The Up-and-Down Procedure (OECD 425) doses single animals in sequence, raising or lowering the dose according to the outcome in the previous animal, and uses statistical estimation to arrive at an LD_{50} with far fewer animals [6, 16, 17]. A limit test at 2000 mg/kg, or 5000 mg/kg in special cases, is allowed when low toxicity is expected, as it normally is for herbal preparations [4, 6].

Older papers, including many from India, use the method of Lorke, in which a first phase with three doses in three animals each is followed by a second phase with more selected doses [32]. It is quick and was widely adopted for plant extracts, but it is less formal than the OECD designs and gives only an approximate LD_{50}. Whichever method is

used, animals are observed intensively during the first hours after dosing, then daily for 14 days. Food is typically withheld overnight before dosing, and a standard volume limit applies, usually 1 mL per 100 g body weight for aqueous vehicles, which becomes a practical ceiling on the dose for poorly soluble or viscous extracts [4, 32, 33].

Published acute studies of T. cordifolia extracts, in the main, describe no mortality and no treatment-related clinical signs at limit doses, for aqueous, hydroalcoholic and alcoholic preparations alike, which has led authors to classify the extracts as practically non-toxic or as category 5 or unclassified under the Globally Harmonised System [1, 10, 11]. Such findings are consistent with the long traditional use of the plant. They tell us that a single large dose is unlikely to be lethal. They do not tell us about effects on specific organs, nor about the consequences of repeated or prolonged exposure, for which acute tests were never intended [4, 34].

Animal characteristics are specified more tightly than many papers acknowledge. Guidelines call for healthy young adult rodents, normally 8 to 12 weeks old, of a commonly used laboratory strain, with the weight variation of animals used in a study not exceeding 20 per cent of the mean. Females are generally preferred in the acute class method because they are often slightly more sensitive, and nulliparous, non-pregnant animals are used [4, 6]. Temperature, humidity, light cycle, cage density and diet need to be controlled and reported, since stress and nutrition alter many of the end points measured later. The Wistar rat is a perfectly acceptable choice, but laboratory-specific reference data, rather than those from other strains, are the proper comparator [25].

Observation of clinical signs deserves more care than it generally gets. Guidance from the OECD lists the signs that should be recorded and the humane endpoints at which animals should be killed to avoid suffering, including changes in the skin and fur, eyes and mucous membranes, respiratory and circulatory function, and behaviour [34]. A structured score sheet and blinded observation help avoid bias, and signs like piloerection, reduced locomotion, tremor and diarrhoea should be documented with the time of onset. Brief sedation after dosing, if it occurs, may reflect the volume and osmolarity of the vehicle rather than the extract, so a vehicle control group is helpful even though guideline tests do not require one [34].

It is worth asking why a large number of papers still report acute toxicity for an herb with such a safe reputation. One reason is institutional, since regulatory and journal requirements call for such data. Another is that LD_{50} values are easy to compare, at least apparently. A third, less comfortable, is that these studies are simple and inexpensive. The scientific return is small, because a plant that is not acutely toxic at 2000 mg/kg gives little information about subtler hazards, and the animals could often be used better in a well-designed repeated-dose study [4, 6, 23].

4. SUBACUTE TOXICITY STUDIES

The repeated dose 28-day study (OECD 407) uses at least three dose groups and a control, with ten animals per group, five of each sex, dosed daily by gavage for 28 days [5]. The highest dose is selected to produce toxic effects but not death, and the lowest is expected to produce no effect. For herbal extracts of low toxicity, a limit dose of 1000 mg/kg per day is allowed when no effects are expected. An optional satellite group can be kept for 14 days without treatment to look for reversibility or delayed effects [5, 7]. Published T. cordifolia studies follow this scheme to a variable degree, and some use fewer animals or only one sex.

During dosing, body weight and food consumption are recorded weekly, and at the end haematology, clinical chemistry, organ weights, macroscopic examination and histopathology are performed. Haematology covers haemoglobin, haematocrit, red and white cell counts with differential, platelets and clotting time; clinical chemistry covers alanine and aspartate aminotransferases, alkaline phosphatase, bilirubin, total protein, albumin, urea, creatinine, glucose and cholesterol, along with electrolytes [5, 24, 35]. Historical control ranges from the same laboratory, or from published databases, are used to place values in context [25, 35].

Without them, a statistically significant change within the normal range can be mistaken for a toxic effect.

Reports of repeated-dose studies on T. cordifolia extracts, again in broad terms, describe no treatment-related deaths, normal or slightly altered body weight gain, haematological indices within control limits, and liver and kidney markers that are unchanged or modestly changed at moderate doses [10, 11, 19]. Several studies, in contrast, describe the extract as hepatoprotective, with reduced enzyme release in toxin-challenged animals, which complicates the reading of toxicity data, since a protective action in one setting does not preclude harm in another [18, 19, 20]. Histopathology is usually reported as normal at the dose levels tested, with occasional mild changes in the liver or kidney described as incidental [5, 23].

Organ weights and histology deserve more attention than they get. At necropsy the liver, kidneys, adrenals, testes, epididymides, thymus, spleen, brain and heart are weighed, and relative weights are normally calculated against body weight, although brain weight is sometimes used as a more stable denominator when body weight itself is changed by treatment [5, 24]. Tissues are fixed in neutral buffered formalin, processed, sectioned and stained with haematoxylin and eosin, and examined at least in the control and highest-dose groups. If lesions are found there, lower groups are examined too. Descriptions of histology in many herbal papers are brief, with no lesion scoring and no statement that the pathologist was blinded to treatment [5, 23].

Blood glucose and lipids, rather than liver and kidney, are the parameters most likely to be influenced by T. cordifolia. Reports of glucose lowering in diabetic rats, together with possible effects on lipid profile, mean that a significant decrease in glucose or cholesterol in treated healthy rats is not automatically an adverse finding. It may be the pharmacology of the extract showing itself. The assessment guidance on adverse and nonadverse effects emphasises that such changes should be judged against their magnitude, dose relationship, associated pathology and reversibility before being labelled toxic [21, 22, 23].

5. INTERPRETING FINDINGS

Sound interpretation begins with a distinction between statistical and toxicological significance. In studies with a handful of animals per group, many comparisons are made across dozens of endpoints, and a few will cross the conventional p value by chance. A change is more convincing when it shows a dose-response relationship, appears in both sexes, is outside the historical range, and correlates with a change in another parameter, for example an enzyme rise with a histological lesion [23, 24, 25]. Papers that list only whether p is below 0.05 give readers little to work with. Reporting means, spread, individual data and control ranges would resolve many of the ambiguities [8, 9].

Liver and kidney biomarkers need particular thought. Alanine aminotransferase is liver-specific in the rat to a greater extent than aspartate aminotransferase, which is also present in muscle and red cells, and alkaline phosphatase can rise with bone growth in young animals or cholestasis. Urea and creatinine are affected by hydration and diet. A pattern across several markers, supported by histology, carries more weight than a single value [24, 35]. Experienced toxicologists also take account of stress from gavage and fasting, which can alter enzymes and glucose in control animals alike [24, 25].

Statistics and sample size are the next consideration. Groups of five or six animals leave little power to detect modest changes, and multiple endpoints inflate false positives unless handled with suitable tests, such as analysis of variance with Dunnett's comparison to control for continuous data. A publication checklist for animal studies asks that authors state the unit of analysis, the method of randomisation and the criteria for exclusion [9]. Replacement, reduction and refinement principles also argue against needless repetition. A reviewer who sees ten near-identical acute studies might reasonably ask whether the next one adds anything [4, 6, 9].

Do animal data predict harm in humans? A review of pharmaceutical compounds found

that rodent and non-rodent studies together identified a majority of human toxicities, but the concordance for individual organs varied and idiosyncratic liver injury was poorly predicted [27]. The point applies to herbal products. A plant extract may show no toxicity in rats at high doses and yet cause rare liver injury in susceptible people, because idiosyncratic reactions depend on genetic and immunological factors absent from healthy laboratory strains [26, 27]. The negative result from rodent studies lowers concern but does not remove it.

This caution is not hypothetical. Compilations of herbal hepatotoxicity list a range of botanicals with documented cases of liver injury, and guidelines on pharmacovigilance of herbal medicines recommend active reporting of adverse events, as well as quality control of the starting material [26, 28]. Whether guduchi itself belongs on such lists is not settled by the sources reviewed here, and where preparations are taken together with other herbs or drugs, attributing injury to one ingredient is difficult. That uncertainty is a reason for good surveillance and product identification, not for dismissal of the concern. Misidentified or adulterated plant material, for example confusion with related Tinospora species, is a recognised risk [11].

6. LIMITATIONS AND FUTURE DIRECTIONS

The published rodent literature on T. cordifolia has several limits. Extracts are poorly characterised, with few papers giving marker compounds, extraction yields or batch variability. Study designs deviate from guidelines, with small groups, a single sex, short observation and incomplete histopathology. Reporting often omits randomisation, blinding and housing conditions, which a publication checklist for animal studies asks authors to address [9]. Doses are sometimes chosen without reference to human exposure. As a result, even when the overall message is of low toxicity, the strength of evidence for any particular product is less than a first reading suggests [11, 23].

Units and dose reporting are a smaller point that causes much confusion. Some papers give the dose as milligrams of extract per kilogram body weight, others as milligrams of dry stem equivalent, and a few as millilitres of decoction, without a conversion. Without the extraction yield, readers cannot relate a rat dose to a human daily intake of powder or juice [10, 11]. Extrapolation between species is usually done by body surface area, which gives a human equivalent dose well below the mg/kg figure for rats, but that approach is a convention rather than a measured relationship, and its limits are often forgotten [23, 27].

Better work is within reach. Studies should use standardised, chemically profiled material from authenticated plant sources with documented identity and handling, and should state the extract-to-drug ratio and the markers measured [11]. Repeated-dose studies should follow OECD 407 or 408 with both sexes, include recovery groups, and measure toxicokinetic exposure to the main constituents so that dose can be related to human use [5, 7]. Targeted immunotoxicity and reproductive endpoints are justified by the plant's reputed actions. Finally, surveillance of human cases through pharmacovigilance systems, with careful causality assessment, will say more about rare harm than any further rat experiment [26, 28].

7. CONCLUSION

The published rodent studies of Tinospora cordifolia extracts broadly agree: single large oral doses are tolerated, and repeated dosing for four weeks does not produce clear organ toxicity at the doses tested, which matches its long traditional use [1, 10, 11]. The experimental designs behind those statements are those of the OECD guidelines for acute and 28-day studies, whose scope and limits are well defined [4, 5, 6]. Where the plant has been tested in toxin models, it has more often protected than harmed the liver [18, 19, 20].

A cautious reading is nevertheless needed. Extract definitions differ, many studies depart from guidelines or report poorly, and rodent studies do not predict rare idiosyncratic injury in people [8, 26, 27]. Better characterised material, complete reporting and active pharmacovigilance would together give a more reliable picture than additional acute

tests, and clinicians and consumers should treat reassurance from animal data as one piece of evidence rather than a clearance [9, 28]. Where new studies are done, following the 28-day or 90-day guideline closely and reporting to the ARRIVE standard will make them directly usable by others [5, 7, 8].

8. ABBREVIATIONS

OECD-Organisation for Economic Co-operation and Development, LD_{50}-Median lethal dose, ARRIVE-Animal Research: Reporting of In Vivo Experiments, ALT-Alanine aminotransferase, AST-Aspartate aminotransferase, TLR4-Toll-like receptor 4, GACP-Good agricultural and collection practices, WHO-World Health Organization, CCl_4-Carbon tetrachloride

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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