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Pharma Research

An International Peer Reviewed, Indexed Journal

[10.62655/s-epub.2026.v18.i01.pp47-54](https://s-epub.in/index.php/trp/index)

IMMUNOMODULATORY ACTIVITY OF TINOSPORA CORDIFOLIA STEM EXTRACT IN CYCLOPHOSPHAMIDE-IMMUNOSUPPRESSED MICE

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Article info

Received date:12-07-2025

Accepted date:20-11-2025

Published date:23-02-2026

ISSN;0975-8216

Keywords:

Tinospora cordifolia, Immunomodulation, Cyclophosphamide, Guduchi, Mouse models

1. INTRODUCTION

Tinospora cordifolia (Willd.) Miers ex Hook. f. & Thoms., known as guduchi or giloy, is a climbing shrub of the family Menispermaceae. In Ayurvedic practice it is classed as a rasayana, a group of preparations thought to build general resistance to disease [1, 2]. Stem decoctions and powders appear in traditional formulae for fevers, jaundice and chronic infections, conditions in which host defence is the obvious common thread [3]. That link is what pushed the plant into laboratories. If guduchi truly strengthens resistance, the effect should be measurable in animals whose immune function has been

Abstract

Tinospora cordifolia (guduchi) stem preparations are used in Ayurvedic practice to support resistance to infection, and experimental work in mice has tried to confirm this using cyclophosphamide-induced immunosuppression as the challenge. This narrative review, which reports no new data, summarises the phytochemistry of the stem, the biology of the cyclophosphamide model, and the published effects of stem extracts on leucocyte counts, antibody formation, delayed-type hypersensitivity, phagocytosis and cytokine balance. Most studies point towards restoration of suppressed immune readouts, with macrophage activation prominent. The evidence is held back by variable extract standardisation, single dose levels, weak blinding and few comparisons with established agents. Reporting standards and chemical profiling need to improve before the plant can be positioned beyond traditional use.

deliberately lowered. This review gathers what published experimental work says about the immunomodulatory activity of the stem extract, with attention to the cyclophosphamide-suppressed mouse. No new experiments are reported.

Immunomodulation is a looser idea than it sounds. A compound may raise a depressed response, damp an excessive one, or leave a healthy system alone, and the same extract can do different things depending on baseline status and dose [4, 5]. Plant products have attracted attention because they contain mixtures that act on several arms of immunity together, from macrophage activation to cytokine release [6]. Mixtures also make the science harder. Which constituent is responsible, and whether it survives extraction, is rarely settled. For this reason the published stem studies are best read as tests of an extract with a defined preparation history rather than of guduchi in some general sense.

The review is organised around the questions a pharmacologist would ask before trusting a claim of this kind. What is in the stem and how is it extracted? Why is cyclophosphamide used to depress immunity, and what does it leave intact? Which readouts have been measured, and do they agree? Where do dose translation and reporting standards leave the interpretation [7, 8]? A synthesis of the main findings is given in table 1, and the distribution of the evidence across the types of immune endpoint is shown in fig. 1. The aim is a measured account that separates what has been repeatedly observed from what rests on a single laboratory.

Table 1: Selected published findings on *Tinospora cordifolia* stem and related preparations in immune models

Model or readout	Reported finding	Ref.
Stem glucan macrophage assay	Polysaccharide fraction stimulated macrophage and lymphocyte proliferation in vitro.	[9, 10]
Stem protein splenocytes	Major protein fraction raised splenocyte proliferation and cytokine release in mouse cells.	[11]
Macrophage activation in vitro	Extract increased phagocytic activity and mediator release in cultured macrophages.	[9, 12]

Model or readout	Reported finding	Ref.
Antibody response sheep red cells	Higher haemagglutination titres reported after extract in suppressed animals.	[1, 13]
Haematological recovery cyclophosphamide	Partial restoration of leucocyte and lymphocyte counts compared with cyclophosphamide alone.	[14, 15]
Delayed hypersensitivity footpad swelling	Increased T-cell dependent responses in extract-treated animals.	[16]
Bacterial infection peritonitis	Improved survival and bacterial clearance after pretreatment in rodents.	[17]
Kupffer cell function liver damage	Enhanced phagocytic activity of liver macrophages in injured rodent liver; colony-stimulating activity in serum.	[18, 19]
Cytokine balance T helper profile	Increased interleukin and interferon- γ release, with Th1-type shift proposed.	[6, 20]
Urotoxicity and antioxidants glutathione	Reduced cyclophosphamide bladder injury linked to glutathione and cytokine changes.	[15, 21]

2. STEM PHYTOCHEMISTRY AND EXTRACTION

The stem is chemically busy. Alkaloids such as berberine, palmatine and magnoflorine sit alongside diterpenoid lactones (for example columbin and tinosporaside), glycosides, steroids and polysaccharides [2, 3]. For immune activity, three families have drawn most interest. A (1,4)- α -D-glucan reported from the stem activated macrophages and lymphocytes in vitro, and an arabinogalactan with immunological activity was described from the same plant. Cordifolioside and related glycosides were linked to phagocytic stimulation, and the alkaloid magnoflorine showed immunosuppressive behaviour in some assays, which is a reminder that the whole extract may contain opposing signals [9, 10]. A reasonable reading is that the stem is a source of several immunologically active constituents that need not all push in one direction.

Extraction choice shapes what ends up in the dose. Aqueous and hydroalcoholic preparations dominate the cyclophosphamide

literature, because they resemble the traditional decoction and extract the polysaccharides and glycosides that are most plausibly responsible for macrophage effects [10, 13]. Alcoholic extracts concentrate alkaloids and terpenoids, and these may influence liver enzymes as much as immune cells [21]. Yields differ with solvent, plant age, season and drying method, and few papers report them. An extract described only as 'stem extract' without solvent ratio, extraction time and marker content cannot be reproduced, and results from one such study cannot safely be added to another.

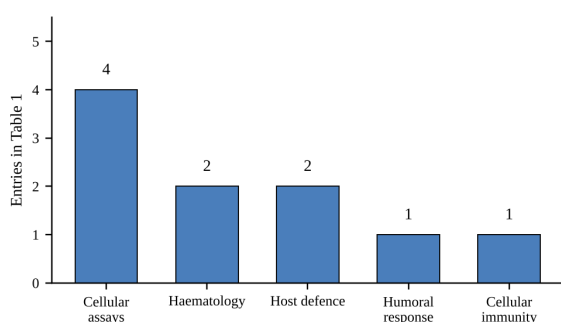


Fig. 1: Number of entries in table 1 by type of immune endpoint

Protein and polysaccharide fractions have added a second route of evidence. Work on the major stem protein reported stimulation of mouse splenocyte proliferation and cytokine release, which means that part of the activity is carried by macromolecules that would be lost in a strongly alcoholic extraction [11]. Similar caution applies to the glucan: its activity depends on molecular size and branching, and crude alcohol precipitation does not guarantee a consistent fraction [9]. The practical consequence is that two preparations both labelled guduchi stem extract could differ in the very component that matters. Fingerprinting by chromatography, together with at least one polysaccharide or alkaloid marker, would make cross-study comparison more honest [2].

Safety and pharmacokinetic data on the individual compounds are thin. Berberine, the best studied alkaloid, has poor oral bioavailability and interacts with drug-metabolising enzymes, so it is a poor guide to what the whole extract does in the animal [21]. Absorption of the large polysaccharides is likely to be minimal,

which suggests that effects, if mediated through the gut, may involve local interaction with mucosal immune tissue rather than systemic exposure. Few studies have measured plasma levels of any stem constituent after oral dosing. Without these figures, statements about dose-response remain empirical, based on administered mass of extract rather than on exposure to an active principle [1].

Standardisation is the obvious remedy, and the tools already exist. High-performance liquid chromatography with diode-array detection can profile the alkaloid fraction and quantify berberine or magnoflorine as markers, while total carbohydrate and glucan content can be followed by simple colorimetric methods and size-exclusion analysis [2, 3]. A reviewer reading a methods section should be able to find the plant part, the solvent, the drying temperature, the yield and at least one marker value. Where activity-guided fractionation has been attempted, the active material has usually been recovered in a polar, high molecular weight pool, which tells formulators what to protect during processing [9, 11].

3. THE CYCLOPHOSPHAMIDE MODEL

Cyclophosphamide is a prodrug activated mainly by hepatic cytochrome P450 enzymes to 4-hydroxycyclophosphamide, which equilibrates with phosphoramidate mustard, the alkylating species that cross-links DNA, and with acrolein, which is responsible for bladder toxicity [22]. Dividing cells are the main casualties. Lymphocyte precursors, activated B and T cells and bone-marrow progenitors proliferate rapidly and are damaged accordingly, so a single dose produces leucopenia, thymic and splenic atrophy and weak antibody responses within days [14, 23]. This predictable and reproducible damage is the attraction of the model for screening immunostimulant plants, and it is cheap to run in standard animal facilities with commercially available inbred or outbred strains.

Protocols follow a few common patterns. A single intraperitoneal injection of cyclophosphamide, or a short course of daily

doses, is given to Swiss albino or BALB/c mice, and the plant extract is administered orally by gavage for one to two weeks, starting before, with or after the cytotoxic drug [14, 15]. White cell counts usually bottom out within several days and then recover spontaneously as the marrow regenerates, which means that the window in which extract effects can be separated from natural recovery is narrow. Sampling times therefore matter. A comparison made at a single late time point may show a difference that merely reflects a slightly faster return to baseline, not enhanced immunity as such [22, 23].

The model is not simply 'less immunity'. Cyclophosphamide discriminates between lymphocyte subsets: regulatory T cells are particularly sensitive at low doses, which is why low-dose schedules can enhance antitumour and vaccine responses in some settings [24]. At the higher doses used to induce immunosuppression in mice, typically given as a single injection or a short series, B-cell and humoral responses fall sharply, while innate effector function is partly preserved [14, 23]. A plant extract that restores antibody titre therefore tells a different story from one that restores neutrophil counts, and papers that report only a total white cell count conceal this distinction.

Timing matters more than many protocols admit. If the extract is given before cyclophosphamide, any protection may reflect altered drug metabolism rather than immune stimulation, because guduchi has been reported to induce detoxification enzymes and raise glutathione (GSH) [15, 21]. If it is given after, the effect is more plausibly one of recovery. Protective actions against cyclophosphamide-related urotoxicity have been described for guduchi and for other botanicals such as *Emblica officinalis*, but these are not immunological results as such and should not be counted as proof of immune enhancement [15, 25]. Clear separation of pre-treatment, co-treatment and post-treatment designs would clarify the interpretation considerably.

Standard readouts have been borrowed from classical immunology. Total and differential

leucocyte counts, haemoglobin and platelet counts follow marrow recovery. Haemagglutination titres, or plaque-forming cell counts in the Jerne assay, measure the antibody response to a challenge with sheep red blood cells (SRBC) [26]. Delayed-type hypersensitivity (DTH), read as footpad or ear swelling, reflects T-cell dependent responses. Carbon clearance and phagocytic index, in the tradition of Biozzi and colleagues, address the reticuloendothelial system [27]. Organ weights and histology of thymus and spleen add structural context. Used together, these measures give a rounded view, but few stem studies have used more than three or four.

Host factors add noise that is easy to overlook. Strain differences in cytochrome P450 activity alter how much active cyclophosphamide metabolite a mouse is exposed to, sex influences both drug clearance and baseline antibody output, and the gut microbiota affects immune tone and may modify how an orally given polysaccharide is handled [14, 22]. Housing, diet and the stress of repeated gavage can shift leucocyte counts by themselves. Because each of these factors varies between laboratories, an effect size in one paper is not directly transferable to another. Concurrent vehicle and cyclophosphamide-only groups, handled identically, are the only safeguard against attributing such variation to the plant [7, 28].

4. REPORTED IMMUNOMODULATORY EFFECTS

Early work established that guduchi was biologically active before the cyclophosphamide model became common. In mice and rats, stem or root preparations raised antibody-forming responses, improved survival in bacterial peritonitis and enhanced the activity of liver macrophages, called Kupffer cells, in damaged liver, and serum from treated animals showed colony-stimulating activity [17, 18, 19]. In vitro, the stem-derived glucan stimulated macrophage and lymphocyte proliferation [10]. These observations supplied the rationale for testing the extract against chemical suppression. Findings drawn from these and later studies are summarised in table 1, grouped by endpoint type, and

counted in fig. 1.

Under cyclophosphamide challenge, the most consistent finding is partial recovery of haematological and humoral parameters. Treated mice are reported to show higher total leucocyte and lymphocyte counts, stronger antibody titres to sheep red blood cells and, in several papers, larger thymus and spleen indices than animals given cyclophosphamide alone [1, 13, 15]. Comparison against established immunostimulants is occasionally included, and the plant extract tends to produce moderate rather than dramatic effects. A related study in a teleost fish showed leaf extract improving non-specific defences, which suggests that conserved innate pathways rather than a mammal-specific mechanism may be involved [29].

Cellular immunity and phagocytosis have been tested in parallel. Delayed-type hypersensitivity responses were increased in extract-treated animals in a number of reports, in keeping with the idea that T-cell dependent reactions are lifted [16]. Macrophage activation is the most prominent mechanism proposed: higher phagocytic activity, nitric oxide release and lysosomal enzyme content were seen after extract exposure in cell and animal systems [9, 12]. Antioxidant and hepatoprotective actions in other injury models support the picture of a plant that influences myeloid cells, although this does not by itself prove the effect operates in the immunosuppressed mouse [21, 30].

Cytokine data point in a similar direction but are less consistent. Stem extract and its fractions increased production of interleukin (IL)-1, IL-6, tumour necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) by macrophages and splenocytes in some experiments, and the protein fraction raised cytokine release from mouse splenocytes [11, 12]. A shift towards T helper 1 (Th1) type activity, in the sense used by Mosmann and Coffman, is frequently suggested, though the evidence for a clean polarisation is thin and several papers measure only one or two mediators [6, 20]. Protection against cyclophosphamide toxicity has also been linked to changes in glutathione and cytokine concentrations, suggesting that immune and

antioxidant effects overlap [15].

Comparisons with reference agents are informative where they exist. Levamisole and other recognised immunostimulants have been used as positive controls in a handful of reports, and the plant extract generally produces smaller changes at the doses tested, while sometimes matching the standard on selected endpoints such as phagocytic index [1, 13]. Dose-response behaviour is mixed. Some experiments describe a rising effect across two or three doses, others a plateau or even a fall at the highest dose, as would be expected if opposing constituents are present [6, 9]. With a single dose group the shape of the curve is simply unknown, which restricts what can be said about potency.

Taken at face value, the studies tell a coherent story: stem extract helps the suppressed mouse regain white cells, antibody and macrophage function, with some support for T-cell dependent responses. Coherence is not confirmation, however. Many of the cited papers come from a small number of laboratories, use different extracts, and measure different endpoints, so independent replication of any single claim is limited [4, 5]. Taken individually, each experiment reads as supportive. Placed side by side, they leave open how large the effect is, what dose is needed, and which constituent does the work. Publication practice adds a further tilt, since experiments that found nothing are rarely written up for plant extracts of traditional repute.

Organ weights and histology provide a structural check on the functional readouts. Cyclophosphamide shrinks the thymus and spleen and depletes cortical lymphocytes, and reports on guduchi describe partial restoration of organ indices and of lymphoid follicle architecture in treated animals [14, 15]. These measures are valuable because they are harder to bias than a titre read by eye, provided the pathologist is blind to group. They also reveal toxicity. An extract that raised the spleen index through congestion or inflammation would look the same on weight alone, so organ weight should always be interpreted alongside cell counts and sections, not in place of them [7].

5. LIMITATIONS AND FUTURE DIRECTIONS

The first limitation is chemical. Without standardisation to named markers, results cannot be linked to a defined product, and batch variation may account for some differences between laboratories [2, 3]. Voucher specimens, solvent ratios, yields and fingerprint chromatograms are inexpensive to report and would transform the usefulness of the literature. Second, doses are rarely justified on a translational basis, so the clinical meaning of a mouse result is unclear [8, 31]. A sensible next step is dose-ranging with at least three levels and a clear human-equivalent calculation, rather than a single dose compared against vehicle.

Design quality is the second concern. Randomisation, blinding of outcome assessors and sample size justification are inconsistently reported, positive controls vary, and statistical analysis is frequently limited to simple tests without correction for multiple endpoints [7, 28]. Where levamisole or another recognised immunostimulant is used as comparator, the plant extract usually performs less strongly, and this should be stated plainly. Studies should also include non-suppressed controls given extract alone, so that readers can tell whether guduchi restores a deficit or merely pushes a healthy system in a particular direction [14, 23].

Quality and regulatory perspectives follow from the same weaknesses. Herbal medicines that make immune-supporting claims are generally expected to rest on reproducible quality attributes, and for a stem extract this means authenticated botanical identity, controlled solvent residues, limits for heavy metals and microbial contamination, and a stability profile, in line with general guidance for stability testing of active substances [4, 5]. None of these are routinely reported in experimental papers, which is understandable for academic work but limits the usefulness of the results to anyone wishing to develop a product. Linking a defined, quality-controlled material to a specific preclinical effect is the sensible bridge towards any later clinical evaluation [1, 3].

Mechanistic depth is limited. Most papers describe what changed, not why. Flow

cytometry of lymphocyte subsets, measurement of a broader cytokine panel, and assessment of macrophage polarisation would show which cell types respond and whether the effect is more than a general rise in leucocyte number [20, 24]. Fractionation guided by activity, with purified polysaccharide and protein tested against alkaloid-rich fractions, would address the question of which constituent matters [9, 11]. Receptor-level studies, for instance on toll-like receptor engagement by the glucan, remain largely speculative and would need dedicated work.

Translation to people is the final hurdle. Clinical evidence for guduchi in immune endpoints is sparse and heterogeneous, and the animal literature cannot substitute for it [1, 4]. Safety needs deliberate study, especially hepatic effects, interaction with immunosuppressants and use in people with autoimmune disease. A realistic research agenda would combine standardised extracts, well-reported randomised animal experiments, activity-guided fractionation and, if the preclinical case holds, carefully designed human studies with defined endpoints. Until then, the stem extract is best described as a promising traditional agent with consistent but modest experimental support.

6. CONCLUSION

Published experiments in cyclophosphamide-suppressed mice, together with earlier cell and rodent work, give a fairly consistent picture. *Tinospora cordifolia* stem preparations help restore leucocyte counts, antibody formation, delayed-type hypersensitivity and macrophage function, and they appear to push cytokine release towards a type 1 pattern [10, 12, 13, 15]. The polysaccharide and protein fractions are the strongest candidates for the active principles, though alkaloids may also contribute and may sometimes act against the trend [9, 11]. The effects look moderate, which fits the traditional role of a rasayana better than that of a potent drug, and the range of endpoints that moved in the expected direction is wider than a single laboratory artefact would easily explain.

Confidence is limited by how the work was done. Extracts are poorly characterised, doses are seldom translated, designs often omit blinding and non-suppressed controls, and mechanistic data are thin [7, 8, 28]. Safety, particularly liver effects and drug interactions, needs equal attention to efficacy, because an extract that stimulates immunity may be unwelcome in people taking immunosuppressants. Better reporting, standardised material and activity-guided fractionation would turn a suggestive literature into a dependable one. Until that happens, the stem extract remains a reasonable subject for further preclinical study but not a basis for firm therapeutic claims [3, 4, 5].

7. ABBREVIATIONS

DTH-Delayed-type hypersensitivity, IFN- γ -Interferon- γ , IL-Interleukin, P450-Cytochrome P450, Th1-T helper type 1, TNF- α -Tumour necrosis factor- α , SRBC-Sheep red blood cells, GSH-Glutathione

8. FUNDING

Nil

9. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

10. CONFLICT OF INTERESTS

Declared none

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