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# Efficacy and Mechanisms of *Tribulus terrestris* in the Management of Erectile Dysfunction: A Systematic Review of Preclinical Studies

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## ABSTRACT

**Background and Purpose:** Erectile dysfunction (ED) is a prevalent male sexual disorder that negatively affects quality of life and interpersonal relationships. Although phosphodiesterase type 5 inhibitors remain the standard treatment, their limitations, including adverse effects, contraindications, and reduced efficacy in certain disease conditions, necessitate the exploration of alternative therapeutic options, including medicinal plants. *Tribulus terrestris* has been reported in several preclinical studies on its potential proerectile effects. However, a comprehensive synthesis of the available preclinical evidence is limited. Therefore, this study systematically reviewed animal studies investigating the efficacy and mechanisms of *T. terrestris* in the management of ED.

**Methods:** A comprehensive literature search was conducted across databases including PubMed, Scopus, Web of Science, and Google Scholar using relevant keywords and Boolean operators. Retrieved articles were screened based on predefined eligibility criteria, and only preclinical studies evaluating the effect of *T. terrestris* on ED-related outcomes in animal models were included.

**Results:** A total of 24 preclinical studies met the inclusion criteria. Approximately 83% of the studies reported significant improvement in erectile function, evidenced by increased intracavernosal pressure, enhanced sexual behaviour, and improved libido. Hormonal modulation was observed in about 92% of the studies, with increases in testosterone and gonadotropin levels. Activation of the nitric oxide cGMP pathway was reported in approximately 75% of the studies, while about 83% demonstrated antioxidant effects and preservation of penile or testicular tissue architecture. Additionally, nearly 96% of the studies reported a favourable safety profile for T. terrestris at therapeutic doses.

**Conclusion:** *T. terrestris* demonstrates promising multi-mechanistic proerectile effects in preclinical models of ED. However, well-designed human clinical trials are required to confirm its efficacy, safety, and clinical applicability.

## Abbreviations

ALP: Alkaline Phosphatase
ALT: Alanine Aminotransferase
AST: Aspartate Aminotransferase
BPA: Bisphenol A
cAMP: Cyclic Adenosine Monophosphate
CAT: Catalase
CC: Corpus Cavernosum
cGMP: Cyclic Guanosine Monophosphate
DHEA: Dehydroepiandrosterone
DHEAS: Dehydroepiandrosterone Sulphate
DHT: Dihydrotestosterone
ED: Erectile Dysfunction
eNOS: Endothelial Nitric Oxide Synthase
FSH: Follicle-Stimulating Hormone
GABA: Gamma-Aminobutyric Acid
GPx: Glutathione Peroxidase
GR: Glutathione Reductase
GSH: Glutathione
GST: Glutathione-S-Transferase
HPG: Hypothalamic-Pituitary-Gonadal
ICP: Intracavernosal Pressure
LAET: Lyophilized Aqueous Extract of Tribulus terrestris
LDH: Lactate Dehydrogenase
LH: Luteinizing Hormone
LPO: Lipid Peroxidation
MAP: Mean Arterial Pressure
MDA: Malondialdehyde
MF: Mount Frequency
ML: Mount Latency
NO: Nitric Oxide
NOS: Nitric Oxide Synthase
Nrf2: Nuclear Factor Erythroid 2-Related Factor 2
PDE5: Phosphodiesterase Type 5
PEI: Post-Ejaculatory Interval / Penile Erection Index
qRT-PCR: Quantitative Reverse Transcription Polymerase Chain Reaction
ROS: Reactive Oxygen Species
SOD: Superoxide Dismutase
TIMP-1: Tissue Inhibitor of Metalloproteinases-1

## INTRODUCTION

Erectile dysfunction (ED) is one of the most prevalent male sexual health disorders globally, characterized by the persistent inability to achieve or sustain an erection sufficient for satisfactory sexual performance (Kundu, 2008; Napolitano *et al*., 2022). Beyond its sexual connotations, ED is becoming more widely acknowledged as an early clinical indicator of cardiovascular disease, metabolic syndrome, and endothelial dysfunction (Müller and Mulhall, 2006). An estimated 40%-50% of 60-70 years old men worldwide are thought to experience ED in one form or another, and prevalence is expected to increase in the future (Delcea, 2019).

Conventional pharmacological management of ED has been revolutionized by phosphodiesterase type 5 (PDE5) inhibitors such as sildenafil, tadalafil, and vardenafil, which enhance cyclic guanosine monophosphate (cGMP) mediated smooth muscle relaxation in penile tissue, thereby improving erectile response (Rosen and Kostis, 2003). However, the high costs, systemic side effects, non-responsiveness in about 30% to 40% of patients, and contraindications, limit the therapeutic success of these agents (De Tejada, 2004). Additionally, PDE5 inhibitors are less effective in people with neurogenic, diabetic, or severe vasculogenic erectile dysfunction because they rely on intact nitric oxide (NO) signalling and sexual stimulation (De Tejada, 2004).

Plant-derived substances that may have aphrodisiac and restorative effects on sexual function have drawn attention in recent years (Mali *et al*., 2022). Among these, *Tribulus terrestris* L. (family Zygophyllaceae), also referred to as caltrop, gokshura, or puncture vine, has attracted a lot of attention from both scientists and ethnopharmacologists (Chhatre *et al*., 2014). It has been traditionally used to treat sexual weakness and increase virility in Ayurvedic, Chinese, and African medicine. It has also been reported in some preclinical studies to modulate androgenic activity, improve libido, and increase erectile capacity (Mahboubi, 2019).

It is believed that the bioactive components of the plant, mainly steroidal saponins (protodioscin, protogracillin, and dioscin), enhance the production of testosterone, the release of nitric oxide, and the relaxation of smooth muscle in corpus cavernosum tissue (Adaikan *et al*., 2001; Gauthaman and Ganesan, 2008). Additionally, *T. terrestris* has neuroprotective, vasodilatory, and antioxidant properties that could help erectile physiology even more (Do *et al*., 2013). Despite these encouraging characteristics, reported results are still inconsistent because of differences in experimental models, dosage, and extract preparation (Ștefănescu *et al*., 2020). Preclinical data clarifying the effectiveness and mechanisms of *T. terrestris* in the treatment of ED have not yet been compiled in a thorough systematic review. Therefore, this study systematically

analyzed the preclinical data currently available on *T. terrestris*, with an emphasis on the drug's efficacy, safety, and molecular mechanisms in relation to ED.

## MATERIALS AND METHODS

**Search Strategy**

This systematic review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Page *et al*., 2021). The review protocol was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD420251182856.

A comprehensive and systematic search strategy was developed to identify all relevant preclinical studies on *T. terrestris* in erectile dysfunction. The literature search was conducted on the 25th and 26th of October 2025 across multiple international databases, including PubMed/MEDLINE, Scopus, Web of Science, Cochrane Library, and Google Scholar. The search strategy combined both Medical Subject Headings (MeSH) and free text keywords related to *T. terrestris*, ED, and preclinical models. Boolean operators “AND” and “OR” were used to optimize retrieval sensitivity and specificity. The key search terms included combinations such as:

i. PubMed/MEDLINE

("*Tribulus terrestris*" OR "*T. terrestris*" OR "puncture vine") AND ("erectile dysfunction" OR “impotence” OR "male sexual dysfunction") AND ("animal model*" OR “preclinical” OR "in vivo" OR "in vitro" OR “rat” OR “rats” OR “mouse” OR “mice” OR “rabbit”*).

Similar search strings were used for other databases.

**Study Selection**

All records retrieved from the database searches were first screened independently by two authors (WCE and VOO) based on their titles and abstracts to assess relevance to the predefined eligibility criteria using the Rayyan software. Discrepancies were resolved by discussions through a third author, CEA. Studies that were clearly unrelated to *T. terrestris*, erectile dysfunction, or preclinical animal research were excluded at this stage. Full-text versions of potentially eligible articles were subsequently retrieved and carefully assessed by the same authors to determine their suitability for inclusion.

**Eligibility Criteria**

The eligibility criteria were defined using the Population, Intervention, Comparison, Outcome (PICO) framework, where P = animal models of ED, I = *Tribulus terrestris* (extracts, fractions, or isolated phytoconstituents), C = control groups receiving vehicle, placebo, or reference drugs (e.g., sildenafil), and O = erectile function or related outcomes including intracavernosal pressure, sexual behaviour parameters, hormonal indices, nitric oxide cGMP signalling, antioxidant markers, and histopathological changes.

Studies were selected if:

- They were preclinical *in vivo* studies using animal models of ED.
- Interventions involved *T. terrestris*, administered as whole plant extract, standardized fraction, or isolated bioactive constituent.
- They included appropriate control groups, such as vehicle-treated, untreated, or reference drug-treated controls.
- They reported outcomes related to erectile function, including physiological (e.g., intracavernosal pressure, sexual behaviour), biochemical (e.g., testosterone, NO, cGMP), or histological parameters.
- They were published in English.

Human clinical trials*, in vitro*-only or *ex vivo*-only studies, reviews, editorials, conference abstracts, and studies involving polyherbal formulations where the specific effects of *T. terrestris* could not be isolated were excluded.

**Data Extraction**

Data extraction was performed systematically using a predesigned extraction form developed in Microsoft Excel. For each eligible study, bibliographic information such as the author’s name, publication year, and country of origin was documented. Information regarding experimental design included the animal species used, sample size, model of ED, and duration of the intervention. Details of the administered preparation, including the extract type (aqueous, ethanol, methanol, or standardized), dosage, route of administration, and frequency, were carefully extracted.

Efficacy outcomes recorded included physiological measures such as intracavernosal pressure (ICP), penile erection index, mounting frequency, and latency to intromission. Biochemical and mechanistic markers such as nitric oxide (NO), cyclic guanosine monophosphate (cGMP), testosterone, luteinizing hormone (LH), and oxidative stress indices (malondialdehyde, superoxide dismutase, glutathione peroxidase) were also extracted. Furthermore, histological findings such as smooth muscle to collagen ratio, endothelial thickness, or evidence of fibrosis were included to correlate functional improvements with structural restoration of penile tissue. Safety and toxicity parameters, including changes in liver or kidney histology and serum enzyme levels, were also recorded where available.

**Data Synthesis**

Due to the heterogeneity in animal species, experimental models of erectile dysfunction, *T. terrestris* preparations, dosage regimens, and outcome measures across the included studies, a quantitative meta-analysis was not feasible. Therefore, a qualitative synthesis of the findings was conducted. The results were organized thematically to summarize the effects of *T. terrestris* on erectile function-related outcomes, including intracavernosal pressure, sexual behaviour parameters, hormonal indices (such as testosterone and gonadotropins), NO cGMP signalling, antioxidant activity, and histopathological changes in penile and testicular tissues. This approach enabled the identification of consistent trends, mechanistic patterns, and variations in outcomes across different experimental models.

**Quality Assessment**

The quality of the included animal studies was assessed using the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines 2.0, which evaluate the transparency, completeness, and methodological quality of reporting in preclinical animal research. This assessment was conducted by two independent authors (WCE and VOO), while discrepancies were resolved by the third author, CEA. Each included study was appraised based on key ARRIVE 2.0 domains, including clarity of study objectives, description of animal characteristics, experimental design, housing and husbandry conditions, ethical approval, outcome measurement, data analysis, and interpretation of results. The overall methodological quality of each study was determined by assessing the extent to which these reporting criteria were adequately addressed. Studies were categorized descriptively based on their level of compliance with the ARRIVE guidelines, and all included studies were retained for qualitative synthesis.

## RESULTS

**Study Selection**

The literature search identified a total of 1,563 records across all databases. These included 99 records from Web of Science, 998 from Google Scholar, 304 from Scopus, 13 from PubMed/MEDLINE, and 149 from Cochrane Library. Prior to screening, 195 records were removed as duplicates or ineligible. The remaining 1,368 records were subjected to title and abstract screening based on the predefined inclusion and exclusion criteria.

Following the title and abstract screening, 1,210 records were excluded. A total of 158 full-text articles were assessed for eligibility. Of these, 134 articles were excluded for various reasons, including outcomes not related to erectile dysfunction mechanisms (n=54), studies that were not preclinical (n=26), review articles, meta-analyses, or non-primary research (n=22), studies not investigating *T. terrestris* (n=16), unavailability of full text or publication in languages other than English (n=12), and clinical or human studies (n=4). Ultimately, 24 preclinical studies met all eligibility criteria and were included in this systematic review. These studies formed the basis for qualitative synthesis of the efficacy, safety, and mechanisms of action of *T. terrestris* in animal models of ED (Figure 1).

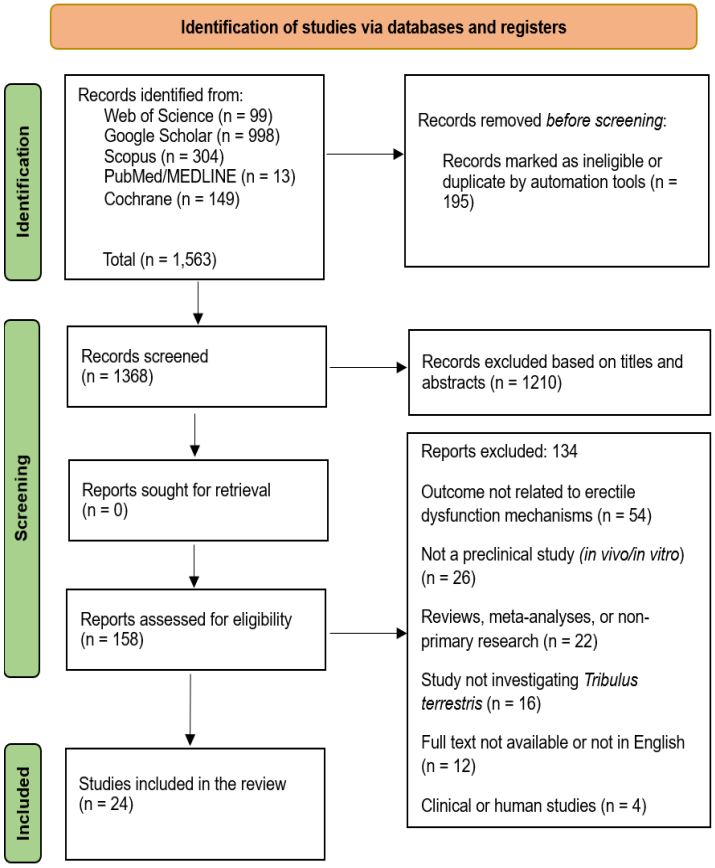


**Figure 1:** ***PRISMA Flowchart of Included Studies.***

**Characteristics of Included Studies**

The majority of the studies were conducted in Asia (n=14, 58.3%) (Al-Obaidi *et al*., 2025; Al-Yawer *et al*., 2008; Do *et al*., 2013; Gauthaman *et al*., 2002, 2003; Gauthaman and Ganesan, 2008; Ghosian Moghaddam *et al*., 2013; Kam *et al*., 2012; Keshtmand *et al*., 2014; Kumar *et al*., 2023; M. Kumari and Singh, 2015; Malekzadeh *et al*., 2024; Meybodi *et al*., 2024; Mohammed *et al*., 2013; Singh *et al*., 2012; Singh and Gupta, 2011; Tyagi *et al*., 2008; Zahra Mohammed Hashim *et al*., 2024), followed by Middle East (n=7, 29.2%) (Aldaddou *et al*., 2022; Al-Obaidi *et al*., 2025; Ghosian Moghaddam *et al*., 2013; Hend *et al*., 2015; Keshtmand *et al*., 2014; Munir *et al*., 2017; Zhang *et al*., 2019), Europe (n=1, 4.2%) (Ștefănescu *et al*., 2021), Africa (n=1, 4.2%) (Ștefănescu *et al*., 2021), and South America (n=1, 4.2%) (Da Silva *et al*., 2024).

Male animals were used in all studies (n=24, 100%). Wistar rats were the most commonly used species (n=14, 58.3%)

(Aldaddou *et al*., 2022; Da Silva *et al*., 2024; Gauthaman *et al*., 2002, 2003; Ghosian Moghaddam *et al*., 2013; Hend *et al*., 2015; Kumar *et al*., 2023; Malekzadeh *et al*., 2024; Mohammed *et al*., 2013; Munir *et al*., 2017; Singh *et al*., 2012a; Singh and Gupta, 2011; Ștefănescu *et al*., 2021; Tyagi *et al*., 2008). while a limited number of studies utilized rabbits (n=2, 8.3%) (Do *et al*., 2013; Kam *et al*., 2012) or primates (n=1, 4.2%) (Gauthaman and Ganesan, 2008).

A wide range of experimental models was used to induce erectile or reproductive dysfunction. Diabetic models predominated, accounting for approximately 41.7% (n=10) of the included studies. Other studies employed chemotherapy agents or toxicant-induced models, including cyclophosphamide, cisplatin, bisphenol A (BPA), cytarabine, and metronidazole (n=5, 20.8%) (Al-Obaidi *et al*., 2025; Keshtmand *et al*., 2014; M. Kumari and Singh, 2015; Munir *et al*., 2017; Hashim *et al*., 2024), hormonal suppression models including castration (n=4, 16.7%) (Gauthaman *et al*., 2002; Gauthaman & Ganesan, 2008; Tyagi *et al*., 2008), chronic drug exposure models including morphine and fluoxetine (n=2, 8.3%) (Ghosian Moghaddam *et al*., 2013; Kumar *et al*., 2023), aging-related models (n=1, 4.2%) (Singh *et al*., 2012).

Regarding sample sizes, the majority employed 5-10 animals per group (n=18, 75%), while fewer studies used smaller (n=2, 8.3%) or larger sample sizes (n=4, 16.7%). Treatment duration ranged from single dose administration (n=2, 8.3%) to 12 weeks (n=1, 4.2%), with the most common durations being 4 weeks (n=6, 25%), 8 weeks (n=4, 16.7%), and 60 days (n=3, 12.5%).

**Extract Type, Dose, and Route of Administration**

Table 2 summarizes the intervention characteristics across the 24 included studies. Regarding extract types, ethanol extracts were the most used preparation (n=15, 62.5%), with concentrations ranging from 70% to 90%. Hydroalcohol extracts (70%) were employed in three studies (12.5%), while aqueous extracts were used in four studies (16.7%). Standardized extracts, particularly those standardized for protodioscin content, were utilized in four studies (16.7%). Other preparations included powder form (n=2, 8.3%), dietary admixture (n=1, 4.2%), commercial supplements (n=1, 4.2%), and furostenol glycoside extracts (n=1, 4.2%).

Dosage regimens varied widely across studies, ranging from as low as 2 mg/kg/day to as high as 500 mg/kg/day. The most used dose ranges were 5-10 mg/kg/day (n=6, 25%), 50-100 mg/kg/day (n=8, 33.3%), and 100-200 mg/kg/day (n=5, 20.8%). Lower doses of 2-2.5 mg/kg/day were employed in three studies (12.5%), while higher doses of 200-500 mg/kg/day were used in three studies (12.5%). The route of administration was predominantly oral (n=22, 91.7%), with only two studies (8.3%) utilizing intraperitoneal injection and one study employing both intravenous and oral routes (4.2%).

**Outcome Parameters**

Outcome parameters assessed varied across studies. Hormonal parameters were evaluated in most studies (n=20, 83.3%), with testosterone being the most frequently measured hormone (n=19, 79.2%), followed by luteinizing hormone (LH) (n=9, 37.5%) and follicle-stimulating hormone (FSH) (n=10, 41.7%). Androgen receptor (AR) expression was assessed in one study (n=1, 4.2%).

Erectile function parameters were measured in twelve studies (n=12, 50%), including intracavernosal pressure to mean arterial pressure ratio (ICP/MAP) (n=6, 25%), corpus cavernosum relaxation (n=4, 16.7%), and penile erection index (n=3, 12.5%). Cyclic nucleotide levels, including cGMP and cAMP, were evaluated in five studies (n=5, 20.8%).

Sperm parameters were assessed in nineteen studies (79.2%), with sperm count being the most frequently evaluated parameter (n=16, 66.7%), followed by sperm motility (n=14, 58.3%), viability (n=9, 37.5%), and morphology/abnormalities (n=5, 20.8%).

Oxidative stress markers were evaluated in twelve studies (n=12, 50%), including malondialdehyde (MDA) (n=5, 20.8%), superoxide dismutase (SOD) (n=5, 20.8%), catalase (CAT) (n=5, 20.8%), glutathione (GSH) (n=3, 12.5%), and glutathione peroxidase (GPx) (n=2, 8.3%). Nitric oxide (NO) levels were measured in four studies (16.7%).

Histopathological evaluations were conducted in seventeen studies (70.8%), assessing testicular architecture (n=13, 54.2%), seminiferous tubule organization (n=10, 41.7%), Leydig and Sertoli cell counts (n=6, 25%), penile tissue structure (n=2, 8.3%), and smooth muscle to collagen ratio (n=1, 4.2%).

Sexual behavior parameters were evaluated in seven studies (29.2%), including mounting frequency (MF), intromission frequency (IF), mounting latency (ML), intromission latency (IL), ejaculatory latency (EL), and penile erection index (PEI).

Additional parameters assessed included body and organ weights (n=6, 25%), liver enzymes (n=3, 12.5%), blood glucose levels (n=3, 12.5%), and reproductive organ weights (n=4, 16.7%).

**Table 1: General Characteristics of Included Preclinical Studies**

| Author & Year | Country | Animal Species and Sex | Sample Size (n) | ED Model | Treatment Duration |
|---|---|---|---|---|---|
| Stefanescu *et al*., 2021 | Romania | Male Wistar rats | 10/group | Diabetic (STZ) | 12 weeks |
| Do *et al*., 2013 | Korea | Male rabbits/SD rats | 8/group | Healthy | 1 month |
| Tag *et al*., 2015 | Egypt | Male Wistar rats | 6/group | Diabetic (STZ) | 60 days |
| Mohseni Meybodi *et al*., 2024 | Iran | Male BALB/c mice | 6/group | Healthy | 55 days |
| Da Silva *et al*., 2024 | Brazil | Male Wistar Kyoto rats | 10/group | Healthy | 40 days |
| Ghosian Moghaddam *et al*., 2013 | Iran | Male Wistar rats | 12/group | Morphine-induced | 4 weeks |
| Al-Yawer *et al*., 2008 | Iraq | Male mice | 20 (experiment), 10 (control) | Healthy | 14 days |
| Al-Obaidi *et al*., 2025 | Iraq | Male albino mice | 4/group | Cyclophosphamide | 14 days |
| Keshtmand *et al*., 2014 | Iran | Male BALB/c mice | 6/group | Cisplatin | 4 days |
| Munir *et al*., 2017 | Pakistan | Male Wistar rats | 8/group | BPA-induced | 4 weeks |
| Hashim *et al*., 2025 | Iraq | Male albino rats | 5/group | Cytarabine | 28 days |
| Zhang *et al*., 2019 | China | Male SD rats | 6/group | Type 2 Diabetic ED | 4 weeks |
| Kumari and Singh, 2015 | India | Male Swiss mice | 5/group | Metronidazole | 28 days |
| Singh *et al*., 2012 | India | Male Wistar rats | 6/group | Aged/sexually sluggish | 14 days |
| Tyagi *et al*., 2008 | India | Male Wistar rats | 6/group | Castration | 14 days |
| Gauthaman *et al*., 2002 | Singapore | Male SD rats | 8/group | Castration | 8 weeks |
| Gauthaman *et al*., 2003 | Singapore | Male SD rats | 10/group | Healthy | 8 weeks |
| Mohammed *et al*., 2013 | Sudan | Male Wistar rats/Cocks | NR | Healthy | 4 weeks |
| Kumar *et al*., 2023 | India | Male Wistar rats | 6/group | Fluoxetine/STZ diabetic | 8 weeks |
| Malekzadeh *et al*., 2024 | Iran | Male Wistar rats | 7/group | Healthy | 60 days |
| Kam *et al*., 2012 | South Korea | Male rabbits/SD rats | 8/group | Healthy | 1 month |
| Singh and Gupta, 2011 | India | Male Wistar rats | 6/group | Healthy/sluggish | Single dose |
| Aldaddou *et al*., 2022 | Saudi Arabia | Male albino rats | 6/group | Nicotine/Lead-induced | 60 days |
| Gauthaman and Ganesan, 2008 | Singapore | Male primates/rabbits/rats | Variable | Castration/Healthy | Single dose to 8 weeks |

**Table 2: Intervention Characteristics and Outcome Parameters of Included Preclinical Studies**

| Author & Year | Extract Type | Dose (mg/kg/day) | Route | Outcome Parameters Assessed |
|---|---|---|---|---|
| Stefanescu *et al*., 2021 | Ethanolic (70%) | 25 | Oral | Blood glucose, insulin, testosterone, LH, FSH, sperm morphology |
| Do *et al*., 2013 | Ethanolic (90%) | 2.5-100 | Oral | ICP/MAP ratio, CC relaxation, cAMP, cGMP |
| Tag *et al*., 2015 | Aqueous | 10 | Oral | Blood glucose, testosterone, FSH, sperm count/viability, MDA, GSH, CAT, GST, testicular histology |
| Mohseni Meybodi *et al*., 2024 | Ethanolic | 5-320 | Oral | Testosterone, AR expression, sperm count/motility/viability |
| Da Silva *et al*., 2024 | Commercial supplement | 100 | Oral | Sperm concentration/motility/viability, testicular morphometry |
| Ghosian Moghaddam *et al*., 2013 | Dietary admix | ~6.25% diet | Oral | LH, FSH, testosterone, estrogen, progesterone |
| Al-Yawer *et al*., 2008 | Powder | 2 | Oral | Testicular histology, Leydig cell count, spermatogenic activity |
| Al-Obaidi *et al*., 2025 | Aqueous | NR | IP | Testosterone, liver enzymes (ALT, AST, ALP), testicular/hepatic histology |
| Keshtmand *et al*., 2014 | Hydroalcoholic (70%) | 100-500 | IP | Body/testis weight, sperm count/motility/viability, NO, tubular diameter |
| Munir *et al*., 2017 | Powder | 20 | Oral | Body/testis weight, testosterone, tubular diameter, Leydig cells, Johnsen's score |
| Hashim *et al*., 2025 | Ethanolic (70%) | 250 | Oral | Liver enzymes, MDA, SOD, CAT, testosterone, FSH, LH, 17β-HSD, sperm motility/integrity/vigor, caspase |
| Zhang *et al*., 2019 | Standardized saponins | 40 | Oral | ICP/MAP ratio, NO, ROS, cGMP, eNOS, TIMP-1, caspase-3/9, smooth muscle/collagen ratio, apoptotic index |
| Kumari and Singh, 2015 | Aqueous | 100-200 | Oral | Body/testis weight, sperm count, testosterone, SOD, CAT, GPx, GR, LPO, LDH, ALP, testicular histology |
| Singh *et al*., 2012 | Aqueous (lyophilized) | 50-100 | Oral | Sexual behavior (mount/intromission latency & frequency, ejaculatory latency), penile erection index, testosterone, sperm count, toxicity markers |
| Tyagi *et al*., 2008 | Furostenol glycoside | 5-25 | Oral | Sexual behavior (MF, IF, ML, IL, EL, PEI), testosterone |
| Gauthaman *et al*., 2002 | Standardized (protodioscin) | 5 | Oral | Sexual behavior (MF, IF, ML, IL, EL, PEI), ICP, prostate weight |
| Gauthaman *et al*., 2003 | Standardized (protodioscin) | 2.5-10 | Oral | Sexual behavior (MF, IF, ML, IL, EL, PEI), ICP |
| Mohammed *et al*., 2013 | Ethanolic | 7.5 | Oral | Testosterone, semen volume/concentration/motility (cocks), testicular histology |
| Kumar *et al*., 2023 | Ethanolic | 100 | Oral | Libido, CC relaxation (ex vivo), blood glucose, sperm density/motility/viability |
| Malekzadeh *et al*., 2024 | Hydroalcoholic (70%) | 2.5-10 | Oral | Testosterone, FSH, LH, Sertoli/Leydig/spermatid cell counts, sperm count/motility |
| Kam *et al*., 2012 | Extract mixture | 2.5-100 | Oral | ICP/MAP ratio, cAMP, cGMP, CC relaxation (in vitro) |
| Singh & Gupta, 2011 | Lyophilized powder | 100 | Oral | Sexual behavior, penile erection index, testosterone, sperm count, body/organ weight, toxicity markers |
| Aldaddou *et al*., 2022 | Methanolic | 50-100 | Oral | Testosterone, LH, sperm count/motility/abnormalities, reproductive organ weights, testicular histology |
| Gauthaman and Ganesan, 2008 | Standardized saponins | 2.5-30 | IV/Oral | Testosterone, DHT, DHEAS, blood pressure, ECG |

**Overview of the Therapeutic Effects of *T. terrestris***

Results from the included studies provide an overview of the therapeutic effects and safety profile observed across the 24 included studies. The therapeutic outcomes were categorized into six major domains: erectile function, hormonal parameters, sperm parameters, oxidative stress markers, histopathology, and safety profile.

**Erectile Function**

Regarding erectile function outcomes, twelve studies (50%) directly measured erectile function parameters. Among these, increased intracavernosal pressure to mean arterial pressure ratio (ICP/MAP) was reported in six studies (25%), improved corpus cavernosum (CC) relaxation was demonstrated in three studies (12.5%), and enhanced penile erection index was observed in three studies (12.5%). Increased sexual behavior parameters including mounting frequency, intromission frequency, and reduced latencies were documented in five studies (20.8%). Twelve studies (50%) did not directly measure erectile function outcomes but focused on other reproductive parameters.

**Hormonal Parameters**

Improvements in hormonal parameter were among the most consistent findings, with twenty-two studies (91.7%) reporting significant increases in one or more hormonal markers. Specifically, increased testosterone levels were observed in nineteen studies (79.2%), elevated luteinizing hormone (LH) in eight studies (33.3%), increased follicle-stimulating hormone (FSH) in nine studies (37.5%), enhanced androgen receptor (AR) expression in one study (4.2%), and increased levels of other androgens including dihydrotestosterone (DHT) and dehydroepiandrosterone sulfate (DHEAS) in one study (4.2%). Two studies (8.3%) did not measure hormonal parameters.

**Sperm Parameters**

Sperm parameter improvements were documented in nineteen studies (79.2%), while five studies (20.8%) did not assess sperm-related outcomes. Among those that evaluated sperm parameters, increased sperm count was reported in fifteen studies (62.5%), improved sperm motility in eleven studies (45.8%), enhanced sperm viability in seven studies (29.2%), improved sperm morphology or reduced abnormalities in five studies (20.8%), increased sperm integrity in two studies (8.3%), and enhanced sperm vigor in one study (n=1, 4.2%). Two studies (8.3%) reported no significant change in sperm parameters despite other positive outcomes.

**Oxidative Stress Markers**

Oxidative stress markers were evaluated in twelve studies (50%), with all reporting favorable antioxidant effects. Decreased malondialdehyde (MDA) levels, indicating reduced lipid peroxidation, were observed in five studies (20.8%). Increased activities of endogenous antioxidant enzymes were reported, including superoxide dismutase (SOD) in five studies (20.8%), catalase (CAT) in five studies (20.8%), glutathione (GSH) in three studies (12.5%), glutathione-S-transferase (GST) in two studies (8.3%), glutathione peroxidase (GPx) in two studies (8.3%), and glutathione reductase (GR) in one study (4.2%). Additionally, decreased reactive oxygen species (ROS) were reported in one study (4.2%), increased nitric oxide (NO) in two studies (8.3%), increased cyclic guanosine monophosphate (cGMP) in two studies (8.3%), increased endothelial nitric oxide synthase (eNOS) in one study (4.2%), and decreased caspase activity indicating reduced apoptosis in two studies (8.3%). Twelve studies (50%) did not measure oxidative stress markers.

**Histopathological Assessments**

Histopathological improvements were documented in seventeen studies (70.8%), while seven studies (29.2%) did not conduct histological assessments. Among those that performed histopathological evaluations, restoration or improvement of testicular architecture and seminiferous tubule organization was the most common finding, reported in thirteen studies (54.2%). Increased or preserved tubular diameter was observed in seven studies (29.2%), enhanced spermatogenesis in five studies (20.8%), increased Leydig cell populations in five studies (20.8%), increased Sertoli cell populations in two studies (8.3%), improved epithelial height in one study (4.2%), increased smooth muscle to collagen ratio in penile tissue in one study (4.2%), decreased apoptotic index in one study (4.2%), and hepatoprotective effects in two studies (8.3%).

**Mechanistic Pathways and Efficacy of *T. terrestris* in ED**

Table 3 provides a comprehensive overview of the mechanisms of action, key efficacy findings, and safety outcomes reported across the 24 included preclinical studies. The mechanisms of action reported across the included studies were diverse and multifaceted. Antioxidant activity was the most frequently reported mechanism, identified in 12 studies (50%), often through modulation of oxidative stress markers or protection against toxin-induced damage. Androgenic stimulation involving the hypothalamic pituitary gonadal (HPG) axis was reported in 8 studies (33.3%), either through direct hormonal measurements or gonadotropin modulation. Nitric oxide (NO) related mechanisms, including endothelium-dependent NO release, eNOS activation, and cyclic nucleotide signaling (cAMP and/or cGMP), were reported in 7 studies (29.2%), primarily in erectile function and corpus cavernosum models. Additional mechanisms included anti-apoptotic effects (n=3, 12.5%), steroidogenic enzyme or androgen receptor upregulation (n=3, 12.5%),

anti-fibrotic activity (n=1, 4.2%), and antagonism of drug-induced HPG axis suppression (n=2, 8.3%).

Notably, 6 studies (25%) did not directly investigate molecular mechanisms but inferred androgenic or reproductive effects based on physiological, hormonal, or histological outcomes.

Hormonal effects, particularly increases or restoration of serum testosterone, were the most consistently reported findings, observed in 22 studies (91.7%). Improvements in sperm parameters, including sperm count, motility, viability, and morphology, were documented in 19 studies (79.2%).

Restoration or improvement of testicular or penile tissue architecture, such as seminiferous tubule thickness, epithelial height, Leydig or Sertoli cell numbers, and smooth muscle collagen balance, was reported in 17 studies (70.8%). Erectile function and sexual behaviour improvements, including increased intracavernosal pressure (ICP or ICP/MAP ratio), enhanced corpus cavernosum relaxation, and improved copulatory behavior indices, were reported in 12 studies (50%). Additional benefits included reduction of oxidative stress markers (n=12, 50%), hepatoprotective effects (n=2, 8.3%), and restoration of hormonal balance in drug suppressed models (n=2, 8.3%). Importantly, one study reported no significant changes in sperm concentration, motility, or viability, despite observing significant improvements in testicular histomorphometry.

**Table 3: Summary of mechanistic pathways, key efficacy findings, and safety outcomes.**

| Author & Year | Mechanism of Action | Key Efficacy Findings | Safety/Toxicity |
|---|---|---|---|
| Stefanescu *et al*., 2021. | FSH linked pathway; antioxidant activity via polyphenols. | High protodioscin extract significantly increased testosterone and improved sperm morphology compared with control ($P$<0.05). | No adverse effects; well tolerated at 25 mg/kg over 12 weeks. |
| Do *et al*., 2013. | Endothelium dependent NO release; cAMP elevation. | *T. terrestris* extract produced significant concentration-dependent relaxation of corpus cavernosum smooth muscle in vitro ($P$<0.05) and significantly increased intracavernous pressure (ICP/MAP) in vivo after one month of oral administration ($P$<0.05). cAMP levels were significantly elevated ($P$<0.05), while cGMP levels did not change significantly. | Not reported |
| Tag *et al*., 2015. | Antioxidant (increased MDA, increased GSH/CAT/GST); androgenic stimulation. | Treatment significantly restored testicular architecture and increased sperm count by 2.73-fold compared with diabetic control ($P$<0.05). | No adverse effects; comparable safety to metformin. |
| Mohseni Meybodi *et al*., 2024. | Upregulation of androgen receptor (AR) gene expression. | Testosterone, sperm count, motility, and viability were significantly increased at 40-80 mg/kg ($P$<0.05), with AR gene expression elevated at 80 mg/kg ($P$<0.05). | No adverse effects; body weight increase correlated with dosing. |
| Da Silva *et al*., 2024. | Hypothesized androgenic support. | *T. terrestris* treatment did not significantly change sperm concentration, motility, or viability compared with controls. Seminiferous epithelium height was significantly higher in treated rats ($P$=0.0069), and tubular lumen surface density was significantly increased ($P$=0.0435). | No adverse effects. |

**Table 3: Summary of mechanistic pathways, key efficacy findings, and safety outcomes (Cont'd)**

| | | | |
|---|---|---|---|
| Ghosian Moghaddam *et al*., 2013. | Antagonism of morphine induced HPG axis suppression. | Morphine addiction significantly reduced LH compared to controls ($P$<0.027). *T. terrestris* treatment in addicted rats significantly increased LH ($P$<0.031). Testosterone was significantly reduced in the addicted group ($P$<0.001) and significantly increased with *T. terrestris* treatment ($P$<0.05). Estrogen was significantly lower in addicted rats ($P$<0.002) and significantly increased after *T. terrestris* ($P$<0.048). Progesterone was significantly increased in *T. terrestris*-treated controls ($P$<0.002). FSH was not significantly affected. | Not assessed. |
| Al-Yawer *et al*., 2008. | LH mediated Leydig cell stimulation. | *T. terrestris* significantly increased seminiferous tubule thickness ($P$<0.004) and the number of interstitial Leydig cells $P$<0.000) compared with control. | No adverse effect. |
| Al-Obaidi *et al*., 2025. | Antioxidant activity (flavonoids and saponins); hepatoprotective effects | Cyclophosphamide elevated liver enzymes and reduced testosterone ($P$<0.05). *T. terrestris* alone maintained normal values and partially prevented enzyme elevation and testosterone loss when co-administered ($P$<0.05). | TT alone showed mild hepatic changes (vascular degeneration and necrosis); co-treatment with cyclophosphamide provided protection. |
| Keshtmand *et al*., 2014. | Antioxidant activity; protection against cisplatin-induced cytotoxicity. | *T. terrestris* hydroalcohol extract significantly increased sperm count, sperm viability, and total and progressive motility compared with the cisplatin group ($P$<0.05). Normal sperm percentage was also significantly higher in treated groups versus cisplatin ($p < 0.05$). Serum nitric oxide did not change significantly. | No adverse effects at tested doses. |
| Munir *et al*., 2017. | Antioxidant activity countering BPA induced oxidative stress. | BPA (bisphenol A) significantly reduced testosterone, seminiferous tubule diameter, Leydig cell number, and Johnsen's score versus control ($P$<0.001). *T. terrestris* co-treatment restored these parameters toward normal levels ($P$<0.001 vs BPA). | TT alone (20 mg/kg) showed no adverse effects. |
| Hashim *et al*., 2025. | Antioxidant / protection against cytarabine-induced reproductive toxicity. | *T. terrestris* significantly restored testosterone, FSH, and LH levels and improved sperm motility after cytarabine exposure ($P$<0.05); liver enzymes were significantly reduced ($P$<0.05). | No adverse effects. |

**Table 3: Summary of mechanistic pathways, key efficacy findings, and safety outcomes (Cont'd)**

| | | | |
|---|---|---|---|
| Zhang *et al*., 2019. | NO/cGMP pathway activation; antioxidant; anti-fibrotic; anti-apoptotic. | Gross saponins of *T. terrestris* significantly increased ICP, ICP/MAP, NO, eNOS, smooth muscle/collagen ratio, and cGMP, and decreased ROS, TIMP-1, and apoptosis compared with diabetic controls ($P$<0.05). Combination with sildenafil further improved cGMP ($P$<0.05). | No adverse effects; synergistic effect with sildenafil. |
| Kumari and Singh, 2015**.** | Antioxidant activity (increased SOD/CAT/GPx/GR, increased LPO). | Metronidazole significantly decreased testicular weight and epididymal sperm count ($P$<0.05), and co-administration with high-dose *T. terrestris* extract (200 mg/kg) completely restored testicular histology and sperm count to control-like levels ($P$<0.05). | No adverse effects. |
| Singh *et al*., 2012. | HPG axis mediated androgenic stimulation. | Chronic administration of *T. terrestris* lyophilized aqueous extract (LAET) produced a significant, dose-dependent increase in mount frequency, intromission frequency, and penile erection index, and significantly decreased mount, intromission, and ejaculation latencies ($P$<0.05) compared with control. Chronic LAET significantly increased serum testosterone ($P$<0.05). Sperm count was not significantly affected. | No toxicity at 500 mg/kg for 28 days; no organ or hematological changes. |
| Tyagi *et al*., 2008. | Protodioscin to DHEA to testosterone conversion. | In castrated rats, *T. terrestris* extract significantly increased intracavernous pressure (ICP) and prostate weight versus castrated control ($P$<0.05) and significantly improved sexual behaviour parameters, including mount and intromission frequencies and decreased latencies ($P$<0.05). | No adverse effects. |
| Gauthaman *et al*., 2002. | Androgenic pathway (DHEA/testosterone/DHT); NO release from nerve endings. | Sexual behaviour parameters and intracavernosal pressure were significantly increased by 28% compared with control in castrated rats ($P$<0.05). | No adverse effects. |
| Gauthaman *et al*., 2003. | Androgenic stimulation; nitric oxide mediated pro-erectile effects. | Sexual behaviour and intracavernosal pressure were significantly improved by 26-43% at 5-10 mg/kg compared with control ($P$<0.05). | No adverse effects; mild, non-significant increase in blood pressure. |
| Mohammed *et al*., 2013. | Phytochemical-driven androgenic support. | There was a significant increase in serum testosterone in rats treated with TT ethanol extract at 7.5 mg/kg, 54.6% higher after 2 weeks and 101.7% higher after 4 weeks ($P$<0.05) compared with control values. In treated cocks, sperm concentration increased significantly ($P$<0.05), while increases in mass and individual sperm motility and rat testosterone were not statistically significant. Histopathology showed enhanced spermatogenesis. | No adverse effects. |

**Table 3: Summary of Mechanistic Pathways, Key Efficacy Findings, and Safety Outcomes (Cont'd)**

| | | | |
|---|---|---|---|
| Kumar *et al*., 2023. | Endothelial NO pathway improvement. | *TT* extract significantly improved corpus cavernosum relaxation, sperm parameters, and libido in diabetic and fluoxetine treated rats compared with control ($P$<0.05). | Not reported. |
| Malekzadeh *et al*., 2024. | HPG axis stimulation; testicular stimulation. | *T. terrestris* extract significantly increased sperm count and motility at 5-10 mg/kg ($P$<0.05), increased serum testosterone at 2.5 mg/kg ($P$<0.05) and FSH at 10 mg/kg ($P$<0.05), and increased Sertoli and Leydig cell counts compared with control. LH changes were not significant. | No adverse effects. |
| Kam *et al*., 2012. | Endothelium dependent NO/cAMP pathway; smooth muscle relaxation. | ICP/MAP ratio and corpus cavernosum relaxation were significantly increased in a dose-dependent manner, with maximal effect at 50 mg/kg ($P$<0.05). | Not reported. |
| Singh and Gupta, 2011. | Gonadotropin-mediated androgenic action | A single dose significantly improved all sexual behaviour parameters, testosterone, and sperm count compared with control ($P$<0.05). | No toxicity; confirmed traditional use as aphrodisiac. |
| Aldaddou *et al*., 2022. | Antioxidant activity; bioactive compounds (diosgenin, phytol). | TT extract protected against nicotine and lead toxicity. There was a significant improvement ($P$<0.05) in sperm count, motility, and morphology, as well as sexual organ weights (testis, epididymis, seminal vesicle, prostate) compared to the nicotine + lead control group. Exact values were not reported. | TT alone showed no adverse effects; mild interstitial edema in some treated groups. |
| Gauthaman and Ganesan, 2008. | Protodioscin-mediated androgen synthesis. | In primates, *T. terrestris* significantly increased testosterone (52%), dihydrotestosterone (31%), and DHEAS (29%) at 7.5 mg/kg compared with baseline ($P$<0.05). In rabbits, dihydrotestosterone was significantly elevated at 5-10 mg/kg ($P$<0.05). | Minimal transient cardiovascular changes (mild BP drop and tachycardia in primates); no significant ECG abnormalities. |

**Evaluation of Quality of the Included Studies**

Quality assessment for the included studies was conducted using the ARRIVE assessment tool 2.0. Full percentages (100%) were recorded for the evaluation of the abstract, background, objectives, interpretation/scientific implications, and generalizability/translation. A score of 75% was recorded for both the ethical statement and housing and husbandry. Less than two-thirds (58.33%) of the studies provided adequate reporting of animal care and monitoring. Additionally, 70.83% of the studies provided a declaration of conflict of interest. Less than half (4.17%) of the studies provided access to the data used in the research. None of the studies reported protocol registration.

Overall, most studies demonstrated acceptable methodological rigor, particularly in outcome measurement and experimental consistency. However, variations were noted in areas such as randomization procedures, blinding, and reporting of allocation concealment, which are common limitations in animal studies. Despite these methodological differences, the consistency of findings across independent studies strengthens confidence in the reliability of the observed effects. A summary of the quality assessment evaluation for each included study is provided in Table 4.

**Table 4: Quality Assessment Using the ARRIVE Tool 2.0**

| Authors and Publication year | Abstract | Background | Objectives | Ethical statement | Housing and Husbandry | Animal Care and Monitoring | Interpretation / scientific implications | Generalizability /Translation | Protocol registration | Data access | Declaration of interest |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Aldaddou *et al*., 2022. | Y | Y | Y | Y | Y | Y | Y | Y | N | N | Y |
| Kumar *et al*., 2023. | Y | Y | Y | Y | N | N | Y | Y | N | N | Y |
| Mohammed *et al*., 2013. | Y | Y | Y | N | N | N | Y | Y | N | N | N |
| Singh and Gupta, 2011. | Y | Y | Y | Y | N | N | Y | Y | N | N | Y |
| Kam *et al*., 2012. | Y | Y | Y | Y | N | N | Y | Y | N | N | Y |
| Zhang *et al*., 2019. | Y | Y | Y | Y | Y | Y | Y | Y | N | N | Y |
| Kumari and Singh, 2015. | Y | Y | Y | Y | Y | Y | Y | Y | N | N | N |
| Singh *et al*., 2012. | Y | Y | Y | Y | Y | Y | Y | Y | N | N | Y |
| Tyagi *et al*., 2008. | Y | Y | Y | Y | Y | N | Y | Y | N | N | N |
| Gauthaman *et al*., 2002. | Y | Y | Y | N | Y | Y | Y | Y | N | N | N |
| Gauthaman and Ganesan, 2008 | Y | Y | Y | Y | N | Y | Y | Y | N | N | N |
| Da Silva *et al*., 2024 | Y | Y | Y | Y | Y | Y | Y | Y | N | N | Y |

**Table 4: Quality Assessment Using the ARRIVE Tool 2.0 (Cont'd)**

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Ghosian Moghaddam *et al*., 2013. | Y | Y | Y | Y | Y | N | Y | Y | N | N | Y |
| Al-Yawer *et al*., 2008. | Y | Y | Y | N | Y | N | Y | Y | N | N | N |
| Keshtmand *et al*., 2014. | Y | Y | Y | Y | Y | N | Y | Y | N | N | Y |
| Al-Obaidi *et al*., 2025. | Y | Y | Y | N | Y | N | Y | Y | N | N | Y |
| Munir *et al*., 2017. | Y | Y | Y | N | Y | Y | Y | Y | N | N | N |
| Hashim *et al*., 2025. | Y | Y | Y | Y | Y | N | Y | Y | N | N | Y |
| Gauthaman *et al*., 2003. | Y | Y | Y | N | Y | Y | Y | Y | N | N | Y |
| Do *et al*., 2013. | Y | Y | Y | Y | N | Y | Y | Y | N | N | Y |
| Tag *et al*., 2015. | Y | Y | Y | Y | Y | Y | Y | Y | N | N | Y |
| Stefanescu *et al*., 2021. | Y | Y | Y | Y | Y | Y | Y | Y | N | N | Y |
| Malekzadeh *et al*., 2024. | Y | Y | Y | Y | Y | Y | Y | Y | N | N | Y |
| Mohseni Meybodi *et al*., 2024. | Y | Y | Y | Y | Y | Y | Y | Y | N | Y | Y |
| **Total** | **100%** | **100%** | **100%** | **75%** | **75%** | **58.33%** | **100%** | **100%** | **0%** | **4.17%** | **70.83%** |

## DISCUSSION

The objective of this review was to systematically analyse the preclinical data currently available on *T. terrestris*, with an emphasis on its efficacy, safety, and molecular mechanisms in relation to ED. This study demonstrates that *T. terrestris* possesses significant potential for restoring male reproductive function and improving erectile physiology. Despite variations in species, experimental designs, and induction methods, a consistent pattern of therapeutic benefits emerged across independent investigations, suggesting reproducible biological effects worthy of clinical translation.

The most striking finding of this review is the consistent elevation of testosterone levels across virtually all experimental models. Twenty-two studies (91.7%) reported significant increases in serum testosterone, often accompanied by elevated LH and FSH levels, indicating hypothalamic pituitary gonadal (HPG) axis stimulation rather than direct androgenic replacement. This interpretation aligns with the review by Neychev and Mitev (2016), who concluded that while *T. terrestris* may not increase testosterone in healthy eugonadal humans, the preclinical evidence suggests HPG axis activation. The review extends these findings by demonstrating efficacy across diverse pathological conditions including diabetes, toxin exposure, drug induced suppression, and aging. What makes these findings particularly noteworthy is the universal response across pathological states. Whether animals were diabetic (Ștefănescu *et al*., 2021; Zhang *et al*., 2019), exposed to chemotherapeutic agents (Keshtmand *et al*., 2014; Hashim *et al*., 2024), or castrated (Gauthaman *et al*., 2002; Tyagi *et al*., 2008), *T. terrestris* consistently restored testosterone levels. This contrasts with human studies by (Kamenov *et al*., 2017) and (Neychev and Mitev, 2016) who found no testosterone increase in healthy eugonadal men, suggesting that the herb may be more effective in hypogonadal or pathological states where the HPG axis is suppressed.

The mechanism appears to involve protodioscin-mediated stimulation of gonadotropin releasing hormone secretion, as proposed by (Adaikan *et al*., 2001; and Gauthaman and Ganesan, 2008). Supporting this, studies in the review consistently showed concurrent increases in LH (33.3%) and FSH (37.5%) alongside testosterone elevation. One study even demonstrated increased androgen receptor expression (Meybodi *et al*., 2024), suggesting dual action involving both hormone production and tissue sensitivity enhancement. This represents an advantage over exogenous testosterone replacement, which suppresses the HPG axis and can lead to testicular atrophy (Corona *et al*., 2016). However, the variable results across studies, particularly the discrepancy between animal and human studies; likely reflect differences in extract standardization. Studies using protodioscin-standardized preparations reported more consistent results than those using crude extracts, highlighting the critical need for phytochemical standardization in future research.

Half of the included studies directly measured erectile function parameters, with all reporting improvements in intracavernosal pressure, corpus cavernosum relaxation, or sexual behavior. These functional improvements were consistently accompanied by enhanced NO signaling, including increased NO levels, upregulated eNOS expression, and elevated cGMP concentrations (Do *et al*., 2013; Kam *et al*., 2012; Zhang *et al*., 2019). This NO mediated mechanism complements the hormonal effects discussed above, as testosterone itself regulates NOS expression in penile tissue (Gur *et al*., 2020).

The efficacy in diabetic models deserves particular attention. Ten studies (41.7%) used streptozotocin induced diabetes, consistently demonstrating restoration of erectile function despite severe metabolic dysfunction. This is clinically significant because diabetic ED is notoriously resistant to conventional treatment, with PDE5 inhibitors showing reduced efficacy in this population (Kouidrat *et al*., 2017). The ability of *T. terrestris* to restore NO production under diabetic conditions suggests it addresses upstream pathological mechanisms, oxidative stress, endothelial dysfunction, and impaired NO synthesis that PDE5 inhibitors cannot.

Moreover, Zhang *et al*. (2019) demonstrated not just functional improvement but structural restoration, with increased smooth muscle to collagen ratios and reduced apoptosis in penile tissue. This suggests disease modifying rather than merely symptomatic effects, distinguishing *T. terrestris* from conventional erectogenic drugs. The finding of potential synergism with sildenafil in the same study opens interesting therapeutic possibilities for combination approaches. Nevertheless, some complexities remain. Gauthaman *et al*. (2003) found greater ICP improvements in castrated rats than in normal animals, again suggesting enhanced efficacy in pathological states. Additionally, the observation that some beneficial effects persisted even with NOS inhibition (Kim *et al*., 1993) indicates that alternative pathways beyond simple NO enhancement may contribute to the herb's erectogenic properties.

Another consistent finding in this review was the antioxidant activity of *T. terrestris*, reported in the majority of studies evaluating oxidative stress. The herb consistently reduced MDA levels while increasing the activities of SOD, catalase, glutathione, and glutathione peroxidase (Hend *et al*., 2015; Kumari and Singh, 2015; Hashim *et al*., 2024). Importantly, *T. terrestris* appears to upregulate endogenous antioxidant enzyme systems rather than simply scavenging free radicals directly; a more sustainable protective mechanism that may involve Nrf2 pathway activation, as seen with other plant polyphenols (Eggler *et al*., 2008). The

clinical relevance becomes apparent in toxin-induced damage models. Five studies demonstrated protective effects against severe reproductive toxicants including cisplatin, cyclophosphamide, BPA, and metronidazole (Keshtmand *et al*., 2014; Kumari and Singh, 2015; Munir *et al*., 2017). In some cases, such as metronidazole toxicity, *T. terrestris* achieved complete recovery of testicular structure and sperm parameters (Kumari and Singh, 2015). Given that oxidative stress contributes to 30-80% of male infertility cases (Hussain *et al*., 2023) and that chemotherapy induced infertility affects many cancer survivors, these protective effects have significant translational potential.

The convergence of functional and structural improvements in testicular tissue is an important finding in this study. Nineteen studies (79.2%) reported enhanced sperm parameters, while seventeen (70.8%) demonstrated histopathological restoration including normalized seminiferous tubule architecture, increased tubular diameter, and proliferation of Leydig and Sertoli cells (Al-Yawer *et al*., 2008; Malekzadeh *et al*., 2024; Munir *et al*., 2017).

This goes beyond symptomatic improvement to suggest actual tissue regeneration. Studies showed higher sperm counts, regeneration of germinal epithelium and restoration of complete spermatogenic cycles (Mohammed *et al*., 2013; Aldaddou *et al*., 2022). Interestingly, while most studies showed parallel improvements in structure and function, (Da Silva *et al*., 2024) reported improved testicular architecture without corresponding changes in all sperm parameters. This suggests that structural restoration may precede functional recovery, or that longer treatment durations might be necessary for complete spermatogenic normalization; an observation with implications for clinical trial design.

Studies employed doses ranging from 2 to 500 mg/kg/day with treatment durations from one to 12 weeks, yet positive outcomes emerged across this wide range. However, closer examination reveals important patterns. Studies using protodioscin standardized extracts achieved significant effects at much lower doses (2.5-10 mg/kg) compared to crude preparations requiring 100-200 mg/kg (Gauthaman *et al*., 2002 vs M. Kumari and Singh, 2015). Where examined, dose response relationships typically showed increasing benefits up to 50-100 mg/kg, with diminishing returns or even reduced efficacy at very high doses (Meybodi *et al*., 2024), suggesting possible receptor saturation or dose-dependent toxicity. This variability highlights a critical limitation in current evidence, which is the lack of standardization across studies. As Ștefănescu *et al*., 2020 emphasized, standardization to specific bioactive compounds is essential for reproducible therapeutic outcomes and rational dose optimization. Regarding duration, acute studies demonstrated immediate behavioral effects (Singh and Gupta, 2011) while chronic treatment produced sustained hormonal and structural changes (Gauthaman *et al*., 2002; Ștefănescu *et al*., 2021). This suggests *T. terrestris* may offer both rapid aphrodisiac effects and long-term restorative properties.

The safety profile was remarkably favorable, with 95.8% of studies reporting no serious adverse effects. This aligns with traditional use patterns and previous safety reviews (Ștefănescu *et al*., 2020). One study reported mild hepatic changes, but notably, co-administration with the hepatotoxin cyclophosphamide actually provided protection rather than exacerbating damage (Al-Obaidi *et al*., 2025). Two studies specifically documented hepatoprotective effects (Hashim *et al*., 2024), suggesting that under most conditions, *T. terrestris* supports rather than harms hepatic function. While the preclinical safety profile supports clinical progression, human studies must include comprehensive monitoring for hepatic, renal, cardiovascular, and endocrine effects, particularly with long-term use. Drug interaction studies are also essential given the herb's effects on multiple physiological systems.

What distinguishes *T. terrestris* from conventional single target therapies is its multi mechanistic action. While PDE5 inhibitors address only the NO-cGMP pathway and require intact baseline function, *T. terrestris* simultaneously modulates hormonal, vascular, antioxidant, and regenerative pathways. This broad mechanistic profile may explain its efficacy across diverse pathological models and suggests potential benefit in complex cases where conventional treatments fail.

**Limitations and Future Directions**

Quality assessment for the included studies was conducted using the ARRIVE assessment tool 2.0. Full percentages (100%) were recorded for the evaluation of the abstract, background, objectives, interpretation/scientific implications, and generalizability/translation. A score of 75% was recorded for both the ethical statement and housing and husbandry. Less than two-thirds (58.33%) of the studies provided adequate reporting of animal care and monitoring. Additionally, 70.83% of the studies provided a declaration of conflict of interest. Less than half (4.17%) of the studies provided access to the data used in the research. Future studies should focus on using well-standardized extracts with clearly defined phytochemical compositions, as this will help ensure consistency and reliability of results. Furthermore, long-term studies assessing both sustained efficacy and safety are particularly important, given the chronic nature of ED and the likelihood of prolonged use of herbal therapies.

## CONCLUSION

*T. terrestris* exhibits consistent proerectile effects across a wide range of animal models of ED. Improvements in

erectile performance were reflected in enhanced intracavernosal pressure, increased penile erection indices, improved sexual behaviour parameters, and restoration of libido and fertility-related outcomes. Additionally, *T. terrestris* acts through multiple complementary pathways that are central to erectile physiology. These include androgenic modulation through increased testosterone and LH levels, activation of the NO cGMP signaling pathway leading to cavernosal smooth muscle relaxation, antioxidant protection against oxidative stress-induced endothelial damage, and preservation of penile tissue structure. *T. terrestris* showed a favorable safety profile and beneficial effects even in pathological conditions where endothelial or hormonal function is compromised.

Finally, the promising preclinical outcomes highlighted in this review support the need for carefully designed human clinical trials, which are essential in confirming safety, efficacy, and clinical relevance, and will ultimately determine the role of *T. terrestris* as a potential therapeutic or adjunct option in the evidence-based management of ED.

## AUTHORS' CONTRIBUTION

WCE conceptualized the study; All authors were involved in the literature review; WCE & VOO extracted the data from the reviewed studies; CCA prepared Figure 1. WCE and VOO prepared Tables 1 & 2; CEA, EJU and AI prepared Tables 3 and 4; CSN Supervised the study; All authors (WCE, VOO, CCA, BOM, CEA, EJU, AI, CSN) wrote the final and first drafts. All authors (WCE, VOO, CCA, BOM, CEA, EJU, AI, CSN) read and approved the final manuscript.

## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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