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Review Article

Effects of phytoecdysteroid exposure on renal function and structure in healthy murine models: a systematic review

Efeitos da exposição a fitoecdisteroides na função e na estrutura renal em modelos murinos saudáveis: uma revisão sistemática

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Abstract

Phytoecdysteroids are bioactive plant metabolites with reported anabolic, antioxidant, anti-inflammatory, and metabolic effects; however, their effects on renal function and structure remain poorly understood. This systematic review evaluated the effects of exposure to phytoecdysteroids on the kidneys of healthy murine models. The review was conducted in accordance with the PRISMA guidelines and registered with PROSPERO (CRD420261412485). Searches were conducted in the PubMed, Scopus, Web of Science, and embase databases, including in vivo experimental studies that evaluated isolated phytoecdysteroids or enriched extracts compared to control groups. Of the 189 records identified, eight studies were included. The studies showed heterogeneity regarding compounds, doses, routes of administration, and duration of treatment. Low and moderate doses did not result in consistent changes in markers of renal function, whereas high doses were associated with increased creatinine levels and structural changes, including glomerulomegaly, podocyte damage, and extracellular matrix deposition. Some studies reported antioxidant effects, although results varied across models. The methodological assessment identified limitations related primarily to the lack of information on randomization and blinding. Taken together, the findings suggest a low potential for renal toxicity at low and moderate doses but indicate a possible risk at high exposures. The heterogeneity and limited number of studies limit the strength of the evidence and underscore the need for standardized experimental protocols.

Keywords: nephrotoxicity, ecdysone, oxidative stress, histopathology, experimental toxicology.

Resumo

Os fitoecdisteroides são metabólitos vegetais bioativos com efeitos anabólicos, antioxidantes, anti-inflamatórios e metabólicos relatados; no entanto, seus efeitos sobre a função e a estrutura renais permanecem pouco compreendidos. Esta revisão sistemática avaliou os efeitos da exposição a fitoecdisteroides em rins de modelos murinos saudáveis. A revisão foi conduzida conforme as diretrizes PRISMA e registrada no PROSPERO (CRD420261412485). Foram realizadas buscas nas bases PubMed, Scopus, Web of Science e Embase, incluindo estudos experimentais in vivo que avaliaram fitoecdisteroides isolados ou extratos enriquecidos, comparados a grupos controle. Dos 189 registros identificados, oito estudos foram incluídos. Os estudos apresentaram heterogeneidade quanto aos compostos, doses, vias de administração e duração dos tratamentos. Doses baixas e moderadas não promoveram alterações consistentes nos marcadores de função renal, enquanto doses elevadas foram associadas ao aumento de creatinina e alterações estruturais, incluindo glomerulomegalia, lesão podocitária e deposição de matriz extracelular. Alguns estudos relataram efeitos antioxidantes, embora os resultados tenham variado entre os modelos. A avaliação metodológica identificou limitações relacionadas principalmente à ausência de informações sobre randomização e cegamento. Em conjunto, os achados sugerem baixo potencial de toxicidade renal em doses baixas e moderadas, mas indicam possível risco em exposições elevadas. A heterogeneidade e o número reduzido de estudos limitam a força das evidências e reforçam a necessidade de protocolos experimentais padronizados.

Palavras-chave: nefrotoxicidade, ecdisona, estresse oxidativo, histopatologia, toxicologia experimental.

1. Introduction

Medicinal plants are defined as those with properties capable of preventing, alleviating, or treating diseases and have been used by humans throughout history (El-Saadony et al., 2025; Firmo et al., 2012). Currently, their use has expanded within healthcare systems as complementary therapies and as important sources of bioactive compounds for the pharmaceutical industry (Figueredo et al., 2014). Among these compounds,

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phytoecdysteroids are plant-derived steroids structurally similar to arthropod molting hormones. In plants, they function as secondary metabolites involved in defense against herbivores and pathogens, whereas in mammals they have attracted attention because of their potential anabolic, antioxidant, anti-inflammatory, and metabolic properties (Báthori et al., 2008).

The biological effects of phytoecdysteroids depend on the specific compound, administered dose, and physiological characteristics of the exposed organism (Canivenc-Lavier and Bennetau-Pelissero, 2023). Consequently, their safety profile has become an important topic in the scientific literature (Patra et al., 2001). In this context, the kidneys are particularly relevant because of their central role in blood filtration and xenobiotic elimination, making them highly susceptible to drug-induced toxicity and structural or functional injury (Miners et al., 2017; Perazella, 2018)

Experimental murine models are widely used to investigate renal injury and evaluate the safety of compounds before clinical studies, as they reproduce important aspects of human kidney physiology and nephrotoxicity (Blanset et al., 2020; Leong et al., 2021). Although several studies have investigated the renal effects of phytoecdysteroids, most have focused on models of induced disease or injury. Consequently, evidence regarding their effects on renal structure and function in healthy animals remains limited, hindering the assessment of their renal safety under physiological conditions. Therefore, this systematic review aimed to synthesize the available evidence on the effects of phytoecdysteroids on kidney structure and function in healthy murine models, providing a comprehensive assessment of their renal safety and identifying gaps to guide future preclinical research.

2. Methodology

2.1. Main Question and Registration on the PROSPERO Platform

The research question was formulated according to the PICO framework (Population, Intervention, Comparison, and Outcomes): "Does phytoecdysteroid exposure affect renal function and structure in murine compared with untreated animals?" The review included murine models (P), exposure to isolated phytoecdysteroids or phytoecdysteroid-enriched plant extracts (I), untreated control groups (C), and renal functional, biochemical, and histological outcomes (O). The review was conducted following PRISMA guidelines (Page et al., 2021), and the study selection process is presented in Figure 1. The protocol was registered in PROSPERO (CRD420261412485).

2.2. Eligibility criteria

Studies were included if they: (i) were in vivo experimental studies; (ii) used healthy murine models; (iii) included an untreated control group; (iv) evaluated isolated phytoecdysteroids or phytoecdysteroid-enriched plant extracts; and (v) assessed renal functional, biochemical, or histological outcomes. No language restrictions were applied.

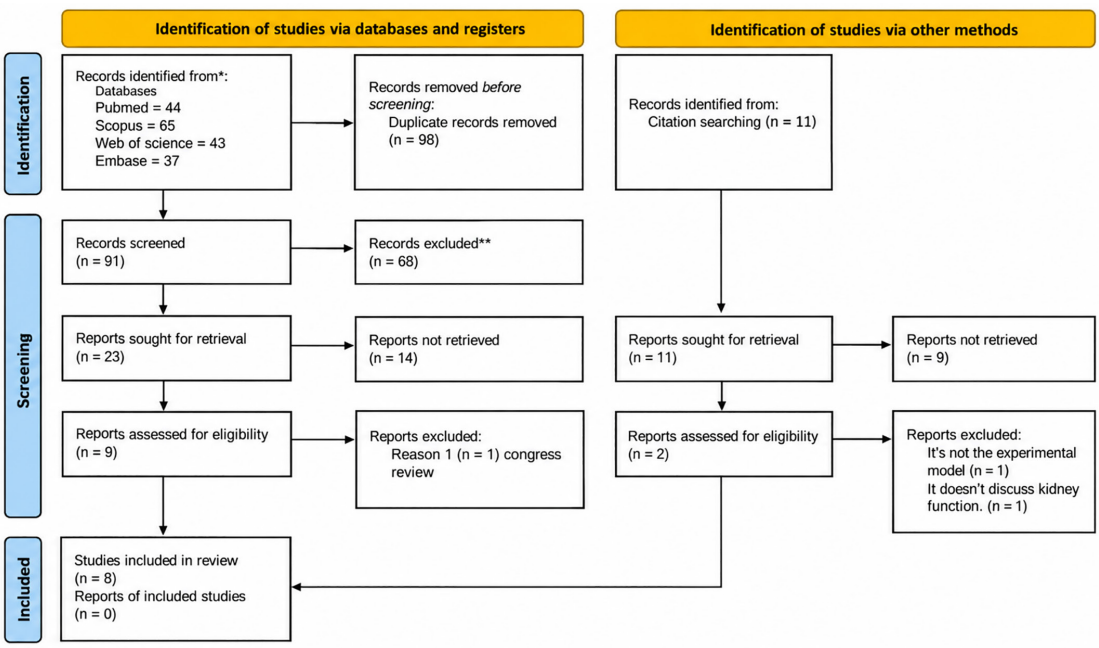


Figure 1. PRISMA 2020 flow diagram for the systematic review. Flowchart illustrating the identification, screening, eligibility, and inclusion steps of the systematic literature search across databases (PubMed, Scopus, Web of Science, Embase) and citation searching, culminating in 8 studies included in the final synthesis. *Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers). **If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools.

Studies were excluded if they were: (i) reviews, conference abstracts, book chapters, scientific notes, or gray literature; (ii) in vitro studies; (iii) lacking a control group; (iv) conducted in animals with pre-existing diseases or induced kidney injury; or (v) without sufficient quantitative data for renal outcome assessment.

2.3. Literature search strategy

The literature search was conducted on May 19, 2026, in the Scopus, PubMed/MEDLINE, Web of Science, and Embase databases, without publication date restrictions. The search strategy was developed based on three main concepts: (i) phytoecdysteroids, (ii) kidney-related outcomes, and (iii) murine models.

The PubMed/MEDLINE strategy was developed using MeSH terms and related free-text terms and subsequently adapted for the other databases according to their specific controlled vocabularies and search fields. The search combined terms related to phytoecdysteroids (e.g., ecdysterone, ecdysone, 20-hydroxyecdysone, turkesterone), renal outcomes (e.g., kidney, renal function, nephrotoxicity), and murine models (e.g., mouse, mice, rat, rodent) using Boolean operators AND and OR. The complete search strategies for each database are provided in Supplementary Table S1.

2.4. Data synthesis and analysis

Study selection and data extraction were independently performed by two reviewers (MSC and ICS), with agreement assessed using the Kappa test ($\kappa = 0.960$). Disagreements were resolved by a third reviewer (FCRD). After duplicate removal, studies were screened by title and abstract, followed by full-text assessment according to the eligibility criteria.

Extracted data included study characteristics (author, country, animal species, strain, sex, age, and weight), intervention details (compound or plant extract, vehicle, dose, route, frequency, and duration), control conditions, and renal biochemical and histological outcomes.

Primary outcomes were renal function markers (serum creatinine, urea, and blood urea nitrogen) and kidney morphological changes. Secondary outcomes included oxidative stress, inflammatory markers, body weight, and kidney weight. Data were synthesized narratively due to heterogeneity among compounds, doses, and evaluated outcomes, which precluded meta-analysis.

2.5. Risk of bias

Risk of bias was assessed according to the SYRCLE Risk of Bias tool for animal studies (Hooijmans et al., 2014). Two reviewers independently evaluated the methodological quality of the included studies. The assessment included six domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, and selective reporting. Two additional items were included: photochemical characterization of the compound and funding source. Each item was classified as "low risk," "high risk," or "unclear risk" when insufficient information was available. Risk of bias results were summarized graphically using Review Manager 5.4 and presented as absolute values and percentages.

3. Results

3.1. Publication characteristics

The search identified 189 records: 44 from PubMed, 65 from Scopus, 43 from Web of Science, and 37 from Embase. After removal of 98 duplicates and screening of titles and abstracts, eight studies met the eligibility criteria. Reference screening of included studies did not identify additional eligible articles (Figure 1).

Characteristics of the included studies are summarized in Table 1. Studies were conducted in five countries, with India accounting for half of the included studies (50.0%; n = 4), followed by the United States, Iraq, Tunisia, and China (12.5%; n = 1 each). Most studies used male animals (75.0%; n = 6), whereas females were used in two studies (25.0%). Animal age was reported in only two studies, both involving animals up to 10 weeks old. Wistar was the most frequently used strain (50.0%; n = 4), followed by C57BL/6 and Sprague-Dawley strains (25.0%; n = 2 each). Animal weight was reported in seven studies (87.5%), ranging from approximately 22 g in C57BL/6 mice to approximately 200 g in other models.

Table 1. Description of the main characteristics of studies evaluating the effects of phytoecdysteroids on the murine kidney.

Study	Country	Sex	Age	Body weight	Model
Lu et al. (2018a)	USA	M	8–10 wk	20–22 g	C57BL/6 mice
Sundaram et al. (2012)	India	M	NR	175–200 g	Wistar rats
Hamden et al. (2008)	Tunisia	M	NR	180–200 g	Wistar rats
Khudhair et al. (2025)	Iraq	F	NR	160–210 g	Sprague–Dawley rats
Naresh Kumar et al. (2013)	India	M	NR	175–200 g	Wistar rats
Lu et al. (2018b)	China	M	10 wk	NR	C57BL/6 mice
Abiramasundari et al. (2017)	India	F	NR	100–120 g	Sprague–Dawley rats
Vedasree et al. (2022)	India	M	NR	180–200 g	Wistar rats

Abbreviations: M, male; F, female; wk, weeks; NR, not reported.

3.2. Treatment characteristics

Most studies evaluated isolated phytoecdysteroids (62.5%; n = 5), whereas three studies (37.5%; n=3) investigated phytoecdysteroid-enriched plant extracts. The main isolated compounds were ecdysone (25.0%; n = 2), 20-hydroxyecdysone (25.0%; n = 2), and turkesterone (12.5%; n = 1) (Table 2). Phytochemical characterization of the plant-derived preparations was reported in two studies (25.0%), using chromatographic and spectrometric approaches, including TLC, FT-IR, LC-ESI-MS, LC-ESI-MS/MS, and HPLC-UV. In *Tinospora cordifolia*, β-ecdysone was used as a reference standard, while *Cyanotis tuberosa* was characterized by the identification of several phytoecdysteroids, including ajugasterone C, dacry-hainasterone, and rubrosterone. In one study (12.5%), the *Ajuga iva* aqueous extract was not phytochemically characterized, and its phytoecdysteroid content was inferred from previous reports. The concentrations of the identified phytoecdysteroids were not quantitatively reported in either characterized extract (Table 3).

All studies administered treatments once daily. Dosages varied considerably, with 5 mg/kg being the most frequently used dose (37.5%; n = 3), while other protocols ranged from µg/g to 1000 mg/kg. Administration routes included subcutaneous injection, intragastric administration, oral gavage, intraperitoneal injection, and oral administration. Treatment duration ranged from 14 to 60 days, with 30 days being the most common period (50.0%; n = 4) (Figure 2).

Table 2. Characteristics of the experimental protocols used in the included studies, according to extract or compound type, vehicle, administration frequency, dose, route of administration, and treatment duration.

Study	Extract	Compound	Dose	Route	Duration (days)
Lu et al. (2018a)	—	Ecdysone	6 µg/g	SC	14
Sundaram et al. (2012)	—	20-Hydroxyecdysone	5 mg/kg	IG	30
Hamden et al. (2008)	*Ajuga iva*	—	50 mg/kg	IP	60
Khudhair et al. (2025)	—	Turkesterone	5 mg/kg	Oral gavage	15
Naresh Kumar et al. (2013)	—	20-Hydroxyecdysone	5 mg/kg	IG	30
Lu et al. (2018b)	—	Ecdysone	6 µg/g	SC	14
Abiramasundari et al. (2017)	*Tinospora cordifolia*	—	100–1000 mg/kg†	Oral	30
Vedasree et al. (2022)	*Cyanotis tuberosa*	—	75 mg/kg	Oral	30

Abbreviations: SC, subcutaneous; IP, intraperitoneal; IG, intragastric; —, not reported.
†100, 250, 500, 750, and 1000 mg/kg.

Table 3. Phytochemical characterization and standardization of plant-derived phytoecdysteroid interventions.

Variable	Abiramasundari et al. (2017)	Naresh Kumar et al. (2013)	Vedasree et al. (2022)	Hamden et al. (2008)
Plant material	*Tinospora cordifolia* extract	*Vitex negundo* extract; isolated compound	*Cyanotis tuberosa* extract	Aqueous crude extract of *Ajuga iva*
Characterization method	TLC, FT-IR, LC-ESI-MS	^1H-NMR, ^13C-NMR, IR, EI-MS, melting point	LC-ESI-MS/MS (Q-TOF), HPLC-UV	Not performed
Reference standard	β-Ecdysone (≥93%)	Spectroscopic data compared with literature	Not reported	Not reported
Phytoecdysteroid profile	Qualitative identification; content not quantified	20-Hydroxyecdysone isolated	Ajugasterone C, dacry-hainasterone, rubrosterone; content not quantified	Content not quantified; inferred from previous literature
Standardization	2.5–3.5% bitter principles; berberine as marker	Not applicable; isolated compound administered	Bioactivity-guided fractionation	Not reported

Abbreviations: TLC, thin-layer chromatography; FT-IR, Fourier-transform infrared spectroscopy; LC-ESI-MS, liquid chromatography–electrospray ionization mass spectrometry; LC-ESI-MS/MS, liquid chromatography–tandem mass spectrometry; HPLC-UV, high-performance liquid chromatography with ultraviolet detection; NMR, nuclear magnetic resonance; EI-MS, electron ionization mass spectrometry. "Not reported" indicates that the information was not provided in the original study; "not applicable" indicates that the intervention was administered as an isolated compound rather than as a plant preparation.

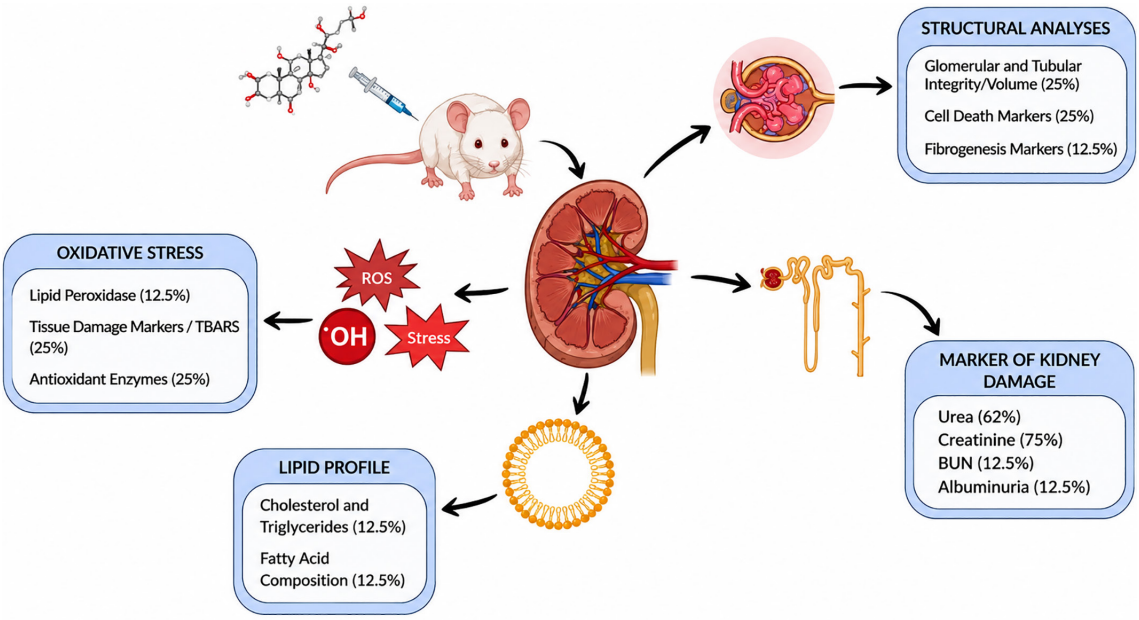


Figure 2. Distribution of analyzed parameters among studies included in the systematic review. Overview of animal model treatments and key renal outcomes evaluated, categorized by structural integrity, biomarkers of kidney damage, lipid profile, and oxidative stress markers. Percentages reflect the proportion of studies assessing each outcome.

3.3. Primary outcomes: renal function and histopathological structural status

Figure 3 summarizes the renal parameters evaluated in murine models exposed to phytoecdysteroids, including kidney injury markers, structural analysis, oxidative stress, and lipid profile.

3.4. Markers of kidney damage and function

Renal function markers included serum or plasma creatinine (75.0%; n = 6), urea (62.0%; n = 5), blood urea nitrogen (BUN) (12.5%; n = 1), albuminuria (12.5%; n = 1), and urinary albumin-to-creatinine ratio (ACR) (12.5%; n = 1). To evaluate potential dose-related effects, doses were grouped into five categories (6 mg/kg, 5–75 mg/kg, 100 mg/kg, 250–750 mg/kg, and 1000 mg/kg). Exposure to 6 mg/kg was associated with increased creatinine, BUN, and ACR levels, whereas urea remained unchanged. At 1000 mg/kg, increased creatinine was observed without changes in urea. Conversely, 100 mg/kg reduced urea levels without affecting creatinine. Doses between 5–75 mg/kg and 250–750 mg/kg did not significantly alter creatinine or urea compared with controls (Table 4).

3.5. Structural and morphological analysis of the kidney

Renal structural assessment included histopathological and morphological analysis (50.0%; n = 4), evaluation of glomerular and tubular integrity (25.0%; n = 2), cell death markers (25.0%; n = 2), and fibrogenesis markers (12.5%; n = 1). Control groups showed preserved renal architecture, whereas treated animals exhibited morphological alterations, including glomerulomegaly and mesangial expansion. Increased expression of α-SMA and desmin was reported in one study (12.5%). Renal ultrastructure was evaluated by electron microscopy in one study, which identified podocyte alterations characterized by reduced density and blurring of podocyte processes. Fibrogenesis markers, including collagen IV and fibronectin, were increased in two studies (25.0%), and cleaved caspase-3 expression was increased in one study.

3.6. Tissue oxidative stress

Oxidative stress was evaluated through markers of cellular damage and TBARS (25.0%; n = 2), antioxidant enzyme activity (25.0%; n = 2), and lipid peroxide levels (12.5%; n = 1). Plant extracts enriched with phytoecdysteroids increased SOD, CAT, and GPX activity and reduced TBARS levels in one study. Another study reported no changes in GPX4 expression or lipid peroxide concentrations.

3.7. Lipid profile

Lipid parameters were assessed in two studies (25.0%). One study evaluated cholesterol and triglycerides, whereas another analyzed fatty acid composition. Phytoecdysteroid treatment reduced total cholesterol and circulating triglycerides and slightly increased HDL-cholesterol levels in one study. Renal lipid composition, including cholesterol, triglycerides, and major fatty acids (palmitic, stearic, oleic, and arachidonic acids), showed no significant differences between groups. An acute toxicity study using 1000 mg/kg reported increased systemic total cholesterol.

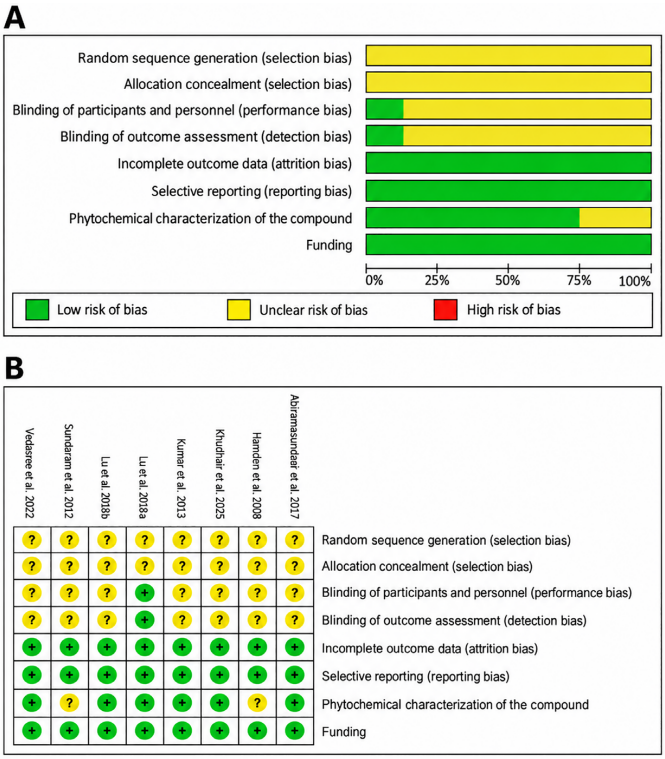


Figure 3. Risk of bias assessment for included studies. Methodological quality evaluation using the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE) tool for studies assessing the effects of phytoecdysteroids on murine kidneys. (A) Overall risk of bias graph presenting the percentage of studies meeting each domain criterion. (B) Risk of bias summary showing the individual judgment for each included study across evaluated domains. Green circles indicate low risk of bias ("yes"), yellow circles with question marks indicate unclear risk of bias ("unclear", due to insufficient reporting), and red circles indicate high risk of bias ("no").

Table 4. Number of studies and the effect of doses on damage markers

Outcome	Low dose(5–75 mg/kg)	100 mg/kg	250–750 mg/kg	1000 mg/kg
Creatinine	NC	↓	NC	↑
Urea	NC	↓	NC	NC
BUN	—	—	—	↑
Albumin/Creatinine ratio	—	—	—	↑

Abbreviations: ↑, significant increase; ↓, significant decrease; NC, no significant change; —, not evaluated or no data available. Numbers indicate the number of studies reporting each outcome. BUN, blood urea nitrogen.

3.8. Secondary outcomes: systemic safety and metabolic homeostasis

Systemic blood pressure was evaluated in 25.0% (n = 2) of the studies, with no significant changes following treatment. Plasma glucose was assessed in 62.5% (n = 5) of studies, using doses ranging from 6 µg/g to 1000 mg/kg, and no differences were observed between treated and control groups. Serum insulin levels were also unchanged in 50.0% (n = 4) of the studies. Histopathological evaluation was performed in one study (12.5%), which reported preserved liver, pancreas, and kidney architecture in treated animals. The same study reported no behavioral changes, hair loss, appetite reduction, or decreased physical activity. Plasma mineral concentrations were assessed in one study (12.5%), with increased magnesium, calcium, iron, and copper levels following treatment with Ajuga iva. Body weight was evaluated in two studies (25.0%). No differences were observed in animals treated with Cyanotis tuberosa, whereas Ajuga iva treatment resulted in a 5.2% reduction in final body weight compared with controls, without changes in liver or kidney weights.

3.9. Assessment of methodological quality

Risk of bias results are presented in Figure 3. None of the included studies fulfilled all SYRCLE criteria. No study reported random sequence generation or allocation

concealment, and only one study described blinding procedures. Phytochemical characterization was performed in six studies (75.0%). No studies reported incomplete outcomes, selective reporting, or private funding sources.

4. Discussion

This systematic review synthesized the available evidence on the renal effects of phytoecdysteroids in murine models. The literature was found to be limited and heterogeneous in terms of experimental models, compounds evaluated, doses, routes of administration, and duration of treatment, limiting direct comparisons between studies. In general, phytoecdysteroids exhibited a favorable renal profile at low and moderate doses, with no consistent changes in key markers of renal function. Some studies also reported a reduction in oxidative stress and an improvement in the systemic lipid profile. However, high doses, especially in acute toxicity protocols, were associated with renal changes, suggesting that the effects are dose-dependent and context-dependent.

Half of the included studies were conducted in India (50.0%; n = 4), while the remainder were carried out in the United States, China, Iraq, and Tunisia (12.5%; n = 1 each). This geographic concentration suggests that preclinical research on the effects of phytoecdysteroids is still limited to a few research groups, possibly related to the historical interest in medicinal plants rich in these compounds (Das et al., 2021). Furthermore, differences among animal models, phytochemical sources, and experimental protocols contribute to the heterogeneity of the studies and should be considered when interpreting the results.

The animal models were predominantly Wistar (50%), followed by C57BL/6 and Sprague-Dawley (25% each). Although widely used in experimental renal studies, these strains exhibit genetic differences that may influence inflammation, oxidative stress, susceptibility to kidney injury, and response to treatments (Chevalier, 2016; Luo et al., 2023). Thus, the intrinsic characteristics of the models used may contribute to the variability observed across studies.

The predominance of male animals (75%; n = 6) also represents a limitation, as sex influences renal processes such as oxidative stress, inflammation, apoptosis, fibrosis, and tissue repair (Bairey Merz et al., 2019). Although the use of males is common to reduce experimental variability associated with the estrous cycle (Prendergast et al., 2014), the low inclusion of females limits the generalizability of the findings and reinforces the need for future studies involving both sexes.

Most studies evaluated isolated phytoecdysteroids (62.5%), while the remainder used enriched plant extracts. Although isolated compounds allow for better pharmacological evaluation and assessment of dose-response relationships, extracts contain other metabolites that may act synergistically, additively, or antagonistically, making it impossible to attribute the observed effects exclusively to phytoecdysteroids (Caesar and Cech, 2019).

The studies also evaluated different molecules, including ecdysone, 20-hydroxyecdysone, and turkesterone. Despite their shared steroidal core, structural differences can alter properties such as metabolism, bioavailability, and biological activity, making direct comparisons difficult (Das et al., 2021; Dinan and Lafont, 2006). The limited number of studies still prevents determining which compound has the greatest nephroprotective potential.

Although the frequency of administration was standardized (once daily), there was wide variation in doses and routes of administration, ranging from protocols of micrograms per gram up to 1000 mg/kg and including different oral and parenteral routes. These differences may affect absorption, metabolism, and bioavailability, limiting comparisons between studies and the establishment of dose–response relationships (Dinan and Lafont, 2006). Thus, more standardized experimental protocols are needed to define optimal conditions for the use of phytoecdysteroids (Das et al., 2021)

The 30-day protocols were the most common (50%), followed by 14-day protocols (25%), both of which were classified as subacute exposures according to OECD TG 407. Variation in treatment duration may influence physiological adaptations and cumulative effects, making comparisons between studies difficult. Renal function markers were the most commonly assessed outcomes, particularly creatinine (75%) and urea (62%). Overall, intermediate doses did not alter these parameters, while 100 mg/kg reduced urea and 1000 mg/kg increased creatinine, BUN, and the albumin-to-creatinine ratio. These findings suggest a dose-dependent response and indicate the potential for a lower frequency of detectable renal alterations at specific exposure levels, although structural and molecular changes may precede alterations in traditional serum markers (Fu et al., 2024).

The functional changes observed in some of the studies were accompanied by histopathological changes consistent with progressive impairment of the glomerular unit. Although half of the studies demonstrated preservation of renal architecture, some experimental models showed glomerulomegaly, mesangial expansion, and ultrastructural changes in podocytes, including blurring and reduced density of the podocyte foot processes along the basement membrane. These changes are recognized as early events in the loss of integrity of the glomerular filtration barrier and may promote the development of albuminuria and the progression of kidney damage. Additionally, increased expression. of desmin and α-SMA, associated with greater deposition of collagen IV and fibronectin, suggests activation of processes related to extracellular matrix remodeling and renal fibrogenesis. Thus, the findings indicate that, although some protocols have demonstrated structural preservation, certain experimental conditions may promote glomerular changes and activation of mechanisms associated with the progression of kidney disease (Agrawal et al., 2021) (Figure 4).

The progression of the observed structural changes may be related to redox imbalance, a key mechanism in kidney injury. Studies using extracts rich in phytoecdysteroids, such as Ajuga iva, have demonstrated increased SOD, CAT, and GPx activity, associated with a reduction in TBARS, suggesting less oxidative damage. However, the absence of changes in GPX4 and lipid peroxides in another study indicates that this response may vary depending on the

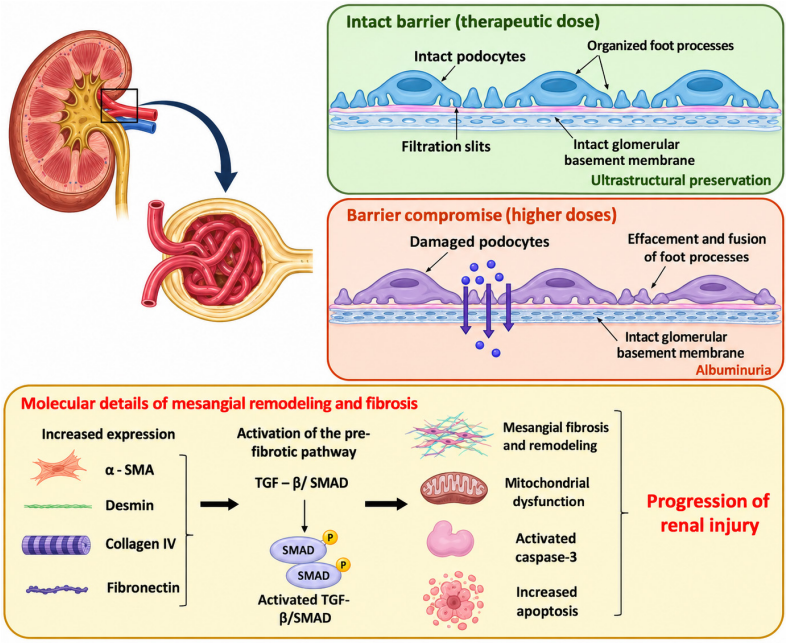


Figure 4. Schematic representation of the dose-dependent renal effects of phytoecdysteroids in healthy murine models. Panel A illustrates the preservation of glomerular architecture observed at low to moderate doses, characterized by podocyte integrity, preserved foot processes, intact filtration slits, and maintenance of the glomerular basement membrane. Panel B depicts renal alterations associated with high-dose exposure, including podocyte injury, foot process effacement and fusion, and impairment of the glomerular filtration barrier, accompanied by albuminuria. Panel C presents the principal histopathological findings reported in the included studies, together with biologically plausible molecular mechanisms proposed from complementary evidence in the literature, including oxidative stress, apoptosis, extracellular matrix deposition, and signaling pathways related to renal fibrosis. The schematic should therefore be interpreted as a conceptual model integrating the findings of this systematic review with current mechanistic knowledge, rather than as an experimentally validated pathway.

experimental model and treatment protocol (Kishi et al., 2024).

The modulation of oxidative stress may also be associated with the observed changes in apoptosis and fibrosis. Studies that identified increased deposition of collagen IV and fibronectin also reported an increase in cleaved caspase-3, suggesting an interaction between cellular damage and renal remodeling. Although specific pathways, such as Nrf2/HO-1, NF-κB, and TGF-β/Smad, have not been directly evaluated, in the included studies, their involvement remains hypothetical and should be interpreted as a mechanistic possibility based on the broader literature rather than as evidence generated by this review (Hassanein et al., 2023; Kishi et al., 2024).

In addition to renal effects, some studies have reported an improvement in the systemic lipid profile, with reductions in cholesterol and triglycerides, while the renal lipid profile remained unchanged. These results suggest possible indirect metabolic effects on renal protection. However, the increase in serum cholesterol and creatinine at high doses (1000 mg/kg) underscores dose dependence and the need to evaluate safety at higher concentrations (Hassanein et al., 2023; Kishi et al., 2024).

Taken together, the results of this review suggest that phytoecdysteroids exhibit potential effects associated with the reduction of oxidative stress, preservation of renal structure, and modulation of processes related to apoptosis and fibrosis. The increase in SOD, CAT, and GPx activity, associated with a reduction in TBARS, is consistent with the antioxidant mechanisms described for these compounds. The experimental evidence synthesized in this review demonstrates alterations in antioxidant enzyme activity, oxidative stress markers, apoptosis, and extracellular matrix deposition. However, because none of the included studies directly investigated intracellular signaling pathways, the involvement of Nrf2/HO-1, NF-κB, or TGF-β/Smad remains hypothetical and is supported only by evidence from previous mechanistic studies. However, the occurrence of changes across different dose ranges underscores the need for further studies to define safe therapeutic concentrations and confirm the molecular pathways involved (Hassanein et al., 2023; Kishi et al., 2024; Ng et al., 2025) (Figure 5).

In addition to renal effects, the included studies evaluated systemic parameters related to the safety of phytoecdysteroids. Blood pressure, assessed in 25% of the studies, showed no significant changes after treatment, suggesting the absence of hemodynamic effects capable of compromising renal function under the experimental conditions evaluated (Ku et al., 2019). Similarly, glycemic homeostasis was preserved, with no significant differences in glucose (62.5%) and insulin (50%) levels between the groups, indicating that the observed renal effects do not

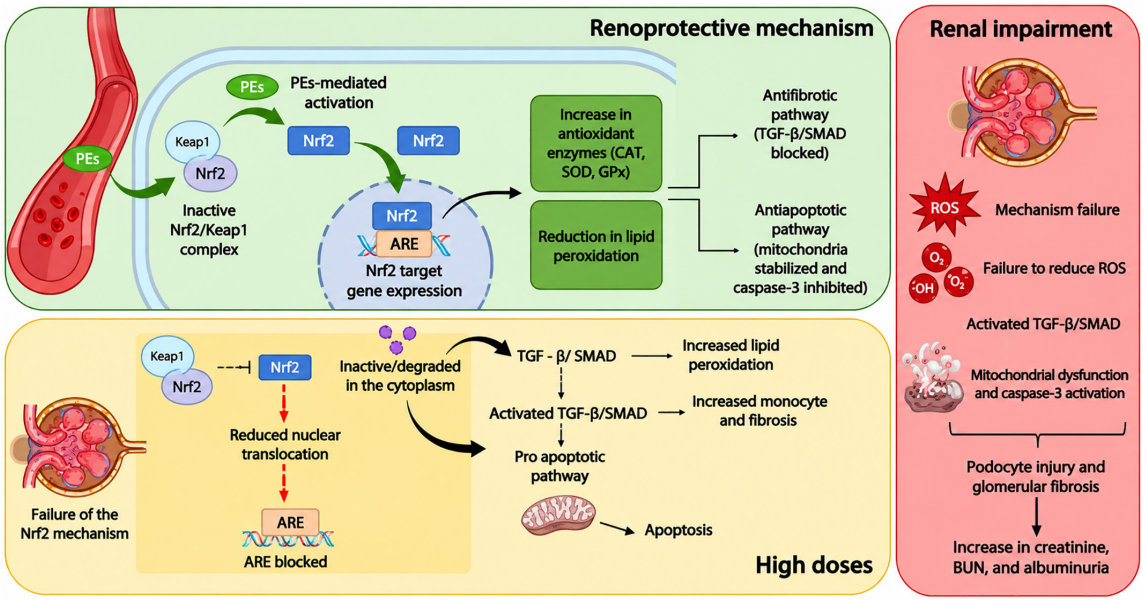


Figure 5. Hypothetical integrated molecular model of the renal effects associated with phytoecdysteroid exposure in healthy murine models. At low to moderate doses (5–75 mg/kg), the included studies reported preserved renal function and structure, accompanied by increased antioxidant enzyme activity (SOD, CAT, and GPx) and reduced lipid peroxidation (TBARS) in some experimental models. Conversely, high-dose exposure (6 µg/g and 1000 mg/kg) was associated with biochemical and histopathological alterations, including increased creatinine levels, podocyte injury, extracellular matrix deposition, and apoptosis. The signaling pathways illustrated (e.g., Nrf2/HO-1, NF-κB, and TGF-β/Smad) were not directly investigated in the included studies and are presented as biologically plausible mechanisms based on the integration of the findings from this systematic review with evidence from the broader literature. Therefore, the proposed model should be interpreted as a conceptual framework rather than an experimentally validated mechanism.

appear to be secondary to changes in glucose metabolism (Alicic et al., 2017; Tuttle, 2018).

Systemic histopathological evaluation, conducted in only one study, demonstrated preservation of the architecture of the liver, pancreas, and kidneys, with no clinical signs of toxicity. Although these findings support a favorable safety profile, the limited number of studies evaluating these parameters precludes definitive conclusions (Dinan and Lafont, 2006; Lafont and Dinan, 2003). Changes in plasma levels of magnesium, calcium, iron, and copper following treatment with Ajuga iva were observed, but their contribution to the antioxidant effects remains uncertain due to the limited evaluation of this outcome(Fridovich, 1981; Ighodaro and Akinloye, 2018).

With regard to body weight, no significant changes were observed in the animals treated with Cyanotis tuberosa, while Ajuga iva caused a slight reduction in final body weight, with no changes in liver or kidney weight and no evidence of organ toxicity. These findings are consistent with metabolic effects previously described for phytoecdysteroids, although they should be interpreted with caution (Gorelick-Feldman et al., 2010).

Overall, the secondary parameters reinforce a favorable safety profile for phytoecdysteroids under the experimental conditions evaluated. However, the low frequency of research on these outcomes highlights the need for more standardized and comprehensive studies on the pharmacological safety of these compounds (Dinan and Lafont, 2006).

5. Conclusion

This systematic review indicates that phytoecdysteroids exhibit a generally favorable renal safety profile in healthy murine models when administered at low and moderate doses. Under these conditions, renal function markers and kidney morphology remained largely preserved, although the available evidence is limited by the small number of studies and their methodological heterogeneity. In contrast, exposure to high doses was associated with biochemical and histopathological alterations, suggesting that renal effects are dose-dependent.

Although some studies reported improvements in oxidative stress markers, the evidence remains insufficient to conclude that phytoecdysteroids exert a direct renoprotective effect under physiological conditions. Future well-designed experimental studies using standardized protocols, rigorous methodological practices, and comprehensive molecular analyses are needed to define the therapeutic safety window of these compounds and to clarify the mechanisms underlying their renal effects.

5.1. Limitations

This review has limitations that should be taken into account when interpreting the results. The small number of eligible studies indicates that research on the renal effects of phytoecdysteroids in healthy murine models remains limited. Furthermore, the heterogeneity of the compounds evaluated, animal models, doses, routes of

administration, exposure times, and outcomes made it impossible to conduct a meta-analysis and restricted direct comparisons between studies.

The assessment of risk of bias revealed insufficient reporting of methodological procedures, such as randomization, allocation concealment, and blinding, thereby reducing confidence in the available evidence. Furthermore, the predominance of studies conducted in male animals and the exclusive inclusion of healthy murine models limit the extrapolation of the findings to females, models of kidney disease, and, most importantly, to clinical practice. An additional limitation is that the molecular pathways illustrated in the proposed mechanistic model were not directly evaluated in the included studies and should therefore be interpreted as biologically plausible hypotheses rather than experimentally confirmed mechanisms.

Despite these limitations, this review represents, to the best of our knowledge, the first systematic synthesis of evidence on the effects of phytoecdysteroids on renal function and structure in healthy mouse models. By identifying gaps in knowledge and highlighting the need for greater methodological standardization, this study provides a basis for future preclinical research. The proposed mechanistic model represents a biologically plausible hypothesis rather than an experimentally confirmed mechanism.

Data Availability Statement

All data analyzed during this study are included in this published article.

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Supplementary Material

Supplementary material accompanies this paper.
Table S1. Full search strategy in PubMed, Scopus and Web of Science including search terms and filters.
This material is available as part of the online article from https://doi.org/10.1590/1519-6984.311680