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SYSTEMATIC REVIEW

Methodological aspects of chemically-induced chronic prostatitis/chronic pelvic pain syndrome models: a systematic review

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Abstract

Background: Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is highly prevalent but poorly understood urological disorder with limited success of available therapies. Chemically induced rodent models of CP/CPPS have become valuable tools for elucidation of its ethiopathogenesis and evaluation of therapies. However, methodological diversity could limit translational relevance. The aim of this systematic review was to assess different methodological aspects of chemically induced CP/CPPS models in rodents. **Methods**: A systematic search was conducted in PubMed and Scopus (2000 up to 13 June 2025), in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Studies were included if they involved *in vivo* models of CP/CPPS induced by chemical agents (*e.g.*, λ-carrageenan, complete Freund's adjuvant). Key data extracted included species and strain, chemical agent type and dose, control group design, pain and morphological assessments and therapeutic interventions. **Results:** Fifty-six studies met our inclusion criteria. Rats were more frequently used than mice, with the domination of Sprague-Drawly rats. λ-carrageenan was the most frequently used chemical agent to induce CP/CPPS (in 62.5% of studies). Pain-related behavior assessments were not done in more than a half of included studies (57%), while mechanical hyperalgesia was assessed more frequently than thermal hyperalgesia. Morphological validation was done in 84% of studies, primarily using hematoxylin and eosin staining and semi-quantitative scoring. Therapies tested were diverse, including herbal extracts (39%), but intervention protocols varied widely. **Conclusions**: Chemically induced CP/CPPS models are valuable for translational research, but significant methodological variability should be ameliorated. Standardized protocols for induction, validation, and treatment assessments are needed to enhance reproducibility and clinical relevance of CP/CPPS models. **The PROSPERO Registration**: The study was registered at PROSPERO (CRD420251106861).

Keywords

Prostate; Animal models; Carrageenan; Complete Freund's adjuvant; Pain

Aspectos metodológicos de los modelos de prostatitis crónica inducida químicamente/síndrome de dolor pélvico crónico: una revisión sistemática

Resumen

Antecedentes: La prostatitis crónica/síndrome de dolor pélvico crónico (CP/CPPS) es un trastorno urológico de alta prevalencia, pero poco comprendido, con un éxito limitado de las terapias disponibles. Los modelos de CP/CPPS inducidos químicamente en roedores se han convertido en herramientas valiosas para la elucidación de su etiopatogenia y la evaluación de terapias. Sin embargo, la diversidad metodológica podría limitar su relevancia translacional. El objetivo de esta revisión sistemática fue evaluar diferentes aspectos metodológicos de los modelos de CP/CPPS inducidos químicamente en roedores. **Métodos**: Se realizó una búsqueda sistemática en PubMed y Scopus (desde el año 2000 hasta el 13 de junio de 2025), de acuerdo con las directrices Elementos de informe preferidos para revisiones sistemáticas y metanálisis (PRISMA). Se incluyeron los estudios que incluían modelos *in vivo* de CP/CPPS inducidos por agentes químicos (*p.ej.*, λ-carragenina, adyuvante completo de Freund). Los datos clave extraídos incluyeron especie y cepa, tipo y dosis de agente químico, diseño del grupo control, evaluaciones morfológicas y del dolor, e intervenciones terapéuticas. **Resultados**: Cincuenta y seis estudios cumplieron con los criterios de inclusión. Se utilizaron ratas con mayor frecuencia que ratones, con predominio de ratas Sprague-Drawly. La λ-carragenina fue el agente químico más utilizado para inducir CP/CPPS (en el 62.5% de los estudios). No se realizaron evaluaciones del comportamiento relacionado con el dolor en más de la mitad de los estudios incluidos (57%), mientras que la hiperalgesia mecánica se evaluó con mayor frecuencia que la hiperalgesia térmica. La validación morfológica se realizó en el 84% de los estudios, principalmente mediante tinción con hematoxilina y eosina y puntuación semicuantitativa. Las terapias probadas fueron diversas, incluyendo extractos de hierbas (39%), pero los protocolos de intervención variaron considerablemente. **Conclusiones**: Los modelos de CP/CPPS inducidos químicamente son valiosos para la investigación traslacional, pero se debe reducir la variabilidad metodológica significativa. Se necesitan protocolos estandarizados para la inducción, la validación y las evaluaciones del tratamiento para mejorar la reproducibilidad y la relevancia clínica de los modelos de CP/CPPS. **Registro PROSPERO**: El estudio se registró en PROSPERO (CRD420251106861).

Palabras Clave

Próstata; Modelos animales; Carragenina; Adyuvante completo de Freund; Dolor

1. Introduction

Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is the most prevalent, but also the least understood type of prostatitis, corresponding to Category III in the National Institute of Health (NIH) classification system [1]. CP/CPPS is characterized by persistent pelvic pain, urinary symptoms and sexual dysfunction in absence of an identifiable infection cause [2, 3] and comprise up to 90–95% of prostatitis cases in clinical practice. The underlying pathophysiological mechanisms of CP/CPPS remain largely unclear despite its high prevalence and significant impact on quality of life [4]. It poses a considerable challenge for effective diagnosis and treatment.

Animal models have been helpful in revealing the pathophysiological mechanisms of CP/CPPS development, as well as in preclinical evaluation of potential therapeutic strategies (both physical and chemical). Over the past decades, various animal models have been developed to replicate the complex clinical picture of CP/CPPS, including autoimmune, stress-induced, infection-based, hormone-related and chemically-induced models [5, 6]. Among these, chemically-induced models have gained increased attention due to their technical simplicity, reproducibility and the ability to inflammatory and pain related features of human CP/CPPS in a control manner [6, 7].

Chemical agents such as λ-carrageenan, complete Freund's adjuvant (CFA), formalin and capsaicin have been used to induce prostatic inflammation and pelvic pain in rodents [8–10]. These substances trigger local amicrobial immune activation, cytokine release and neurogenic inflammation, thereby generating pro-inflammatory environment that results in nociceptive sensitization. λ-carrageenan and CFA models have been particularly useful for studying the early immune responses and chronic pain pathways associated with prostatitis [11, 12]. Despite the growing number of studies using these models, methodological variability persists regarding the type of chemical agent, dosage, method of administration, species and strain of rodents, pain and inflammation evaluation methods. These diversities limit the comparability of results and their translational utility.

To date, narrative reviews have broadly summarized the characteristics of all available CP/CPPS models, including those based on infection, autoimmunity, hormone imbalance and chemical irritation [5, 6]. However, there is a lack of systematic evaluation focused on methodological aspects and features of experimental design of chemically induced CP/CPPS models. Having in mind their increasing use in translational research, it is essential to critically appraise these models to ensure alignment with clinical disease phenotypes.

The aim of the present systematic review was to fill this gap by providing a comprehensive methodological analysis of rodent studies employing chemically induced CP/CPPS models. Our focus was on dissecting key features of experimental design, including the type of chemical agent, administration protocol, rodents' strain and group formation, use of sham comparators, pain assessment methods and methods of histological validation, as well as treatments studied.

2. Materials and methods

2.1 Protocol and registration

This systematic review was prospectively registered with PROSPERO (ID: CRD420251106861). The study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [13], and the completed checklist has been included as **Supplementary material 1**.

2.2 Eligibility criteria

PICOS (Population, Intervention, Comparator, Outcomes, Study design) framework was used to develop the inclusion and exclusion criteria.

Inclusion criteria were as follows:

● Population: male rodents (rats or mice) used as animal models for CP/CPPS.

● Intervention: induction of prostatitis/pelvic pain by chemical agents (*i.e.*, λ-carrageenan, complete Freund's adjuvant (CFA), formalin, capsaicin, doxycycline).

● Comparator: Sham or intact animals treated or not with vehicle.

● Outcomes: Primary outcomes were histological verification of prostatic inflammation and/or pain behavior (*i.e.*, mechanical/thermal hyperalgesia/allodynia). Secondary outcomes included prostate index, pro-inflammatory cytokines.

● Study design: Original *in vivo* studies using rodent models. Only peer-reviewed full-length articles published in English were included.

Exclusion criteria were as follows:

● *In vitro* or *ex vivo* studies.

● Models based on autoimmune, hormonal, infection, or stress-induced mechanisms without chemical induction.

● Reviews, editorials, conference abstracts or commentaries.

● Studies not published in English.

2.3 Databases and search strategy

We have conducted a full search of two electronic databases PubMed and Scopus. The research included publications from 2000 up to 13 June 2025.

The search strategy was designed using Boolean logic adapted for each database. The search strings were as follows:

("chronic prostatitis" OR CPPS) AND (rat OR rats OR mice OR mouse OR rodents) AND (carrageenan OR capsaicin OR doxycycline OR formaldehyde OR agar OR lipopolysaccharide OR "complete Freund").

2.4 Study selection

All records identified through the database searches were imported into reference manager and duplicates were removed. Two independent reviewers screened the titles and abstracts. Full-text screening was conducted for all studies judged eligible. Discrepancies were resolved by consensus, and if not possible, in consultation with a third reviewer.

2.5 Data extraction

Data were extracted independently by two reviewers using a standardized data extraction form. Extracted data included.

● General study information: full citation details including authors, study title, journal and year of publication.

● Animal characteristics: species, strain, number of animals in groups and number of groups.

● Prostatitis induction design: chemical agent used, dose, volume, site and method of administration.

● Pain assessments: methods of mechanical and/or thermal hyperalgesia/allodynia verification.

● Morphological assessments: histological evaluation of prostate tissue and type of staining, grading of inflammation, prostate index calculation.

● Characteristics of control group: sham or intact animals treated or not with vehicles.

● Treatment studied: type of treatment, route of administration and duration.

● Proinflammatory cytokines assessments.

Discrepancies were resolved by consensus or consultation with a third reviewer.

2.6 Data synthesis and analysis

Extracted data were qualitatively and descriptively analyzed and frequencies are calculated and presented in percentages. Studies were grouped based on the type of chemical agent used. Results were presented in figures, and a narrative summary of the key methodological aspects were performed.

3. Results

3.1 Study selection and overview

A total of 208 studies were initially identified through predefined databases search. Upon removal of duplicates and application of inclusion and exclusion criteria, 56 studies were included in the final analysis. The study selection process is illustrated in the PRISMA flow diagram (Fig. 1).

General overview of the included studies (n = 56) is presented in **Supplementary Table 1**.

3.2 Animal species and strains used

Among the included studies, rodent species used for chemically induced CP/CPPS models were rats and mice (Fig. 2A). Rats were more frequently used than mice, with 93% of studies using rats. The most common rat strain was Sprague-Dawley, while predominant mouse strain was C57BL/6 as shown in Fig. 2B. This distribution reflects common choices in pain and inflammation research due to strain-specific responses to chemical agents.

3.3 Methodological characteristics of model induction

Various chemical agents were used to induce CP/CPPS (Fig. 3A) with λ-carrageenan being the most frequently used (62.5%). On the second place was CFA with 25% of all studies included in the review and formalin/formaldehyde on third place with frequency of 5%. Dosages and volumes of

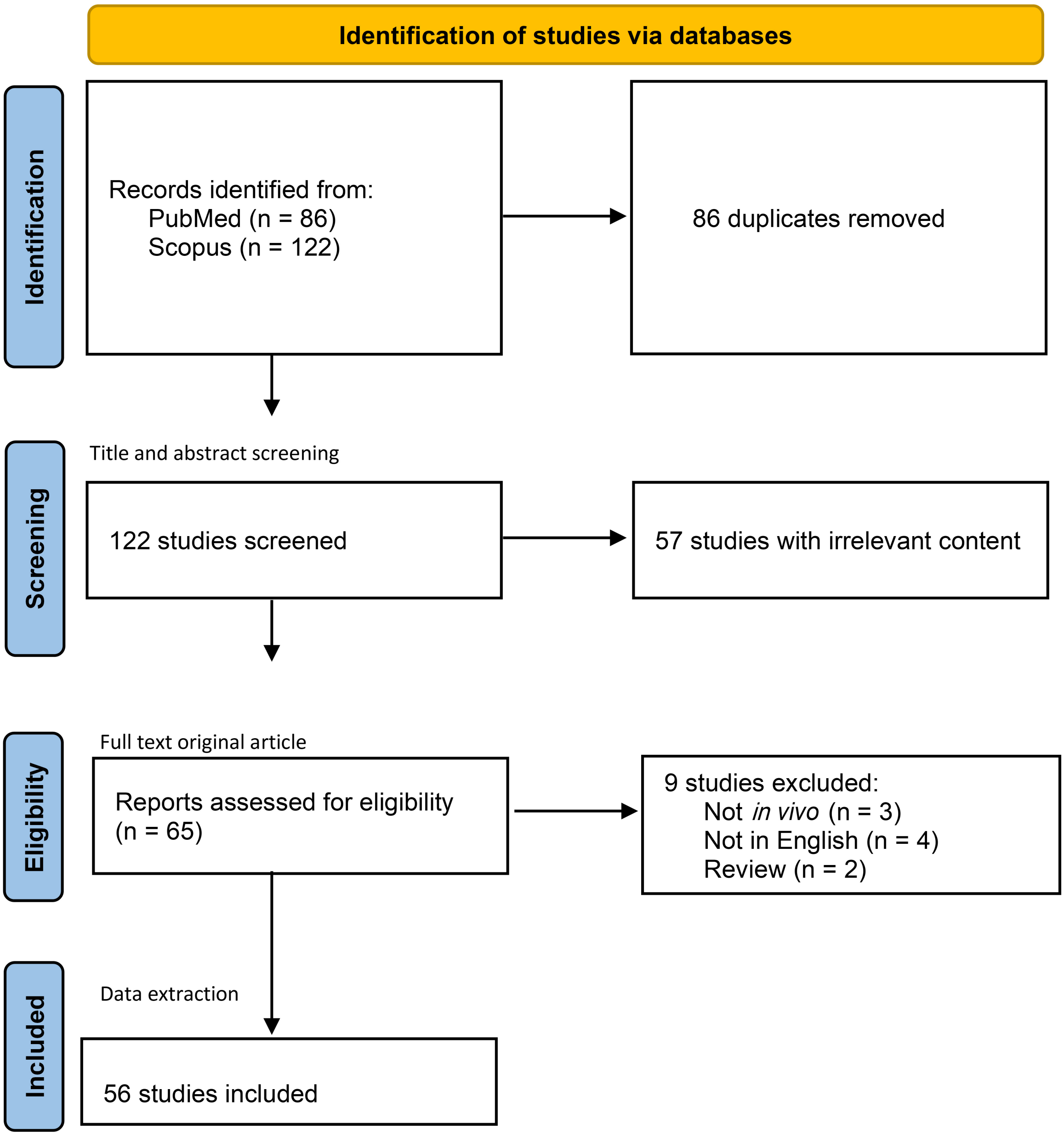

FIGURE 1. PRISMA flow diagram.

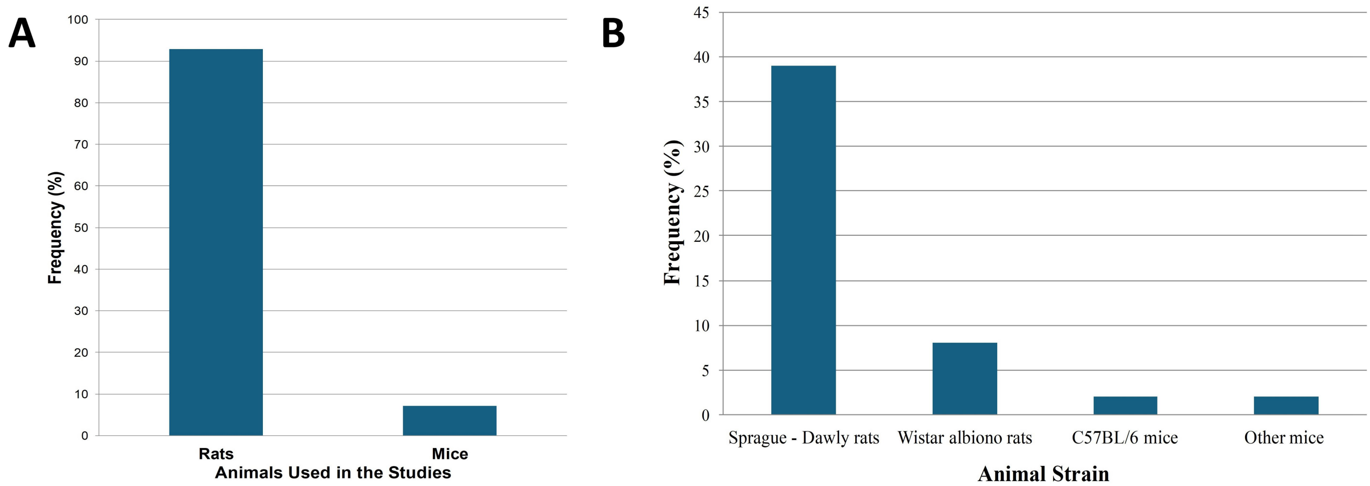

FIGURE 2. Species used in the chemically-induced models of CP/CPPS. Frequency of laboratory rodents used in chemical-induced model of CP/CPPS (A) with frequency of rat and mice (B) strains in included publication (n = 56 publications).

administration varied across the studies (Fig. 3B,C). The most frequent dose of λ-carrageenan was 1% (in 66% of includes studies) with not neglected number of studies using 3% (in 23%). Application volume for λ-carrageenan varied from 20 to 200 μL with median volume of 50 μL (50 to 100 μL) and mode of 100 μL. CFA was administered most frequently (in 69% of studies) in dose of 100 μL. Control groups (Fig. 3D) typically consisting of vehicle-treated sham operated animals or just sham operated animals, while intact/naïve animals were used in neglected number of included studies. The number of animals per group ranged from 2 to 50 with median number of 8 (6–10) and mean $\pm$ SD of 9.35 $\pm$ 6.75 animals per group and number of experimental groups per study varied widely (Fig. 3E) what reflects heterogeneity in experimental design.

3.4 Functional validation trough pain assessment

Pain-related behavior assessments were not done in more than half of the studies included (57%) (Fig. 4A). Determination of mechanical hyperalgesia/allodynia was more frequent (25%) than determination of thermal hyperalgesia/allodynia (12.5%) while only in minor number of studies both mechanical and thermal pain assessments were done. Mechanical pain sensitivity was assessed using dynamic von Frey aesthesiometers or von Frey filaments in majority of cases (Fig. 4B), while thermal sensitivity was evaluated using radiant heat or hot plate test (Fig. 4C). These functional readouts provide evidence of peripheral and central sensitization relevant to CP/CPPS.

3.5 Morphological validation of chemically induced CP/CPPS models

Morphological evaluation was performed in a substantial number (84%) of studies to confirm successful model induction (Fig. 5A). In less than half of included studies (46%), prostate index (ratio of prostate weight to total body weight) was calculated as a morphological indicator (Fig. 5B), although this method was less consistently applied. Histopathological assessment was most frequently conducted using hematoxylin and eosin (H&E) staining (57%), with some studies (30%) combining this with immunohistochemistry (IHC) for enhanced tissue characterization (Fig. 5C). In addition to qualitative histological analysis, in majority of included studies (58%), H&E-stained sections were further quantified using semi-quantitative scoring systems to assess glandular structure, inflammatory infiltration and tissue integrity (Fig. 5D).

These approaches underscore the heterogeneity in morphological validation methods and highlight the need for standardized scoring criteria to improve comparability and reproducibility.

3.6 Applications in therapeutic and mechanistic studies

A large proportion of the included studies used chemically-induced CP/CPPS models to test therapeutic interventions or investigate disease mechanisms (Fig. 6A). The treatment studied was diverse, ranging from conventional pharmacological agents (in 34% of included studies) to herbal extracts (39%) and non-pharmacological modalities (Fig. 6B). Routes of administrations (Fig. 6C) included oral gavage as a predominant route (56.5%), and intraprostatic injections (20%), with other routes like intraperitoneal injections were less frequent (in less than 10% of included studies). Duration of treatment varied substantially, and duration depended on the type of chemical agent used (Fig. 6D).

This diversity reflects the translational utility of chemically induced models for preclinical evaluation of novel therapies. However, the variability in intervention protocols also underscore the need for harmonization of treatment paradigms, including timing, dosage and administration routs, to ensure the generation of robust and comparable results.

3.7 Evaluation of inflammatory markers

Inflammatory markers were assessed in more than a half of the included studies (64.3%, Fig. 7A). Tumor Necrosis Factor α (TNFα), Interleukin (IL)-1β and IL-6 were the most frequently assessed (in 46.4%, 46.4% and 25% of included studies that evaluated inflammatory markers, respectively, Fig. 7B). Other inflammatory markers reported to be evaluated are IL-2, IL-8, IL-10, IL-17A, IL-18, Tumor Growth Factor β1 (TGF-β1), Interferon γ (IFNγ), C-X-C ligand (CXC).

4. Discussion

This systematic review provides a comprehensive synthesis of different methodological aspects of chemically-induced CP/CPPS models in rodents. Our review is based on 56 original studies that were included in analysis. The growing use of chemically-induced CP/CPPS models for elucidating disease mechanisms and testing therapeutic strategies are notable. However, significant heterogeneity was detected in chemical agents used, validation strategies in regard to detection of hyperalgesia and prostate inflammation, what could limit cross-study comparability and translational validity.

Our findings revealed that rats, predominantly the Sprague-Dawley strain, are the most frequently used species in chemically-induced CP/CPPS model, representing 93% of included studies. This choice of researchers could be justified as appropriate if we have in mind the similarities in morphology of human and rat prostate. Namely, the rat prostate shares key cellular architecture and developmental origins with the human prostate, although it is organized into four lobular units (ventral, dorsal, lateral, and anterior lobes). Furthermore, the dorsolateral rat lobe is considered anatomically and functionally analogues to the human peripheral zone [14] and is the most common site for intraprostatic injections.

Among chemical agents, λ-carrageenan was the most widely used inducer [15–49] in 62.5% studies, followed by CFA [50–63] in 25% of included studies, while capsaicin [64], lipopolysaccharide (LPS) [65, 66], formalin/formaldehyde [67–69], doxycycline [70], has been employed less frequently.

Our review pointed out variability in the choice of chemical agent, doses and administration volumes. For instance, λ-carrageenan was most often used at 1% concentration, but

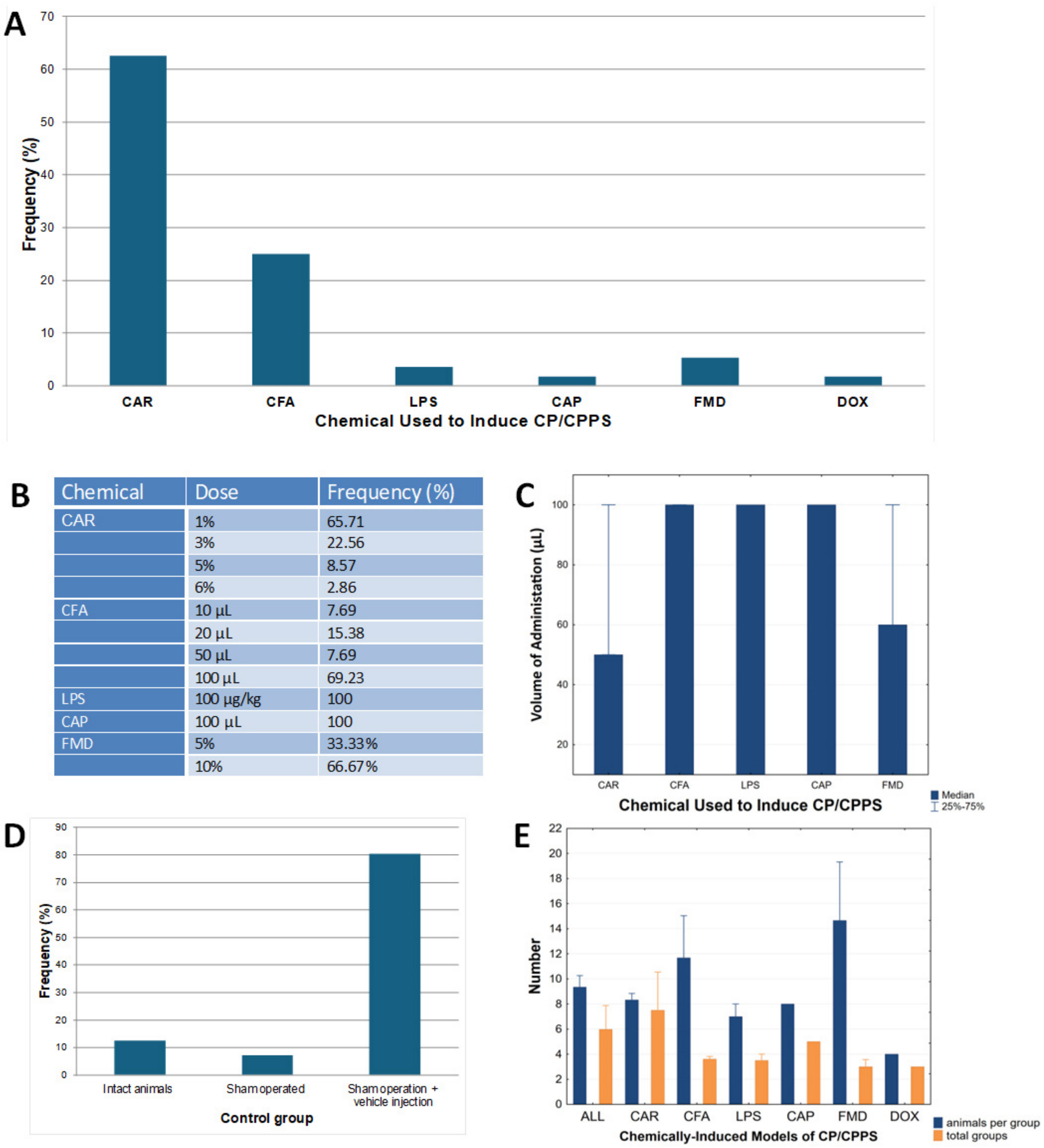

FIGURE 3. Methodological aspects of chemically-induced CP/CPPS in rodents. Type of chemical used (A), its dose (B) and volume of administration (C) in included publications. Characteristics of the control group included in the studies (D), as well as number of animals per group and total numbers of groups included (E). CAR: λ-carrageenan; CFA: complete Freund's adjuvant; LPS: lipopolysaccharide; CAP: capsaicin; FMD: formaldehyde; DOX: doxycycline; ALL: all models together; CP/CPPS: chronic prostatitis/chronic pelvic pain syndrome.

doses range from 0.5% to 3% and volumes from 20 to 200 μL. Such variations underline the lack of standardized induction protocols, which could make the extrapolation of results more complicated.

Having in mind that these chemical agents were delivered by intraprostatic injections, potential technical concerns could exist about chemical extravasation causing peritoneal inflammation if leakage occurs during administration. In order to mitigate this risk, recommended procedural safeguards should be followed: (i) reducing volume (*e.g.*, 50 μL of 3% λ-carrageenan instead of 100 μL) for intraprostatic injection; (ii) performing injections under laparotomy followed by layered closure of the abdominal wall and skin; and (iii) employing slow, controlled injection techniques to reduce backflow. These could ensure no tissue damage and peritoneal inflammation as reported in included studies [44].

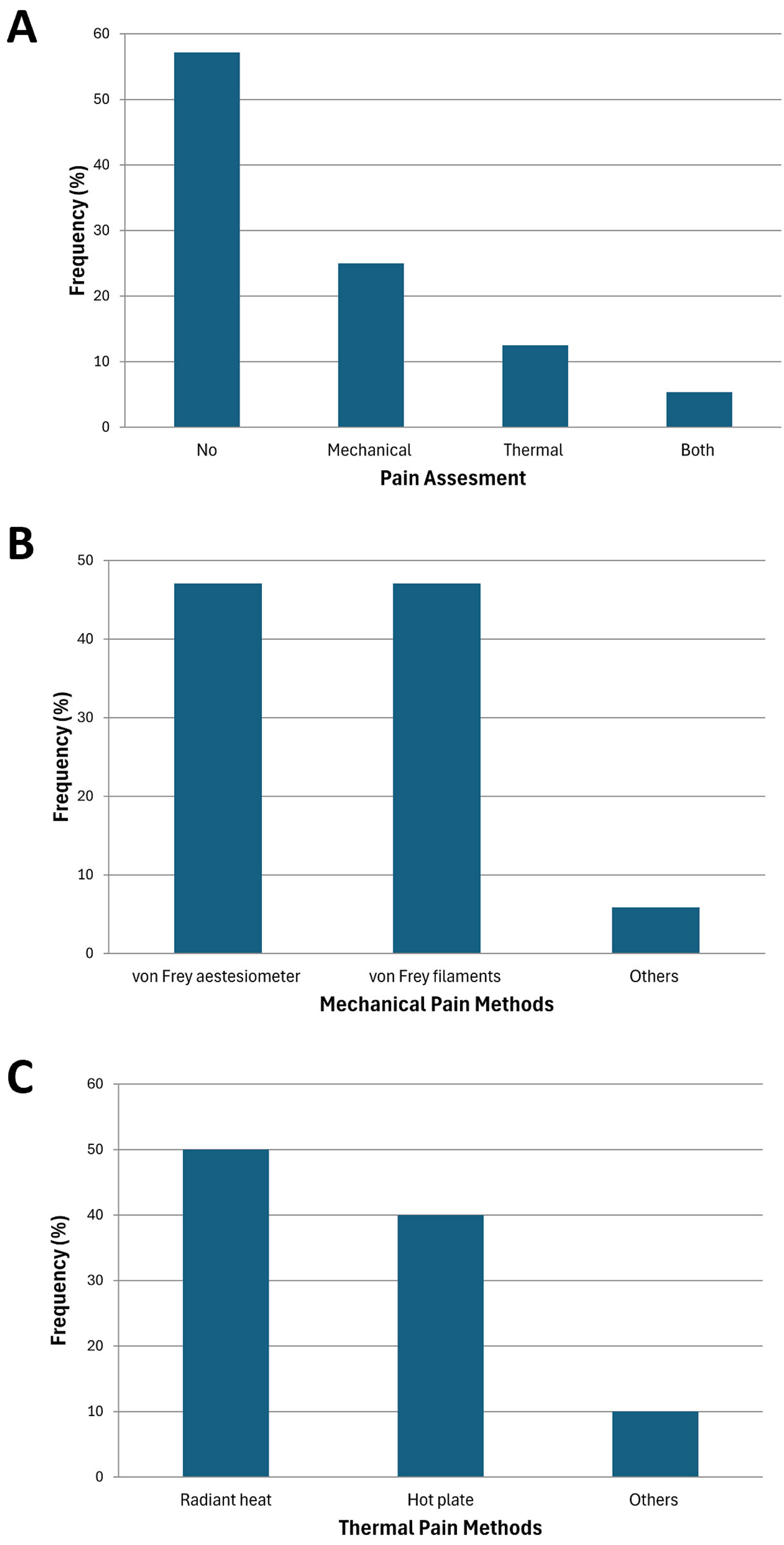

FIGURE 4. Functional validation: assessment of pain threshold in animals with chemically-induced CP/CPPS. Frequency of hyperalgesia/allodynia verification during CP/CPPS in included publications (A) with methods for mechanical (B) and thermal (C) pain assessments.

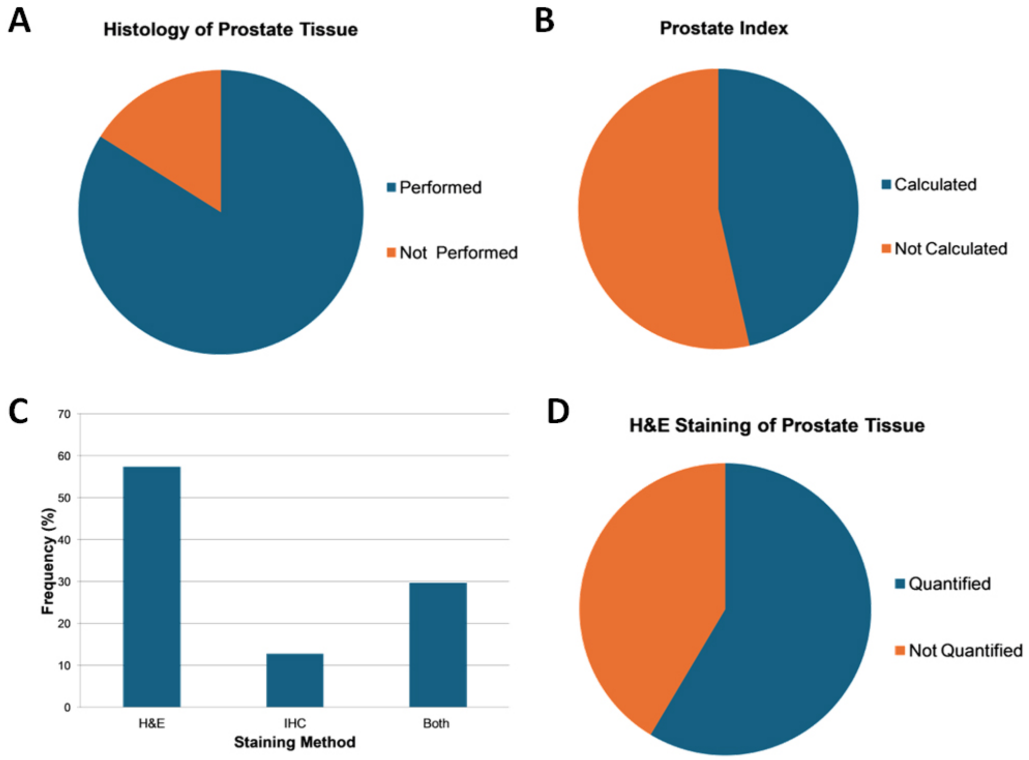

FIGURE 5. Morphological validation of chemically-induced CP/CPPS models. Morphological validation of the established model included histological evaluation (A) and/or calculation of prostate index (B) as a ratio of prostate weigh and total weigh of the animal. Histological evaluation included hematoxylin and eosin (H&E staining), immunohistochemistry (IHC) or both (C). H&E staining of prostate tissue in some cases were quantified (D).

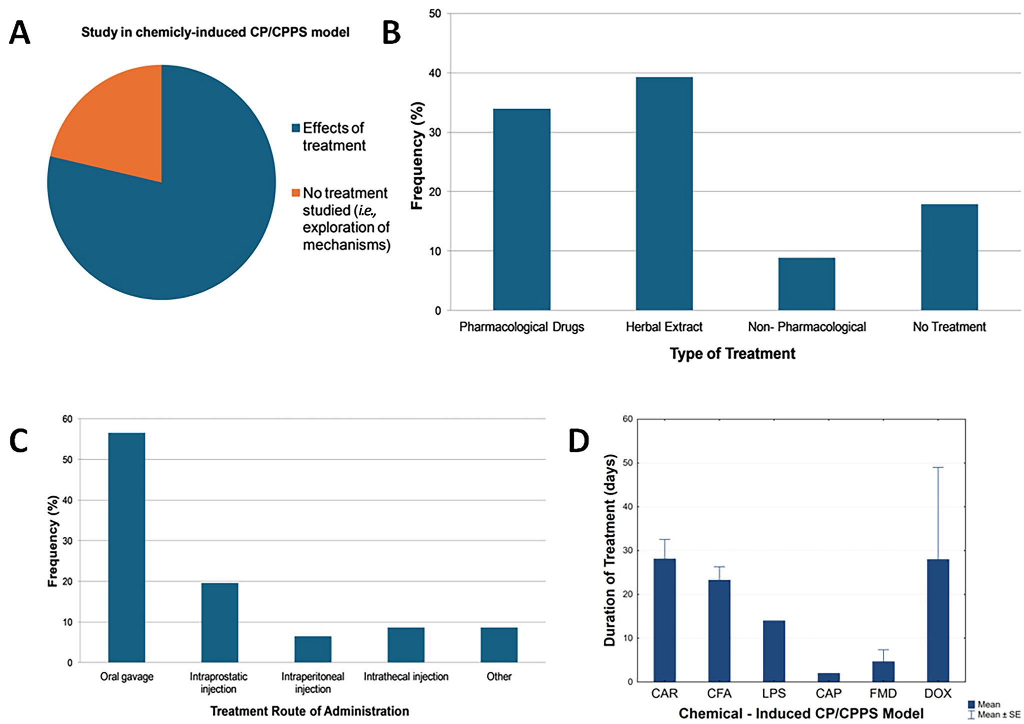

FIGURE 6. Treatments studied using chemically-induced CP/CPPS models. Frequency of studies using chemically-induced CP/CPPS models to examine the effects of treatment or mechanisms of diseases (A). Type (B) and rout of administration (C), as well as duration (D) of treatment studied. Treatments were classified as pharmacological drugs, herbal extracts or non-pharmacological treatments. Duration values are expressed as means ± standard error CAR: λ-carrageenan; CFA: complete Freund's adjuvant; LPS: lipopolysaccharide; CAP: capsaicin; FMD: formaldehyde; DOX: doxycycline; CP/CPPS: chronic prostatitis/chronic pelvic pain syndrome.

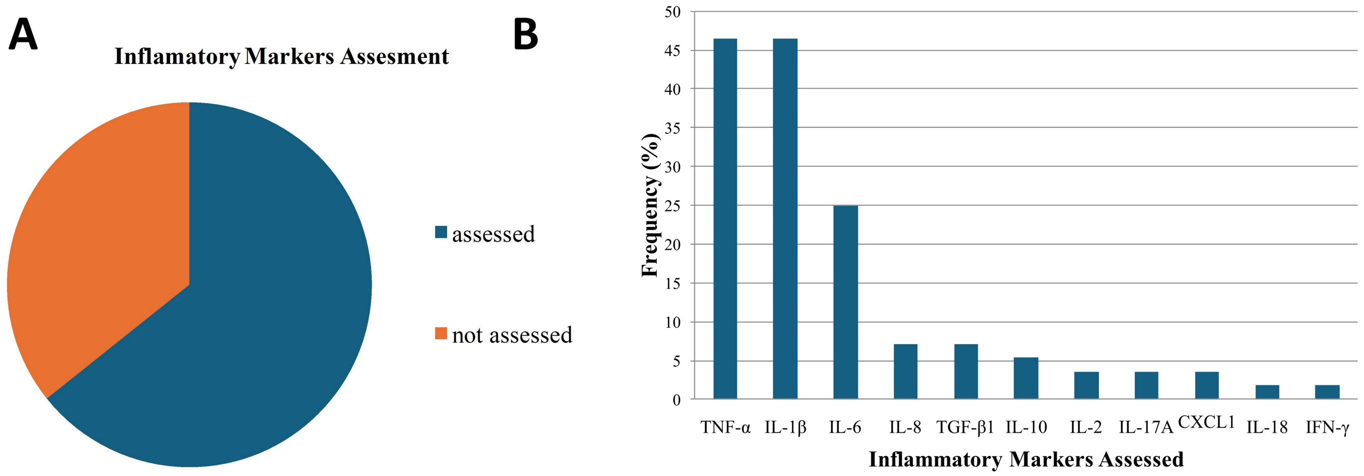

FIGURE 7. Frequency (A) and type (B) of inflammatory markers assessed in chemically-induced CP/CPPS models. Frequency of examined studies assessing the inflammatory markers, including their types TNFα (Tumor Necrosis Factor α), Interleukins (IL), Interferon γ (IFNγ), C-X-C ligand (CXC), Transforming Growth Factor β1 (TGF-β1).

λ-carrageenan, a polysaccharide derived from red seaweed, is widely used to model peripheral inflammation due to its ability to stimulate Toll-like receptor (TLR4) and activate pro-inflammatory signaling pathways [71, 72]. When injected into the prostate, λ-carrageenan induces rapid-onset inflammation and persistent pelvic pain, making it suitable for acute and subacute studies. We and many other studies have found robust functional and morphological validity of CP/CPPS induced by λ-carrageenan [8, 33, 35, 41].

CFA is an emulsion of heat-killed Mycobacterium tuberculosis in mineral oil, triggers strong type 1 T helper (Th1) and macrophage-mediated immune responses, leading to inflammatory states [73]. Intraprostatic CFA reliably produces sustained mechanical allodynia [74].

In Table 1 (Ref. [44, 46, 64, 73–75]) we summarized reported success rates, clinical translational value, and proposed mechanisms for major chemically-induced models of CP/CPPS.

Chemically-induced models of CP/CPPS differ not only in their induction protocols but also in their success rates, mechanisms, and translational relevance. For example, carrageenan reliably induces acute prostate inflammation and pain, with reported success rates of 100% under standard protocols [44, 46]. Its strength lies in reproducibility and cost-effectiveness, which explains its frequent use in analgesic and anti-inflammatory drug testing. However, carrageenan models are less suitable for studying long-term chronicity.

On the other hand, prostate antigen-based models show high and reproducible induction of chronic, immune-mediated prostatitis [75]. They are particularly applied when the research objective is to explore autoimmune pathways, chronic inflammatory cascades, and immune-targeted therapies. Their drawback is greater complexity and strain dependency.

Capsaicin, through Transient Receptor Potential Vanilloid 1 (TRPV1) activation, is chosen primarily for studies of neurogenic inflammation and sensory pathway modulation. Although success is high, its translational value is more focused on pain signaling rather than the immune aspects of CP/CPPS.

Other chemicals (formalin, doxycycline) induce inflammation with relatively high short-term success but are used less frequently due to nonspecific tissue injury and limited translational precision. Hence, the rationale for model selection should be strongly hypothesis-driven: researchers studying innate immunity or acute inflammatory cascades often prefer carrageenan; those focused on autoimmunity and chronic inflammation employ CFA or Experimental Autoimmune Prostatitis (EAP) protocols; while investigations into pain neurobiology often apply capsaicin or formalin models.

These differences explain why no single chemical model fully replicates human CP/CPPS. Actually, each model interrogates distinct pathophysiological axes (innate inflammation, neurogenic pain, or autoimmunity), making them complementary tools for translational research [76].

Pain-related behavior assessments were absent in more than half of the included studies, which is concerning given that pelvic pain is the hallmark of CP/CPPS. Among those that evaluated nociception, mechanical hyperalgesia/allodynia was more frequently measured than thermal sensitivity. The von Frey filament test, either in its manual or electronic form (dynamic aestesiometer), was the primary method for quantifying mechanical thresholds. These tests assess the withdrawal reflex or abdominal retraction reflex upon mechanical stimulation [77, 78]. However, manual von Frey test is subject to potential operator bias and variability in application force, whereas electronic aesthesiometer offer better reproducibility. Despite their utility, a number of studies lack report on habituation period, blinding of observer, which are essential for minimizing bias.

Thermal hyperalgesia was assessed in fewer studies (12.5%), commonly using radiant heat or hot plate test. While these paradigms measure latency to nocifensive responses (licking, jumping), their relevance to deep pelvic pain remains uncertain. Future models should consider visceral pain assessments, such as abdominal withdrawal reflex or referred hyperalgesia maps, which may better mimic symptoms seen in patients.

Micturition-related symptoms, sexual dysfunction and erectile dysfunction are common part of CP/CPPS mosaic requiring

TABLE 1. Summary of the major chemically-induced CP/CPPS models.

Chemical agent	Translational significance	Proposed mechanisms	Key references
λ-Carrageenan (intraprostatic injection; *e.g.*, 1–5% solutions)	Models robust prostate inflammation with measurable mechanical/thermal hyperalgesia—widely used to study pelvic pain mechanisms and analgesic interventions. Less consistent for very long-term chronicity beyond several weeks unless protocol altered. Success rate is 100% in several rodent studies (*e.g.*, 3% or 5% intraprostatic injections produced inflammation and pain in all treated animals; sustained pain for $\geq$2 weeks in some reports).	Local activation of innate inflammatory cascade with inflammatory cell infiltration, COX-2/prostaglandin upregulation, vascular permeability; produces peripheral sensitization and downstream central sensitization	[44, 46]
Capsaicin (intraprostatic injection)	Useful when the experimental question targets neurogenic inflammation, sensory afferent activation (C-fibers), and TRPV1-mediated pain signaling (*i.e.*, neuroimmune cross-talk). Reported as a robust, reproducible model in the original description—authors report consistent induction of neurogenic prostatitis and pain behavior in treated rats.	Direct TRPV1 activation on sensory C-fibers, neurogenic inflammation (substance P, CGRP release), peripheral sensitization and central changes	[64]
Complete Freund's Adjuvant (CFA)	-Intraprostatic CFA reliably produces sustained mechanical allodynia with success rate 100%. -With prostate antigen also widely used to induce EAP (success depends on antigen protocol and strain from 75 to 100%) appropriate for autoimmune/inflammatory pathway studies and therapeutic testing targeting immune mechanisms.	-Triggers strong Th1 and macrophage-mediated immune responses, leading to inflammatory states -Strong adjuvant effect; adaptive immune activation to prostate antigens (T cell infiltration, Th1/Th17 responses)	[73–75]

COX-2: Cyclooxygenase-2; TRPV1: Transient Receptor Potential Vanilloid 1; CGRP: calcitonin gene-related peptide; EAP: Experimental Autoimmune Prostatitis; Th1: type 1 T helper; Th17: type 17 T helper cells.

effective treatments [79, 80]. Model of LPS-induced CP/CPPS was valuable in examining micturition deficiencies [65]. as well as some other models [81, 82]. Sexual dysfunction has been examined mostly in non-chemically-induced models of CP/CPPS and EAP model has been demonstrated to be valuable model to evaluate sexual faction impairment related to CP/CPPS [83]. Using this model, it was shown that impaired erectile function in CP/CPPS is probably result of endothelial dysfunction, oxidative stress, as well as apoptosis and *corpus cavernosum* smooth muscle cell dysfunction [84–87].

Histopathological confirmation was conducted in 84% of the included studies, most often by H&E staining. These analyses revealed varying degrees of epithelial disruption, glandular atrophy, stromal edema, and immune cell infiltration as hallmark features of prostatitis. Prostate index was calculated in 46% of studies, while just over half applied semi-quantitative scoring systems for grading inflammation.

While H&E staining offers valuable morphological insights, the increased use of immunohistochemistry (IHC) in 30% of studies enhances cellular specificity. However, the scoring systems were rarely standardized or validated across the studies. Therefore, development and consensus on quantitative histopathological criteria, similar to the Gleason score in prostate cancer, would significantly improve model comparability and reproducibility.

Chemically-induced CP/CPPS models have been used to test wide spectrum of interventions. Herbal extracts (39%) and pharmacological agents (34%) dominate the field, with oral gavage being the most common route. Non-pharmacological approaches, including acupuncture or low-frequency electrical stimulation were reported in minority of the studies. It should be noted that the therapeutic landscape for CP/CPPS is heterogeneous: α-blockers, anti-inflammatory agents retain roles in selected patients, while pelvic-floor physical therapy, neuromodulation techniques, and psychological interventions address non-inflammatory and central sensitization components. Herbal and nutraceuticals (*e.g.*, quercetin,) have shown symptomatic benefit. Contemporary guidelines therefore recommend a phenotype-directed, multimodal approach (*e.g.*, UPOINT system assessing Urinary symptoms (U), psychosocial dysfunction (P), organ-specific symptoms (O), infection-related symptoms (I), neurological/systemic conditions (N), tenderness of skeletal muscles (T)) rather than reliance on any single therapy; mechanistic differences among therapies (innate inflammation *vs.* neurogenic pain *vs.* immune-mediated disease *vs.* myofascial contributors) explain why combining modalities often produces superior outcomes for many patients [88].

While no single model can fully reproduce the large complexity of the CP/CPPS syndrome, based on our own experience and reviewed included studies, we consider the carrageenan-induced model to be the most practical and reliable among chemically-induced models herein evaluated. It offers a good balance between technical feasibility, reproducibility of pelvic pain behaviors, and histological confirmation of localized prostatic inflammation, as also supported by prior studies [15–49]. Also, it has been the most frequently applied among chemically-induced CP/CPPS models. At the same time, immune-mediated models, like EAP provide important mechanistic insights into autoimmunity-related aspects of CP/CPPS, highlighting the complementary value of different approaches [75]. Different models should be considered complementary rather than mutually exclusive due to their specific advantages and disadvantages.

This review poses certain limitations. First, only English-language publications were included, which may present language bias. Second, we did not perform meta-analysis due to qualitative nature of data of interest. However, our descriptive synthesis reveals key trends and gaps in current methodologies.

To enhance reproducibility and translational value of chemically-induced CP/CPPS models, we recommend: (i) standardization of induction protocols, (ii) mandatory pain behavior assessment using validated methods in blinded fashion, (iii) use of sham controls with clear reporting on vehicle and surgical manipulation, (iv) inclusion of both morphological and functional endpoints, (v) adoption of Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines [89]. By addressing these methodological challenges, the filed can progress toward mora consistent and clinically relevant animal models that better mimic the complexity of human CP/CPPS.

5. Conclusions

Based on the presented findings, it could be concluded that chemically- induced CP/CPPS models are valuable for translational research. However, heterogeneity was detected in chemical agents used, validation strategies in regard to detection of hyperalgesia and prostate inflammation, what could limit cross-study comparability and translational validity. Theretofore, these methodological variabilities should be ameliorated. In order to enhance reproducibility and clinical relevance of CP/CPPS models, it is necessary to have standardized protocols for induction, validation, and treatment assessments.

AVAILABILITY OF DATA AND MATERIALS

The data presented in this study are available on reasonable request from the corresponding author.

AUTHOR CONTRIBUTIONS

NŠ, NE and DH—designed and conceptualizes study. DM, MV, EĐ, ARM and OS—provided additional help in data synthesis and qualitative analysis. Review of the included study were done by all authors. All authors were involved as reviewers. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at `https://files.intandro.com/files/article/2005871424042876928/attachment/Supplementary%20material.zip`.

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