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Karanjin in Preclinical In Vivo Models: A Systematic Review of Therapeutic Efficacy, Mechanistic Pathways, Pharmacokinetics, Safety and Formulation Strategies

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Research Article

Keywords: Karanjin, preclinical, animal models, pharmacokinetics, nanoformulation

Posted Date: October 1st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11080572/v1

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Additional Declarations: No competing interests reported.

Abstract

Karanjin is a furanoflavonoid which is isolated from the seeds of South Asian traditional plant known as *Pongamia pinnata*. In recent years, it has been reported to exert various pharmacological effects in preclinical studies across models of inflammatory, metabolic, dermatologic, skeletal, neurological and cancer. Previous studies have mainly investigated activities rather than testing whether efficacy is connected to causal mechanism, systemic exposure, reproducibility, and safety. This systematic review aimed to analyse *in vivo* therapeutic potential, mechanisms, pharmacokinetics, safety profile and formulation strategies along with translational gaps needed for clinical evaluation. The literature review was conducted through databases like PubMed, Web of Science, ProQuest and Scopus up to 30 March 2026, and the data was obtained for systematic analysis. 16 studies met the eligibility criteria, and the data was synthesised qualitatively by disease domain, with study design, sample size, dosing, mechanistic pathways and reporting transparency. The review was conducted in accordance with PRISMA 2020 and was registered in the PROSPERO (CRD420261422803). The risk of bias (RoB) was evaluated through SYRCLE's RoB tool for the *in vivo* studies. Therapeutic benefits were reported in models of various diseases such as inflammatory, neurological, cancer, metabolic, skeletal, gastrointestinal, respiratory, and dermatological but most domains were represented by single studies and mechanistic depth varied substantially. All the studies included in this review reported at least one beneficial result, yet most domains contained only one or two small studies. Only the acute-lung-injury study used an *in vivo* pathway perturbation to test causal involvement of TLR4, otherwise most other mechanistic claims arose from downstream biomarkers or accompanying cell experiments. No efficacy study quantified plasma or target-tissue karanjin in the same animals. Oral pharmacokinetic evidence indicated that there was a strong relationship between dose and systemic exposure but short elimination half-life and limited oral absorption. The acute and subacute toxicity study also suggested that there was acceptable short-term safety in experimental animals. Overall, from this systematic review, karanjin appears to be a promising multitarget preclinical drug candidate for future use, but needs further efficacy testing, better mechanistic proof, optimisation of pharmacokinetics, safety testing and ARRIVE-compliant reporting before clinical translation can be seriously considered.

1. Introduction

Natural products remain an important source of bioactive scaffolds for drug discovery and has provided humanity with some of the best medicine, like vincristine, vinblastine, quinine, etc. In the modern age, the demand for plant-based products, like pure phytochemicals, has increased in medicine, nutraceuticals, pharmaceuticals, biotechnological and other allied health sectors (Nasim, Sandeep, and Mohanty 2022). A large number of therapeutic drugs for the treatment of human health complications used in the developing and developed countries such as India, the United States of America, Europe, etc., were mainly derived from herbal drugs (Riaz et al. 2023; Gnanaraj et al. 2022). Further, preference is given to many plant based products in daily routine life to treat various kinds of human complications based on the belief of their safety profile compared to other available allopathic drugs in the world (Chaachouay and Zidane 2024).

The evolutionary refinement of plant secondary metabolites has yielded structurally diverse compounds with inherent biological activity, optimised protein-binding capacity and drug-like properties that synthetic chemistry often struggles to replicate *de novo* (Latif and Nawaz 2026). Phytochemicals have attracted considerable attention in recent years. Notably, many bioactive phytochemical compounds have been utilised lately in the in the field of medicinal chemistry (Yang and Ling 2025). Among these phytochemicals, flavonoids are one of the most studied polyphenolic classes, showing various bioactivities like antioxidant, anti-inflammatory, anticancer, antiproliferative, neuroprotective and metabolic effects (Hasnat et al. 2024). The therapeutic effects of flavonoids are shown by several clinically active compounds namely quercetin, genistein, and epigallocatechin gallate, which have served as drug candidates as well as molecular probes for analysing disease pathophysiology (Stachelska, Karpiński, and Kruszewski 2025).

In the flavonoid superfamily, furanoflavonoids constitute a specialised subgroup characterised by the fusion of a furan ring to the flavonoid backbone. This structural modification confers lipophilicity, increased membrane permeability and redox-modulating efficiency that differentiate furanoflavonoids from their conventional flavone and flavanol counterparts (Singh et al. 2016). The furan moiety participates in π-stacking interactions with aromatic amino acid residues and can act as an electron-rich center for radical scavenging, making furanoflavonoids effective modulators of oxidative stress and inflammatory signalling (Rao et al. 2009). In traditional Chinese Medicine and Ayurveda medicine, plants rich in furanoflavonoids have been utilised for dermatological ailments, gastrointestinal diseases and inflammatory conditions (Roy et al. 2019).

Pongamia pinnata, also known as karanja, pongam, or Indian beech tree, is a medium-sized leguminous tree native to the Indian subcontinent and widely found throughout tropical and subtropical Asia, Africa and Australia (Fugare et al. 2021). The plant has been documented in Ayurvedic medicine for its therapeutic ability and its various parts like seeds, seed oil, bark, leaves and roots have been traditionally used for skin diseases, rheumatoid arthritis, haemorrhoids, diarrhoea and sepsis (Jeelani et al. 2023). The plant's seed oil has been utilised topically for eczema, psoriasis, scabies and wounds, while bark and leaves have been used to manage febrile and inflammatory disorders (Pulipati et al. 2018).

Phytochemical analysis of *Pongamia pinnata* has shown a complex secondary metabolite profile dominated by furanoflavonoids, flavanols, chalcones and terpenoids (Sattar et al. 2024). Karanjin, a principal furanoflavonoid constituent is shown to be extracted from the seeds of the plant at 0.5-2% concentrations (Aina Akmal Mohd et al. 2020; Likhar and Fegade 2026). The percentage of karanjin extraction depends on the geographical location and extraction methods (Singh et al. 2021). Karanjin was first isolated in the early twentieth century and later characterised as a methoxylated furano flavonoid with a benzopyran core linked to a furan ring at the C-8 position (Likhar and Fegade 2026). This structural architecture provides hydrophobicity (logP ~ 3.8), may facilitate cellular uptake and interaction with membrane-bound and intracellular protein targets.

The pharmacological potential of karanjin is likely due its chemical structure. The conjugated double-bond system spanning the flavonoid A- and C-rings, combined with the electron-donating methoxy substituent at C-3 and the phenyl B-ring, generates a planar π-system capable of intercalating into DNA and interacting with ATP-binding pockets of kinases (Likhar and Fegade 2026). The furan ring enhances reactivity toward electrophilic centers and aids hydrogen bonding with enzymatic active sites (Bhatt, Limaye, and Rangan 2025). Moreover, the phenolic hydroxyl groups and the enone system provide radical-

scavenging capacity, enabling karanjin to participate in redox cycling and chelate transition metal ions that catalyse reactive oxygen species (ROS) formation (Rekha et al. 2021) (Singh et al. 2021). This polypharmacological profile is useful for complex, multifactorial diseases like cancer, neurodegeneration and metabolic disorders, where single-target interventions often fail due to multi-pathway involvement and complex mechanisms (Gnanaraj et al. 2022).

Last few years have witnessed karanjin to be examined in both *in vitro* and *in vivo* models. For instance, cell line study examining cytotoxicity against lung adenocarcinoma (A549), hepatocellular carcinoma (HepG2), leukemia (HL-60), breast cancer (T47D, MDA-MB-468, SKBR3) and prostate cancer (PC3) cell lines proposed mechanisms like G2/M cell cycle arrest, caspase-dependent apoptosis and PI3K/Akt pathway suppression (Guo et al. 2015; Yu et al. 2022; Roy et al. 2019). However, *in vitro* findings usually fail to translate to organism level efficacy due to pharmacokinetic limitations, off-target effects and limited representation of tissue-level pharmacology.

Therefore, a growing body of *in vivo* animal studies has also begun to validate karanjin's therapeutic potential across neurological, oncological, metabolic, inflammatory, gastrointestinal, respiratory, dermatological and skeletal disease models. These studies report improvements in behavioral outcomes, tumor burden reduction, glycemic control, joint inflammation reduction, colonic mucosal healing, lung injury reduction, bone mass preservation and psoriatic lesion attenuation (Alex et al. 2017; Shiven et al. 2024; Mujtaba et al. 2024; Tamrakar et al. 2008; Patel and Trivedi 2017; Bose et al. 2014). Preclinical studies suggest karanjin modulates inflammatory signalling and may target disease-specific pathways across metabolic, gastrointestinal, pulmonary, dermatologic, bone, neurological and oncologic models (Dinesh Kumar and Kanika 2022; Tirgar, Vekaria, and Raval 2025). However, this evidence is scattered across various models and outcomes and translational interpretation is limited by heterogeneity and variable reporting of randomization/blinding in animal studies. This systematic review therefore aims to synthesise and characterise all available animal studies for isolated karanjin in various disease models, to map mechanistic pathways, to assess pharmacokinetic properties, safety and formulation strategies and to identify key methodological and translational gaps that must be addressed before clinical application.

This broad scope was chosen because karanjin is an emerging bioactive compound with limited but diverse preclinical evidence across multiple disease models. Existing reviews have described karanjin's chemical characteristics and pharmacological activities, including its proposed neuroprotective potential. However, an assessment of therapeutic promise also requires examination of whether reported animal benefits are supported by reliable study designs, experimentally substantiated mechanisms, and pharmacokinetic and safety evidence relevant to the tested interventions. The present systematic review addresses this question by examining animal studies of isolated karanjin and its formulations alongside the available evidence on exposure and tolerability. Its contribution lies in distinguishing reported efficacy from the evidence needed to support therapeutic development, while identifying unresolved questions concerning mechanistic attribution, formulation effects, and reproducibility. This compound-centred synthesis provides a basis for prioritising the experiments required to advance karanjin beyond exploratory preclinical findings.

2. Methods

2.1 Review Question

To what extent do animal studies of karanjin connect therapeutic effects with experimentally supported mechanisms, relevant drug exposure, and adequately characterised safety?

2.2 Protocol and Registration

The systematic review was conducted according to the PRISMA 2020 guidelines (Page et al. 2021). A review protocol was developed and registered in the PROSPERO database under CRD420261422803.

2.3 Search Strategy

A comprehensive literature search was conducted in scientific databases (PubMed, Scopus, Web of Science, ProQuest) for articles published up to March 2026. The search was performed with a database-specific strategy using both controlled terminology and free terms which included "karanjin" or "karanja" or "*Pongamia pinnata*," AND "*in vivo*," or "animal model," or "animal studies" AND/OR "pharmacological" or "efficacy" or "preclinical" or "biological studies" AND "anticancer," or "anti-inflammatory," or "antioxidant," or "neuroprotective," or "anti-diabetic" or "cardioprotective" or "dermatological" or "topical" or "toxicity" or "pharmacokinetic". The exact search strings, platforms, limits and search date are reproduced in Supplementary File S1 in accordance with PRISMA reporting guidelines. Database searches were supplemented by hand-searching reference lists of included studies and relevant review articles, as well as forward citation tracking using Google Scholar. The search was restricted to studies written in English language. The retrieved records were checked in EndNote 21 with duplicate removal via both automatic matching and manual verification before export to Rayyan for screening of titles and abstracts by two independent reviewers. Disagreements were resolved by discussion, and unresolved conflicts were adjudicated by a third reviewer. The final search was conducted on March 30, 2026.

3. Eligibility Criteria

3.1 Inclusion Criteria

The PICO framework (Table 1) was followed for inclusion criteria. The population consisted of live animals used as models for inducing human diseases, like rodents, fishes or any other mammals in animal testing. Intervention types were defined by using karanjin as the candidate drug. Study designs were eligible if it was presented as karanjin formulations, such as liposomal or ethosomal nanogels, which should be compared against either the drug itself or its carrier. Comparators may include either the standardised/control drug or the model disease itself. Results to be measured include any type of treatment/therapeutic outcome, such as pharmacological, toxicological and in vivo formulation-related outcomes.

Table 1
PICO Framework

Components	Description
Population	Animal disease models (rats, mice, zebrafish) and healthy animals used for pharmacokinetic and toxicological studies
Intervention	Karanjin (isolated compound or formulated)
Comparator	Control / disease model, standard drug
Outcomes	*In vivo* efficacy, anticancer, anti-inflammatory, antioxidant, neuroprotective, anti-diabetic, metabolic, dermatological, toxicity outcomes

3.2 Exclusion Criteria

Studies were excluded if they were reviews, *in vitro* studies, in silico studies, conference abstracts, retracted publications, full text could not be retrieved, studies not in English language, studies carried out on the isolated Pongamia extracts other than karanjin.

3.3 Study Selection and Data Extraction

Two independent reviewers screened titles and abstracts, followed by full-text review of the articles selected. Discrepancies were resolved through discussion with a third reviewer. Extracted data included first author and year, compound, animal species, sex, sample size per group, disease model, karanjin dose, route, frequency, duration, reported mechanisms of action and key findings. Given substantial heterogeneity in animal species, disease models, dosing, formulations, comparators, and outcome measures, meta-analysis was not considered, and findings were synthesized narratively by therapeutic domain.

4. Risk of Bias (RoB) Assessment

Methodological quality and RoB were evaluated using the SYRCLE (Systematic Review Centre for Laboratory Animal Experimentation) RoB tool, specifically designed for animal intervention studies (Hooijmans et al. 2014). The tool assesses ten domains: (1) sequence generation, (2) baseline characteristics, (3) allocation concealment, (4) random housing, (5) blinding of caregivers/investigators (performance bias), (6) Random outcome assessment (7) Blinding of outcome assessor, (8) incomplete outcome data, (9) selective outcome reporting, (10) other sources of bias (e.g., inappropriate influence of funders, inappropriate experimental unit, major internal data inconsistency, contamination). Each domain was rated as "low risk," "unclear risk," or "high risk" based on explicit reporting in the publication. A low-risk judgment was assigned when the study provided a clear indication and sufficient information of an adequate method; high risk judgement was only assigned only where there was a clear indication of an explicitly inadequate method; and unclear risk was assigned when reporting was insufficient to make a definitive judgment. Statements that animals were “randomly allocated” or “randomly divided” without specifying the method used to generate the random sequence were rated as unclear rather than low risk. In the same way, absence of information regarding allocation concealment, random housing, or blinding was judged as unclear rather than high risk. For random outcome assessment, studies in which all the animals were included in outcome assessment were determined as low risk, whereas studies assessing only a subset of animals without describing random selection were considered unclear (Hooijmans et al. 2014).

5. Results

5.1 Study Selection

The systematic search identified 1,265 records from four different databases namely PubMed, Scopus, Web of Science and ProQuest, yielding 1,060 unique records after duplicate removal. Title screening removed 884 records that were irrelevant and did not meet eligibility criteria. Of the 176 records screened, 111 were excluded based on further title and abstract screening. 65 records were assessed for full-text eligibility and n = 49 were excluded for the following reasons: *in silico* only (n = 13), review/editorials (n = 7), no *in vivo* animal data or *in vitro* study (n = 12), crude extract studies without isolated karanjin (n = 2), conference abstracts (n = 6) and other reasons (n = 9). Sixteen publications met the inclusion criteria and were included in the qualitative synthesis. The PRISMA flow diagram is presented in Fig. 1.

Additional animal studies identified during the verification were screened against the prespecified eligibility criteria. An older alloxan-diabetes rat report was not added because the full text could not be obtained for eligibility and data verification. A common-carp genotoxicity study evaluated environmental pesticide exposure rather than a human-disease or therapeutic model and a gastroprotective rat publication was excluded because it had been retracted. Studies of crude Pongamia extracts, karanja oil, complex polyherbal preparations, insect or larval toxicity and cell-only anticancer activity remained outside the review's intervention or population criteria.

5.2 Study Characteristics

The sixteen included studies were published between 2008 and 2026 (no lower publication year limit was applied and 2008 was the earliest eligible study identified), with a notable concentration of publications (n = 10) between 2021–2026, reflecting recent interest in this compound. The studies utilised mice (n = 6 studies), rats (n = 11) and zebrafish (*Danio rerio*, n = 1). Two publications used more than one rodent species. Mouse strains included C57BL/6 (n = 2), BALB/c (n = 1) and Swiss albino (n = 2), rat strains included Sprague-Dawley (n = 5) and Wistar (n = 5). Sex distribution was male-only (n = 5), female-only (n = 3), both sexes (n = 5) and unspecified (n = 3). Sample sizes varied from 3 to 12 animals per experimental group. The common route of administration was oral gavage (n = 11), followed by topical application (n = 3), intraperitoneal injection (n = 1) and immersion in water (zebrafish, n = 1). Dosing ranged from 5 mg/kg to 2000 mg/kg for systemic administration and 2.5–5% w/v for topical formulations while the acne study used a 3% w/v ethosomal gel and reported topical dose levels of 0.05, 0.5 and 5 mg/cm2. A comprehensive summary of study characteristics is provided in Table 3.

5.3 Risk of Bias Assessment

The SYRCLE RoB assessment analysed methodological limitations across the sixteen included animal studies (Hooijmans et al. 2014). Sequence generation was determined as low risk in only two studies whereas one study was considered high risk because the authors explicitly reported use of a non-random allocation procedure for the behavioral experiment (Logeshwari et al. 2025) (Patel and Trivedi 2017; Lubau et al. 2026; Ma et al. 2025), with the other thirteen studies providing insufficient information to determine whether randomization was truly random (Unclear RoB). Baseline characteristic similarity was reported or inferable in only two reported studies but unclear in 14 (Ansari et al. 2021; Lubau et al. 2026; Ma et al. 2025). Allocation concealment was not clear in most of the studies and only reported in one study and high risk in Logeshwari et al. (2025) (Tirgar, Vekaria, and Raval 2025; Lubau et al. 2026; Logeshwari et al. 2025). Random housing to prevent cage-level confounding was mentioned in only two studies Rekha et al. (2021) and Mujtaba at al. (2024) (Rekha et al. 2021; Ma et al. 2025; Mujtaba et al. 2024). Blinding of caregivers and investigators was explicitly reported in two toxicology studies but unclear in the remainder studies (Lubau et al. 2026; Naresh Shejawal 2014). Random outcome assessment was judged as low risk in 12 of the 16 included studies as these studies evaluated the complete experimental cohort. Blinding of outcome assessors was reported in four studies. Incomplete outcome data was generally low risk of bias (Shiven et al. 2024). Selective reporting was also considered low risk of bias based on the consistent group sizes except 3 studies(Tirgar, Vekaria, and Raval 2025; Bose et al. 2014; Tamrakar et al. 2008). Two studies were determined as high risk for other sources of bias because of material internal inconsistencies between reported numerical or molecular outcomes.

Table 2
SYRCLE Risk of Bias Assessment for Included *In Vivo* Studies

Study	Disease/Model	Sequence generation	Baseline characteristics	Allocation concealment	Random housing	Blinding of caregivers/investigators	Random outcome assessment	Blinding of outcome assessor
		1	2	3	4	5	6	7
Ansari et al 2021 (Ansari et al. 2021)	Topical inflammation, acne and dermal irritation	U	U	U	U	U	L	U
Logeshwari et al., 2025 (Logeshwari et al. 2025)	Neurotoxicity	H	U	H	U	U	U	L
Zhang et al., 2024 (Zhang et al. 2024)	Acute lung injury	U	U	U	U	U	L	U
Tirgar et al., 2025 (Tirgar, Vekaria, and Raval 2025)	Breast cancer (DMBA)	U	U	U	U	U	L	U
Zhang et al., 2021 (Zhang et al. 2021)	Colon carcinogenesis (DMH/DSS)	U	U	U	U	U	L	U
Saini et al., 2017 (Saini, Lakshmayya, and Bisht 2017)	Amnesia (diazepam)	U	U	U	U	U	L	U
Bose et al., 2014 (Bose et al. 2014)	Arthritis (AIA)	U	U	U	U	U	U	U
Lubau et al., 2026 (Lubau et al. 2026)	Acute/subacute toxicity	L	L	L	L	U	L	U
Tamrakar et al., 2008 (Tamrakar et al. 2008)	Diabetes (STZ/db/db)	U	U	U	U	U	U	L
Trivedi et al., 2017 (Patel and Trivedi 2017)	Colitis (TNBS)	U	U	U	U	U	L	U
Ma et al., 2025 (Ma et al. 2025)	Osteoporosis (OVX)	L	L	U	U	U	L	U
Rekha et al., 2021 (Rekha et al. 2021)	Inflammation (carrageenan)	U	U	U	L	U	L	U
Shiven et al., 2024 (Shiven et al. 2024)	Tape-stripped mouse-tail psoriasis	U	U	U	U	U	L	U
Mujtaba et al., 2024 (Mujtaba et al. 2024)	Psoriasis (IMQ)	U	U	U	L	U	L	U
Shejawal et al., 2014 (Naresh Shejawal 2014)	Pharmacokinetics	U	U	U	U	L	U	L
Yi et al., 2015 [40]	Pharmacokinetics	U	U	U	U	U	L	L

L = Low risk of bias, H = High risk of bias, U = Unclear risk. (1) Sequence generation, (2) Baseline characteristics, (3) Allocation concealment, (4) Random housing (5) Blinding of caregivers/investigators (6) Random outcome assessment (7) Blinding of outcome assessors (8) Incomplete outcome data, (9) Selective outcome reporting, (10) Other sources of bias.

Note

Scale was adapted according to the SYRCLE Risk of Bias tool for *in vivo* animal studies (Hooijmans et al. 2014).

6. Therapeutic Domain Synthesis

6.1 Neuroprotective Disorders

Two studies in this systematic review explored the neurobehavioural and neurocognitive effects of karanjin. A study by Saini et al. (2017) utilised diazepam-induced amnesia model to evaluate karanjin's neuro cognitive effects (Saini, Lakshmayya, and Bisht 2017). Karanjin at 25–50 mg/kg administered orally improved memory shown by water maze test in diazepam induced mice. Likewise, Logeshwari et al. (2025) exposed adult zebrafish to bisphenol A (BPA) (4 mg/L) to induce neurotoxicity and found karanjin (5–10 mg/L) prevented cognitive deficits (Logeshwari et al. 2025). Karanjin improved novel-tank and t-maze behaviors and restored normal acetylcholine levels by inhibiting acetylcholinesterase activity as well as reversed hippocampal degeneration and oxidative stress.

6.2 Cancer

Two studies investigated karanjin in oncologic models. Zhang et al. (2021) used male Wistar rats in a dimethylhydrazine/dextran-sodium-sulfate model of colon carcinogenesis and administered karanjin orally at 50, 100, or 200 mg/kg for 18 weeks. Histology showed reduced hyperplasia/dysplasia and better mucin/goblet-cell preservation. Karanjin reduced aberrant crypt foci and colon lesions, increased catalase, glutathione and superoxide dismutase, enhanced apoptosis and altered p53, Bcl2, BAX and PCNA expression (Zhang et al. 2021). A second study by Tirgar et al. (2025) used DMBA-induced mammary tumourigenesis female Sprague-Dawley rat model (Tirgar, Vekaria, and Raval 2025). Karanjin administered at 50–200 mg/kg orally reduced tumor volume and changed the level of tumor markers positively like SMAR1 (increased), CDP/Cux, ER/PR/HER2 (reduced). This suggests karanjin interfered with cancer cell proliferation and survival signals in this model.

6.3 Diabetes (Metabolic Disease)

A study by Tamrakar et al. (2008) in the characteristics Table 3 reported that karanjin lowered blood glucose level significantly in diabetic rats and db/db mouse (Tamrakar et al. 2008). In STZ rats, single dose of 50 and 100 mg/kg lowered blood glucose by 11.7 and 20.7% respectively compared with controls while in db/db mice 100 mg/kg dose for 10 days reduced glucose level by 30.6%. In addition to blood glucose levels karanjin also improved lipid profiles by reducing cholesterol and triglycerides. The study also demonstrated inhibition of protein tyrosine phosphatase-1B (PTP1B) in a parallel enzyme assay and proposed PTP1B as a possible target.

6.4 Arthritis and Inflammation

One of the studies by Bose et al. (2014) found that karanjin 10–20 mg/kg administered orally reduced Freund's adjuvant-induced arthritis (AIA) in male Wistar rats (Bose et al. 2014). They observed significant, dose-dependent reductions in urinary hydroxyproline and glucosamine (markers of joint collagen breakdown) and in serum TNF-α, along with improved paw swelling, arthritis scores and histopathology. In parallel, in LPS-stimulated macrophages karanjin (4–6 μg/mL) suppressed NF-κB activation and downstream NO/ROS production, explaining the lower TNF-α secretion. Thus, karanjin's *in vivo* anti-arthritic effect correlates with *in vitro* anti-inflammatory signalling.

Another study by Rekha et al. (2021) evaluated karanjin in acute inflammation models, carrageenan-induced paw edema and xylene-induced ear edema in Wistar rats (Rekha et al. 2021). Karanjin demonstrated significant anti-edema activity. Karanjin was administered orally at 50 mg/kg and inhibited paw and ear oedema by approximately 34.1% and 51.1%, respectively. This study also included parallel in vitro antioxidant and soy lipoxygenase-1 experiments, in which Karanjin inhibited soy LOX-1.

6.5 Bone Remodeling

A study by Ma et al. (2025) have seen the effect of karanjin in ovariectomised C57BL/6 female mice and analyzed the bone protective effects (Ma et al. 2025). The micro-CT analysis showed that karanjin at 15–50 mg/kg given intraperitoneally twice weekly for six weeks restored trabecular bone mass and microarchitecture compared to OVX control. They reported no liver or kidney toxicity at reported doses.

6.6 Gastrointestinal Inflammation (Colitis)

In a study by Patel & Trivedi (2017) karanjin administered orally at 100 and 200 mg/kg in a mouse model of TNBS induced colitis improved colon ulcer (Patel and Trivedi 2017). The 200 mg/kg group significantly improved ulcer and fibrosis and reduced MPO and TNF-a levels. The 100 mg/kg however showed moderate improvement as compared to 200 mg/kg. Thus, karanjin is suggested to show anti-colitic effect at higher doses.

6.7 Respiratory/Lung Injury

Zhang et al. (2024) examined the therapeutic effect of karanjin in male C57BL/6 mice with lipopolysaccharide (LPS)-induced sepsis-associated acute lung injury (Zhang et al. 2024). Karanjin administered orally at 50, 100 and 200 mg/kg attenuated acute lung injury by reducing lung tissue edema and neutrophil infiltration. This improvement was dose-dependent with 200 mg/kg showing the most beneficial effect. Moreover, it lowered serum TNF-α and IL-6 levels and provided stronger pathway evidence of TLR4 activation. Histology also confirmed restoration of alveolar structure.

6.8 Dermatological Studies (Formulation)

Three studies from this systematic review evaluated karanjin in dermatological models. Shiven et al. (2024) developed a karanjin-loaded liposomal lotion (2.5% and 5% w/v) and tested it against a plain karanjin lotion (5% w/v) in a tape-stripped mouse tail model (Shiven et al. 2024). The liposomal formulation achieved 16.09% greater drug activity than conventional lotion, with enhanced skin permeation and retention. Mujtaba et al. (2024) formulated a karanjin-loaded ethosomal nanogel and evaluated it in an IMQ-induced psoriasis model in Sprague-Dawley rats (Mujtaba et al. 2024). The nanogel demonstrated 64% drug permeation, significant epidermal retention, reduced psoriasis severity scores and improved histopathology.

Ansari et al. (2021) evaluated a 3% karanjin-loaded ethosomal gel in Wistar rats (Ansari et al. 2021). In a testosterone-induced sebaceous-gland enlargement model (n = 5 per group), testosterone 1.42 mg/kg intramuscularly was administered for 14 days, followed by 7 days of topical treatment at reported dose levels of 0.05, 0.5 and 5 mg/cm2. K-EGF reduced both the number and size of sebaceous-gland units, with a more pronounced qualitative effect than the standard herbal and clindamycin gels. Short-term local tolerability was supported by a Draize score of 1.73 after 0.3 g was applied for 24 h (n = 3) and by histology showing intact dermis and skin appendages after twice-daily application for 2 days (n = 3).

7. Pharmacokinetics and Toxicity

Three studies from the Table 3 Shejawal et al. (2014), Yi et al. (2015) and Lubau et al. (2026) analyzed karanjin pharmacokinetics and toxicity (Lubau et al. 2026; Naresh Shejawal 2014; Yi, Wang, and Yi 2015). Shejawal et al. (2014) utilised HPLC to examine plasma levels after oral administration of karanjin at 10 mg/kg in female Sprague-Dawley rats and reported time of maximum concentration (T_{max}) = 2.3 h and elimination half-life ($T_{1/2}$) = 3.8 hours. However, the maximum plasma concentration C_{max} was only 0.498ug/ml at 50 mg/kg (1.5 μM), indicating limited bioavailability.

Yi et al. (2015) administered isolated karanjin orally to male Sprague-Dawley rats at 5, 10 and 20 mg/kg (Yi, Wang, and Yi 2015). Mean Cmax values were 187.5 +/- 25.2, 347.0 +/- 38.2 and 772.0 +/- 121.0 ng/mL. Tmax was approximately 1.5 h, terminal half-life ranged from 1.9 to 2.2 h and concentrations remained detectable up to 12 h. Cmax and AUC were dose-related, while dose-normalised exposure and elimination parameters did not differ significantly.

Lubau et al. (2026) conducted comprehensive oral toxicity evaluation (Lubau et al. 2026). In Sprague-Dawley rats, a single 2000 mg/kg dose caused no deaths or clinical signs of distress. In 28-day dosing, 250 mg/kg/day caused no significant changes in body weight, food or water consumption, organ or blood changes. 500 mg/kg/day led to slight AST/ALT increases but still within normal range. No gross histological damage was reported at 250 mg/kg.

Table 3
summarizing key information from the selected studies.

Author and Year	Animal Model	Study Design/ Methodology	Sex and Species	Doses Used	Sample Size	Mechanism of Action/ Pathway/Target	Parameters	D (I C
Logeshwari et al 2025 (Logeshwari et al. 2025)	Adult Zebrafish (*Danio rerio*)	Behavioral, biochemical and histopathological assessment	Both males and females	5 mg/L, 10 mg/L Karanjin	10	Neuroprotective via antioxidant action	Acetylcholinesterase activity, antioxidant levels (GSH, SOD), memory function	N ir
Zhang et al 2024 (Zhang et al. 2024)	Mice (C57BL/6)	LPS was used to induce sepsis-associated ALI in mice	Male	50, 100, 200 mg/kg Karanjin orally	6	Anti-inflammatory, antioxidant, TLR4 pathway suppression	Liver function, oxidative stress markers, histopathology, MPO activity, inflammation,	A s H n ir lu
Tirgar et al 2025 (Tirgar, Vekaria, and Raval 2025)	Rats (Sprague-Dawley)	Tumorigenesis study (DMBA-induced breast cancer)	Female	50, 100, 200 mg/kg/day Karanjin orally	6	Antioxidant and anti-inflammatory modulation	Tumor size, gene expression, oxidative stress markers	B n
Zhang et al., 2021 (Zhang et al. 2021)	DMH/DSS-induced colon carcinogenesis in Wistar rats	18-week oral efficacy study; histology, TUNEL, immunohistochemistry and antioxidant assays	Male Wistar rats	50, 100 and 200 mg/kg orally	5	p53/Bcl2/BAX-mediated apoptosis; reduced PCNA; antioxidant defense	Aberrant crypt foci, colon lesions, CAT, GSH, SOD, TUNEL, p53, Bcl2, BAX and PCNA	C ir c
Trivedi et al. 2017 (Patel and Trivedi 2017)	Balb/c mice	TNBS-induced colitis model (rectal administration of TNBS solution in 50% methanol)	Male and Female Balb/c mice	100 mg/kg and 200 mg/kg Karanjin, Sulfasalazine as reference drug (100 mg/kg)	6	Anti-inflammatory, antioxidative	Body weight, stool consistency, colon length and weight, MPO activity, oxidative stress markers (MDA, NO, CAT, SOD, GSH)	K tr T c
Saini et al 2017 (Saini, Lakshmayya, and Bisht 2017)	Swiss albino mice	Diazepam-induced amnesia; behavioral tests (EPM & MWM)	Male	25 mg/kg, 50 mg/kg	6	Neuroprotective, antioxidant effect	Transfer latency (EPM), escape latency (MWM)	d ir n
Bose et al 2014 (Bose et al. 2014)	Adjuvant Induced Arthritis (AIA) model in male Wistar rats.	AIA model with biochemical, radiographic, histopathological and parallel mechanistic analysis	male	Oral, 10 mg/kg/day and 20 mg/kg/day for 21 days.	6	Anti-inflammatory via NF-κB inhibition	Arthritis score, urinary hydroxyproline, glucosamine, radiographic and histopathological analysis, ROS, NO	A a
Lubau et al., 2026 (Lubau et al. 2026)	Sprague-Dawley rats	Acute and subacute oral toxicity study (single dose and 28-day repeated dose)	Male and Female	5 mg/kg, 500 mg/kg, 2000 mg/kg (acute); 5 mg/kg, 50 mg/kg, 250 mg/kg (subacute)	3 per group (acute); 8 per group (subacute)	Anti-inflammatory, antioxidative	Body weight, food intake, water intake, organ weights, serum ALT, AST, ALP, histopathology	A s d K

Author and Year	Animal Model	Study Design/ Methodology	Sex and Species	Doses Used	Sample Size	Mechanism of Action/ Pathway/Target	Parameters	D (I C
Tamrakar et al. (2008) (Tamrakar et al. 2008)	Streptozotocin-induced diabetic rats and hyperglycemic	Oral anti-hyperglycemic efficacy study in STZ-diabetic rats and db/db mice	Male and Female	Oral, 50 and 100 mg/kg	6	α-Glucosidase inhibition, AMPK activation, PPAR-γ modulation; hypolipidemic	Blood glucose, cholesterol, triglycerides, PTP1B enzyme inhibition	A h
Ma et al., 2025 (Ma et al. 2025)	C57BL/6 mice Ovariectomy (OVX)-induced osteoporosis model in mice	Randomised controlled trial in mice with OVX surgery to induce osteoporosis	Female	15 mg/kg, 50 mg/kg karanjin i.p	5	Inhibits osteoclastogenesis by blocking JAK2 phosphorylation and promotes osteogenesis via activation of Wnt/ β-catenin signalling	Bone volume fraction (BV/TV) -Trabecular thickness (Tb.Th) - Molecular markers (e.g., JAK2, β-catenin	K a ir t a b n a a
Rekha et al 2021 (Rekha et al. 2021)	Wistar rats	Anti-inflammatory paw and ear edema models	male	50 mg/kg Karanjin orally	6	Anti-inflammatory, antioxidant	Paw volume, ear thickness, antioxidant levels, histopathology	C ir e x e n
Shiven et al 2024 (Shiven et al. 2024)	Wistar rats, Swiss albino mice	Tape-stripped mouse tail psoriasis-like model	Male and female	Karanjin 2.5% w/v, 5% w/v liposomal lotion; plain Karanjin lotion 5% w/v	6	Anti-inflammatory, Formulation-enhanced local delivery; no direct molecular target established	Skin permeation, skin retention, inflammatory markers, histopathology	K li v l n
Mujtaba et al., 2024 (Mujtaba et al. 2024)	Sprague-Dawley rats	Karanjin-loaded ethosomal nanogel formulation development	Not reported	3% (w/w) karanjin-loaded ethosomal nanogel	6	Anti-inflammatory and epidermal retention via ethosomal delivery systems	Particle size, ZP, PDI, entrapment efficiency, *in vitro* release, drug retention	*I* p e I p
Shejawal et al., 2014 (Naresh Shejawal 2014)	Sprague-Dawley rats	oral pharmacokinetic study using validated RP-HPLC	Female	Karanjin (10 mg/kg via oral administration)	3	Anti-inflammatory, antioxidant, reduces NO and cytokine levels	Pk: Cmax- 0.498 +/- 0.01 ug/ml, Tmax – 2.3 h, t1/2- 3.78 h, elimination with 24 h	B f p k c a
Ansari et al., 2021 (Ansari et al. 2021)	Wistar albino rats; carrageenan paw edema, testosterone-induced sebaceous-gland enlargement and dermal irritation	Topical *in vivo* efficacy and local-tolerability study; only live-animal arms extracted	sex not reported for the *in vivo* arms	K-EGF 3% w/v; 0.3 g for 24 h in Draize test; reported anti-acne dose levels 0.05, 0.5 and 5 mg/cm2 for 7 days; applied 1 h before carrageenan (amount not reported)	n = 5/group (anti-acne); n = 3 (Draize); n = 3 (skin histology); paw-edema group size not reported	Local anti-inflammatory action and suppression of testosterone-induced sebaceous-gland enlargement; carrier contribution cannot be separated from karanjin	Paw volume and % inhibition; Draize score; skin histology; sebaceous-gland number and size	C ir e te ir s e s d
Yi et al., 2015 (Yi, Wang, and Yi 2015)	Sprague-Dawley rats	Dose-ranging oral pharmacokinetic study using validated LC-MS and	Male Sprague-Dawley rats	5, 10 and 20 mg/kg orally	6	Dose-dependent systemic exposure and elimination	Cmax, Tmax, AUC and terminal t1/2	C p

Author and Year	Animal Model	Study Design/ Methodology	Sex and Species	Doses Used	Sample Size	Mechanism of Action/ Pathway/Target	Parameters	D (I C
		noncompartmental analysis						

8. Discussion

8.1 Summary of Included Studies

Overall, this systematic review synthesised the effects of karanjin in 16 animal studies. It evaluated the therapeutic efficacy, mechanistic basis, pharmacokinetic properties, formulations and safety profile of isolated karanjin across eight distinct disease domains. The results suggest that karanjin demonstrates beneficial effects in neuroprotection, cancer, metabolic regulation, inflammation, bone health, gastrointestinal and dermatology. Across these studies, karanjin is suggested to behave as a broad-spectrum anti-inflammatory compound across multiple organ systems. In arthritis and colitis, it suppresses TNF-α and oxidative mediators, in lung injury, it blocks TLR4/NF-κB signalling, in cancer and osteoporosis, it modulates redox-sensitive proliferative pathways (STAT/ JAK, Wnt). This coherence is biologically plausible given karanjin's structure as a phenolic furanoflavonoid. Outcome measures range from blood glucose to behavioral scores to histology, making direct comparisons impossible. Therefore, quantitative pooling or efficacy ranking was not performed. It was concluded that the preclinical evidence is suggestive but not definitive.

Pharmacological rationale behind the use of karanjin is very compelling. As a furanoflavonoid, karanjin has hydrophobic and hydrogen bonding properties that make it possible to have interactions with many different proteins. The phenolic groups help karanjin in its antioxidant properties. Due to its ability to perform these functions, karanjin may be considered as a polypharmacological molecule and not as a molecule targeted at one site only (Raghav, Mahanty, and Rathinasamy 2019).

8.2 Therapeutic Domains

8.2.1 Anti-Inflammatory Effects

Chronic inflammation underlies numerous pathological conditions and karanjin has shown potent anti-inflammatory properties in multiple experimental models. In TNBS-induced colitis models, karanjin administration reduces macroscopic colon damage, decreases inflammatory cell infiltration and restores normal mucosal architecture (Patel and Trivedi 2017). The treatment significantly lowers myeloperoxidase, nitric oxide and malondialdehyde levels, while increasing antioxidant enzymes like catalase, superoxide dismutase and glutathione.

In the adjuvant-induced arthritis model in rats, karanjin was reported to reduce paw edema, suppress joint inflammation and attenuates cartilage loss (Bose et al. 2014). **Figure. 2.** illustrates the proposed mechanistic insights for anti-inflammatory behavior of karanjin (Bose et al. 2014). These therapeutic effects were often associated with inhibition of NF-κB nuclear translocation and decreased production of pro-inflammatory cytokines like TNF-α and IL-6. Suppression of the TLR4 signalling pathway in lipopolysaccharide-induced lung injury model further supports karanjin's anti-inflammatory potential (Zhang et al. 2024).

The most consistent finding reported in inflammatory studies of this review is karanjin's inhibition of NF-κB signalling and its upstream regulator TLR4 (Zhang et al. 2024). In adjuvant arthritis, karanjin inhibited NF-κB p65 nuclear translocation, along with reduced serum TNF-α, thus providing joint protection (Bose et al. 2014). In TNBS-colitis model, karanjin reduced MPO and NO while normalizing antioxidant enzymes, along with NF-κB-dependent iNOS and pro-inflammatory cytokine reduction(Patel and Trivedi 2017). In LPS-induced lung injury model, TLR4 pathway attenuation was accompanied by reduced TNF-α and IL-6, proposing the MyD88-dependent signalling cascade (Zhang et al. 2024). This mechanistic finding could be explained by the IKKβ kinase pathway, which phosphorylates IκBα to release NF-κB, contains a cysteine-rich activation loop susceptible to redox modulation and electrophilic attack by flavonoid metabolites (Fig. 2**)** (Hinz and Scheidereit 2014; Edwards et al. 2020). Nevertheless, the mechanism is less reliable than the phenotypic consistency. TLR4/NF-κB signalling was measured most directly in the lung and arthritis studies, whereas its involvement in other models was often inferred from downstream cytokines or NO. The macrophage experiments reported by Bose et al. (2014) support a possible role of NF-κB pathway in the anti-inflammatory mechanism of karanjin, but an *in vitro* observation does not prove that NF-κB inhibition mediated the *in vivo* joint benefit (Bose et al. 2014).

Moreover, the anti-inflammatory effects in the studies of this review performed on distinct organ systems like joint, gut, lung suggests that karanjin acts as a systemic immunomodulator rather than a tissue - specific compound. This is therapeutically promising for chronic inflammatory diseases (rheumatoid arthritis, inflammatory bowel disease, chronic obstructive pulmonary disease) but on the other hand raises safety concerns of immunosuppression and infection susceptibility. Notably, no included study evaluated immune competence (like delayed-type hypersensitivity, bacterial clearance assays), leaving this question unanswered. Additionally, the anti-inflammatory therapeutic effects were observed at higher doses (100–200 mg/kg), which may be difficult to achieve safely in humans during clinical translation.

8.2.2 Neuroprotective Activity

The neuroprotective role of karanjin in the two included studies of this review suggests that it can ameliorate cognitive deficits through restoration of cholinergic pathway and inflammatory profiles. In zebrafish exposed to bisphenol A, a neurotoxicant, karanjin treatment reverses behavioral deficits and restores cognitive function (Logeshwari et al. 2025), **fig. 3** presenting the proposed mechanistic insights underlying the neuroprotective behavior of karanjin

in BPA-induced neurotoxicity model. These improvements correlate with normalization of acetylcholinesterase activity and reduction in oxidative damage within neural tissues. Histopathological analysis demonstrates preservation of neuronal network following karanjin administration. This zebrafish BPA-neurotoxicity model is particularly interesting given growing concerns about environmental endocrine disruptors and developmental neurotoxicity. Karanjin's ability to restore AChE activity and preserve hippocampal structure aligns with the cholinergic hypothesis of memory dysfunction and suggests potential translation in neurodegenerative conditions such as Alzheimer's and Parkinson's disease. In another study conducted on mice models of chemically induced amnesia, karanjin improves memory retention and behavioural outcome in maze-water tests (Saini, Lakshmayya, and Bisht 2017). The neuroprotective effects are associated primarily to antioxidant activity and modulation of cholinergic signalling. While mechanistic exploration remains preliminary, the available data indicate that karanjin attenuates neurotoxicity by mitigating oxidative stress and inflammatory mediators in neural tissue. However, there are some limitations in both these studies that may halt its clinical translation. First, neither study examined pathway involvement through pharmacological blockade like, atropine challenge or genetic manipulation like AChE knockout. Moreover, although karanjin has moderate lipophilicity, (logP = 3.8), brain penetration of orally administered karanjin is uncharacterised, due to which efficacy of drug to CNS is not certain. Additionally, both the models employed (diazepam-induced amnesia, BPA neurotoxicity) are pharmacological or toxicological models rather than neurodegeneration models (e.g., APP/PS1 transgenic mice, Aβ25-35 injection). Future studies should examine the effects of karanjin in genetic AD models, assess cerebrospinal fluid concentrations and employ multi-electrode array or functional MRI tests to confirm circuit-level protection.

8.2.3 Anticancer Activity

In the two reported cancer studies in this review, karanjin exhibits significant anticancer activity across different animal models. In the DMH/DSS colon-carcinogenesis model, oral karanjin reduced aberrant crypt foci and colon lesions, increased endogenous antioxidant defenses, promoted TUNEL-positive apoptosis, increased p53 and BAX, reduced Bcl2 and limited proliferative signalling measured by PCNA (Zhang et al. 2021). In mice models of chemically induced breast cancer, karanjin administration significantly reduces tumor volume and weight. The upregulation of SMAR1 and downregulation of ER/PR/HER2 in the DMBA model is mechanistically important (Tirgar, Vekaria, and Raval 2025). SMAR1 (scaffold/matrix attachment region binding protein 1) functions as a tumor suppressor by recruiting histone deacetylases and regulating p53 stability, its upregulation by karanjin suggests epigenetic modulation. On the other hand, suppression of HER2 and hormone receptors indicates interference with mitogenic signalling pathways that drive breast cancer proliferation, **fig. 4** demonstrating the proposed mechanism underlying the anticancer activity of karanjin in DMBA-induced breast cancer model (Tirgar, Vekaria, and Raval 2025). These results indicate that G2/M cell cycle arrest and caspase activation are consistent with DNA damage checkpoint activation, possibly through ROS-mediated ATM/ATR signalling pathway. Histopathological analysis revealed increased apoptotic characteristics and low proliferating index. Moreover, the anticancer efficacy was found to be attained without any adverse effects on health at therapeutic levels, indicating a good therapeutic index.

It was demonstrated using *in vitro* experiments conducted on lung adenocarcinoma (A549), hepatocellular carcinoma (HepG2), leukemia (HL-60), breast cancer (T47D, MDA-MB-468, SKBR3) and prostate cancer (PC3) that karanjin causes a decrease in cell viability in a dose- and time-dependent fashion. The mechanism by which karanjin exhibits its cytotoxic activity is primarily by causing cell cycle arrest at G2/M phase. (Guo et al. 2015). The *in vivo* findings in this review are directionally compatible with cell studies in cancer showing G2/M arrest, apoptosis, ROS modulation and PI3K/Akt-associated role of karanjin (Guo et al. 2015; Yu et al. 2022). Yet these cell data should be used as mechanistic basis, not pooled with animal efficacy. Two additional observations complicate a uniformly anticancer narrative. First, transcriptomic analysis in MCF-7 cells found partial estrogen-like activity of karanjin (Bhatt, Limaye, and Rangan 2025). Second, karanjin interferes with ABCB1, ABCC1 and ABCG2 transporter function at low micromolar concentrations (Michaelis et al. 2014). Transporter modulation could potentially enhance intracellular exposure to some anticancer drugs, but it could also alter the pharmacokinetics and toxicity of co-administered medicines.

It seems that apoptosis is the main mechanism responsible for the cytotoxic effects of karanjin. There is evidence of increase in the number of apoptotic cells after the administration of this compound. It has been found that there is an activation of caspase-8, caspase-9 and caspase-3, which means that there is participation of the two mechanisms of apoptosis – intrinsic and extrinsic. The inhibition of the pathways of oncogenesis, such as KRAS and PI3K/Akt, has been detected in cancer cells. High concentration of intracellular ROS is evidence of the oxidative stress triggering apoptosis in cancer cells.(Panda et al. 2019).

Inspite of this, there is a need for further research on safety and efficacy of karanjin as an anticancer agent. With regard to the PK information, the dose administered for efficacy studies up to 200 mg/kg might not yield clinically significant drug exposure in plasma. Pharmacokinetics in tumors would help to establish that the biological effects demonstrated in such experiments are due to pharmacological reasons. Furthermore, lack of survival data, metastatic data and combination studies such as with paclitaxel or doxorubicin preclude evaluation of clinical translatability. In future studies, researchers can use PDX models and GEMMs.

8.2.4 Metabolic Regulation

In one of the study the hypoglycemic and hypolipidemic effects are observed in STZ-diabetic rats and db/db mice. Potential and proposed mechanisms include α-glucosidase inhibition (delaying carbohydrate absorption), AMPK activation (enhancing peripheral glucose uptake), preservation of pancreatic β-cell mass and PPAR-γ modulation (improving insulin sensitivity). A later cellular study by the same group showed that karanjin can promote GLUT4 translocation through a PI3K-independent mechanism, providing a plausible peripheral glucose-uptake hypothesis (Jaiswal et al. 2011). Tamrakar et al. (2008) study used traditional instead of modern metabolic phenotyping standards and lacks critical assessments like intraperitoneal glucose tolerance tests (IPGTT), hyperinsulinemic-euglycemic clamps, HOMA-IR calculations and pancreatic immunohistochemistry (Tamrakar et al. 2008). The 30% glucose reduction in db/db mice is clinically meaningful but must be contextualised against the natural disease progression in this model, which exhibits spontaneous glycemic fluctuations. Future metabolic studies should incorporate continuous glucose monitoring, body composition analysis (DEXA or MRI) to comprehensively assess metabolic syndrome-type outcomes.

8.2.5 Bone Remodelling

The OVX-osteoporosis study by Ma et al. (2025) included in this review examines bone remodelling (Ma et al. 2025). Mechanistically, karanjin suppressed osteoclastogenesis by inhibiting JAK2 phosphorylation (thereby reducing downstream STAT signalling and RANKL expression) while simultaneously promoting osteoblastogenesis via Wnt/β-catenin pathway activation. By simultaneously inhibiting osteoclastogenic JAK2/STAT signalling and activating osteoblastic Wnt/β-catenin, karanjin addresses both sides of the bone remodeling condition (**fig. 5)**, a dual action shared with only a few approved osteoporosis therapies (e.g., romosozumab, which inhibits sclerostin to promote formation, but does not directly inhibit resorption). The μCT data showing restored BV/TV and trabecular thickness are interesting, though biomechanical validation (e.g., femoral three-point bending, vertebral compression) is necessary to confirm that increased bone quantity translates to improved bone quality and fracture resistance. This study shows good mechanistic depth, combining high-resolution imaging with molecular pathway analysis. The use of intraperitoneal administration rather than oral likely achieved higher systemic exposure, which may explain efficacy but again complicates translation to oral dosing. The intraperitoneal route used in this study complicates translation, as oral bioavailability is poor and osteoporotic patients require convenient long-term dosing (Al Shoyaib, Archie, and Karamyan 2019). An orally bioavailable formulation or injection would be recommended. Furthermore, the study duration was not clear in the study, osteoporosis trials typically require 4 - 6 weeks in rodents to capture meaningful bone turnover. Long term studies should assess bone formation rates (dynamic histomorphometry with calcein labelling) and evaluate whether karanjin's effects persist reduces with chronic administration (de Bakker et al. 2015). The absence of estrogen or bisphosphonate comparators although it used β-estradiol as a positive comparator and the lack of biomechanical testing limit comprehensive assessment of bone quality. High-quality follow-up should report acquisition and segmentation according to established micro-CT guidance, include dynamic histomorphometry and serum turnover markers and add femoral bending or vertebral compression tests (Bouxsein et al. 2010).

8.2.6 Dermatology: Formulation Studies

The three formulation studies carried out on karanjin and dermatology discussed in this review provide a major translational insight concerning karanjin – for any topical applications, the medicinal value of karanjin lies not in its intrinsic properties but rather on the basis of the formulation used. The problem that is addressed in these studies is the poor water solubility and poor dermal permeation of karanjin. This is a major breakthrough in the formulation of the compound, since the use of vesicles such as liposomes or ethosomes provided better skin penetration and retention (Shiven et al. 2024; Mujtaba et al. 2024). Another study by Ansari et al. 2021 in the testosterone model, the 3% K-EGF reduced sebaceous-gland number and size and showed short-term local tolerability (Draize score 1.73; intact dermis and appendages) (Ansari et al. 2021). These results are significant since inhibition of sebaceous gland hyperplasia will decrease sebum production, which is one of the main factors of acne formation. Nevertheless, anti-acne efficacy was estimated by means of qualitative histology, treatment duration was only 7 days, information on dose-dependency was insufficient and there were no quantitative morphometry data, sebum content evaluation, inflammation biomarkers, microbial load assessment, or lesion counting. Moreover, there was no control experiment using plain karanjin *in vivo*, which prevented isolation of drug and vehicle effects. Ethosomes represent soft phospholipid vesicles that contain ethanol and fluidize lipids of the stratum corneum, increasing penetration of lipophilic substances, such as karanjin, into the viable epidermis. Design of the study in these papers was progressive and included an improved methodology needed for phytochemical drug development. Nevertheless, some deficiencies are present. Firstly, systemic bioavailability was not quantified and it is significant since psoriasis often requires administration of large body surface area and chronic usage. Secondly, contact sensitization (type IV hypersensitivity) was not assessed. Thirdly, although IMQ model can be useful for screening purposes, it does not replicate the whole spectrum of psoriasis pathophysiology, especially the IL-23/IL-17 axis, which became the target for modern treatment (Rudzikaitė-Fergizė et al. 2024). Future studies should evaluate karanjin in IL-23–driven genetic models and assess Th17 cytokine modulation. Moreover, the absence of clinical scoring standardization (e.g., PASI analog) and long-term safety data for topical karanjin limits readiness for clinical applications. The next step should be a factorial design that compares vehicle, free karanjin, blank vesicles and karanjin-loaded vesicles at matched drug doses. Studies should measure skin-layer concentrations, systemic exposure, standardised psoriasis-like scores, cytokines in the IL-23/IL-17 axis, sensitization, phototoxicity, repeat-dose dermal toxicity and durability after treatment withdrawal. On the present evidence, topical dermatology is in the near-term development path, but not yet a clinically validated indication.

9. Pharmacokinetics and Toxicology

Pharmacokinetic studies in this review indicate that karanjin possesses low systemic exposure following oral administration, with a reported C_{max} of 0.498 ± 0.01 μg/mL and $AUC_{0-\infty}$ of 1.492 ± 0.01 μg·h/mL. Karanjin shows a terminal elimination half-life of 3.78 ± 0.30 hours, with plasma concentrations declining to near undetectable levels within 24 hours post-administration, indicating relatively rapid clearance and limited oral bioavailability. The half-life suggests the need for dosing optimisation to maintain therapeutic levels (Naresh Shejawal 2014). Moreover, elimination half-life of 3.78 hours necessitates twice-daily or thrice-daily dosing to maintain therapeutic concentrations, which poses adherence challenges for chronic disorders. Yi et al. reported dose-dependent C_{max} values of 0.187 +/- 0.03, 0.347 +/- 0.04 and 0.772 +/- 0.12 microg/mL after 5, 10 and 20 mg/kg isolated karanjin, with Tmax approximately 1.5 h and $T_{1/2}$ approximately 1.9-2.2 h (Yi, Wang, and Yi 2015). Because neither experiment included intravenous dosing, an absolute oral-bioavailability percentage is unavailable. Available pharmacokinetic data demonstrate dose-related systemic exposure after oral administration of Karanjin, with Tmax of approximately 1.4–1.5 h and a terminal half-life of approximately 1.9–2.2 h. Absolute oral bioavailability remains unknown because an intravenous reference arm has not been reported. Acute toxicity assessments reveal no mortality at doses up to 2000 mg/kg and subacute studies demonstrate stable biochemical and histopathological parameters across major organs (Lubau et al. 2026). The pharmacokinetic data from Shejawal et al. (2014) represent a critical inflection point for translational potential. A C_{max} of 0.498 μg/mL (~1.5 μM) following 10 mg/kg oral administration is substantially lower than the concentrations typically required for *in vitro* cytotoxicity (IC_{50} values often in the 10–50 μM range against cell lines) (Naresh Shejawal 2014). This disconnect termed the *in vitro - in vivo* efficacy gap suggests that either *in vivo* efficacy is driven by tissue accumulation and local concentrations exceeding plasma levels or active metabolites contribute to pharmacological effects occur at supra-pharmacological doses that may not be clinically achievable.

Differences between the studies may reflect the botanical matrix, dose, sex, bioanalytical sensitivity, sparse versus serial sampling, or genuine kinetic variability. They should not be interpreted as a definitive formulation advantage without a head-to-head study. The short plasma half-life also does not, by itself, justify a twice- or three-times-daily regimen. Dosing frequency depends on the concentration–effect relationship, target residence, active metabolites, tissue persistence and therapeutic window, none of which has been defined

Formulation strategies to improve bioavailability such as novel nanodrug delivery systems like solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), self-emulsifying drug delivery systems (SEDDS), liposomes, polymeric nanoparticles (nanospheres and nanocapsules) and conventional strategies like cyclodextrin inclusion complexes, or prodrug approaches are urgently required to be investigated, **fig. 6** illustrates the proposed formulation strategies for improving bioavailability of karanjin (Elkordy et al. 2021; Rajendran et al. 2026; Meunier et al. 2025) . Notably, none of the included efficacy studies employed these advanced formulations, the formulation studies on karanjin were restricted to topical dermatological applications. For systemic conditions, PK-guided dose or formulation-enabled exposure should be prioritised. Overall, these findings collectively indicate a wide safety margin in preclinical models. However, comprehensive ADME profiling, metabolite identification and chronic toxicity evaluations remain incomplete and should be performed in future studies (Hong and Lyu 2025).

10. Safety Profile and Therapeutic Index

The toxicology data from this review indicate a wide therapeutic index in rodents. The absence of mortality at 2000 mg/kg (acute dose) and the NOAEL of ≥250 mg/kg/day (subacute dose) provide a safety margin of at least 2.5-fold to 25-fold relative to efficacious doses (10–100 mg/kg) (Lubau et al. 2026). This margin is safe but must be interpreted cautiously. Rodent toxicology does not always predict human toxicity due to species differences in metabolism, transporter expression and target engagement (Conour et al. 2013). The slight ALT/AST increase at 500 mg/kg/day, while within normal ranges, may signal early hepatic stress that could progress with longer exposure durations. Rekha et al. likewise reported no mortality in acute testing (Rekha et al. 2021). In contrast, the colon-carcinogenesis publication described mortality at doses above approximately 600 mg/kg in BALB/c mice (Zhang et al. 2021). Differences in species, formulation, vehicle, purity, observation methods, or reporting could explain the discrepancy. A 28-day rodent study is useful for hazard identification but cannot establish safety for chronic inflammatory, metabolic, oncological, or osteoporosis indications. OECD TG 407 specifies core design and reporting expectations for repeated-dose studies, while longer programs are required for chronic use. Moreover, the absence of genotoxicity, reproductive toxicity and cardiovascular safety data represents significant limitations that must be addressed prior to Investigational New Drug (IND) application (Cho et al. 2003).

Potential drug interactions deserve particular attention because karanjin modulates major efflux transporters (Li et al. 2022). This could change exposure to anticancer agents, antimicrobials, immunosuppressants, or CNS drugs. Future toxicology should use chemically defined material, analytically verified formulations, both sexes, exposure confirmation and recovery groups. A therapeutic index can be estimated only after the efficacious and toxic unbound exposures are established in comparable systems.

11. Methodological Quality, Reproducibility and Risk of Bias

According to the SYRCLE RoB assessment, there is no clear pattern in the literature of karanjin preclinical research. The prevalence of "unclear risk" in randomization, allocation concealment, blinding and random housing domains is more likely to be caused by poor reporting and not misconduct in itself. Still, poor reporting is indicative of quality of work and affects reproducibility, which is one of the basic principles of NIH Principles and Guidelines for Reporting Preclinical Research and ARRIVE guidelines 2.0 (Percie du Sert et al. 2020).

Lack of explanation of sample size choice is especially alarming. Studies that use small groups of 3 - 12 animals may lack the statistical power to detect even moderate effects and thus lead to either type I or type II errors. "File drawer problem," which refers to the tendency to hide negative or inconclusive results, might result in overestimation of effectiveness of karanjin in the existing literature. It is strongly recommended for future researchers to register the protocols (for example, through the Open Science Framework or Animal Study Registry) and publish both positive and negative results with effect size and confidence interval.

12. Strengths and Limitations of Current Review

This is the first systematic review to comprehensively map the *in vivo* preclinical evidence for isolated karanjin across multiple disease domains while simultaneously evaluating pharmacokinetics, formulation advances and safety. The use of the SYRCLE tool provides standardised quality appraisal, and the PICO-structured eligibility criteria ensure reproducibility. By maintaining a compound-centered (rather than disease-centered) scope, diverse mechanistic approaches and translational gaps that would be hard to compile in disease specific reviews were identified.

The primary limitation of the present review is the quality and quantity of available data. In most experiments, there were small N (3-6 animals per group), a lack of replicate cohorts and incomplete blinding. Lack of possibility to conduct a meta-analysis because of heterogeneity results in the lack of numerical accuracy. The RoB evaluation (**Table 2**) demonstrates unclear or moderate risk in several domains. The science needs more precise adherence to the ARRIVE guidelines such as stating randomization and blinding and having larger N calculated by power analysis.

Another issue is dosage translation. Some studies use extremely high doses (e.g. 200 mg/kg in mice or 2000 mg/kg in rodents for toxicity testing (Lubau et al. 2026). While these identify a biological effect, they may not be clinically realistic. The bioavailability data suggest that oral dosing yields low plasma levels, thus, some positive results might require formulation changes in practice. Topical studies (acne, psoriasis) already address this, but systemic indications do not. Bridging studies are needed: e.g. PK in disease models, dose– response curves and comparisons with standard drugs.

A third limitation is selective mechanistic focus. Many studies emphasise one marker (TNF-α, AChE, etc.) as proof of mechanism, but rarely do they comprehensively map out the pathway. For instance, (Rekha et al. 2021) might show reduced NF-κB but not reported on upstream TLR4 or downstream IL-1β. This approach might overinterpret the significance of one finding (Zhang et al. 2024). Thus, while there is a narrative of anti-NFκB action, it is supported by partial data in each model. Confirmation of key pathways (e.g. via inhibitor studies, genetic knockouts) would strengthen the claims.

Publication bias is another important aspect to take into account. Since there are only about ~16 articles, it is not possible to build a formal funnel plot, yet there is an obvious anecdotal bias – there are very few "no effect" publications. Every study included here claims some kind of effect of karanjin. One should consider a possibility of small-study bias (file drawer phenomenon) in preclinical research. Review readers should be informed about this possibility because true efficacy may be even lower.

In summary, this systematic review suffers from heterogeneity which prevents pooling, incomplete reporting on internal validity (randomization/blinding etc.), lack of some full texts due to the "no paywalled access assumed constraint" and indirectness of animal model to human clinical outcome.

13. Conclusion

Thus, according to this systematic review, karanjin can be seen as promising preclinical candidate with coherent multi-domain bioactivity. There is preliminary evidence to claim its efficacy in animal models of inflammatory arthritis, colitis, acute lung injury, breast cancer, osteoporosis, diabetes, cognitive impairment and psoriasis, and mechanisms include NF-κB/TLR4 inhibition, JAK2/STAT regulation, Wnt/β-catenin activation, cholinergic restoration and induction of antioxidant enzymes. Pharmacological studies have shown low oral bioavailability and fast elimination of karanjin; toxicological studies have indicated the safety margin for short term rodent exposure. Nevertheless, current body of literature does not provide sufficient support for any claims regarding the clinical efficacy and translational research should be done after robust and unbiased preclinical studies.

14. Future Directions

The future research on karanjin should emphasise its mechanistic basis. For example, one or two model systems should be selected, and their mechanism of action should be studied in more details (for instance, with help of pathway inhibitors, gene expression profile, etc.). There should be correlation between exposure and effect size via dose PK-PD studies. In case of oral administration, there is a need in the improvement of karanjin bioavailability with the use of some formulation technology (for example, nano emulsion or cyclodextrin complexation). The authors should encourage the use of IACUC grade standards for karanjin and report purity and solvent/vehicle used. It will help with reproducibility of experiments.

The future research should put emphasis on ARRIVE compliant methodology, robust bias mitigation measures (randomization, blinding, allocation concealment), standardization of models/outcomes per domains to make meta-analysis possible, PK/PD bridging (dose-exposure-response); chronic toxicity and metabolite profiling, better reporting of compound identity (purity, supplier, CAS); as well as investigation of transporters/CYP interaction liability before clinical translation (Percie du Sert et al. 2020).

Declarations

Funding:

This research was supported by Sunway University, Malaysia.

Author Contribution

M.Y.H. conceptualized the review, developed the methodology, conducted the literature search, screened studies, extracted and analysed the data, performed the risk-of-bias assessment, prepared the figures and tables, and wrote the original manuscript. A.R.H. contributed to study screening, data extraction, validation, risk-of-bias assessment, and manuscript revision. Y.S.W. and M.Y.F.C. contributed to the study design, methodological validation, interpretation of findings, supervision, and critical revision of the manuscript. M.S. contributed to the interpretation of the pharmacological and formulation evidence, supervision, and critical manuscript revision. K.R. contributed to conceptualization, methodology, supervision, project administration, and critical revision of the manuscript. M.F.S. contributed to conceptualization, validation, supervision, project administration, and critical revision of the manuscript. All authors reviewed and approved the final manuscript and agree to be accountable for the work.

Acknowledgements:

This research work was supported by the Sunway University with funding provided by the Sunway University Malaysia. The authors also acknowledge Prof Mahendran Sekar and Assoc Prof. Yuan Seng Wu for their contribution to the completion of the systematic review manuscript.

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Figures

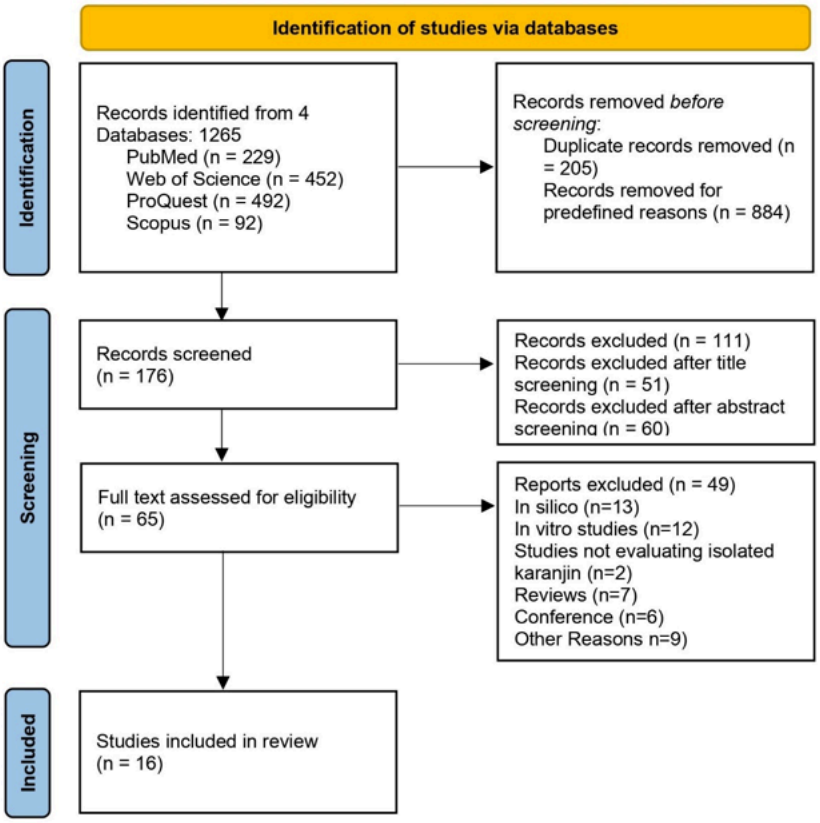

Figure 1

Preferred Reporting Items for Systematic Reviews (PRISMA) flow diagram showing the study selection process (Page et al. 2021).

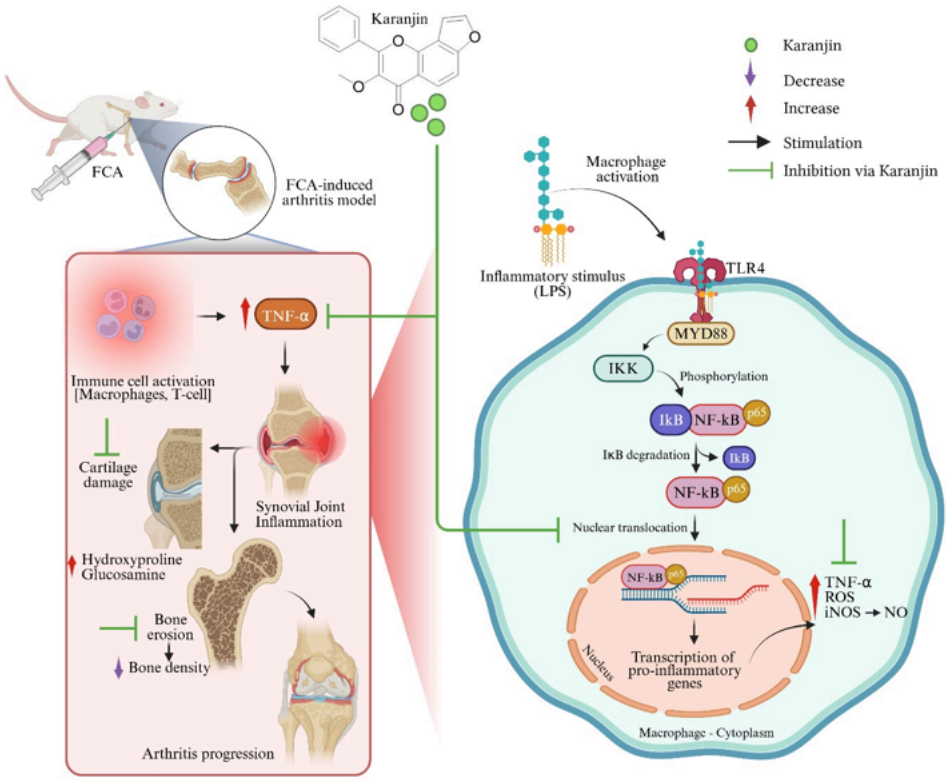

Figure 2

Proposed anti-inflammatory and anti-arthritic mechanism of karanjin based on combined *in vivo* and *in vitro* evidence. FCA-induced arthritis rat model was integrated to evaluate the effect of karanjin against inflammation. Karanjin facilitated to reduce the serum TNF-α levels, resulting in reduced cartilage damage, bone erosion, joint pain and inflammation. Consequently, it helped to hinder the arthritis progression. For investigating the underlying mechanism, an *in vitro* inflammatory model (LPS-stimulated macrophages) was utilised. Karanjin inhibited the nuclear translocation of NF-κB p65, thereby suppressed the transcription of inflammatory cytokines. As a result, the production of TNF-α, ROS and NO reduced, which eventually help in suppressing the inflammation and may underlie its protective effects against arthritis progression.

Abbreviations: FCA: Freund's complete adjuvant, LPS: Lipopolysaccharide, TLR4: Toll-Like receptor 4, MYD88: Myeloid differentiation primary response 88, IKK: IκB kinase, IκB: Inhibitor of nuclear factor kappa B, NF-κB (p65): Nuclear factor kappa light chain enhancer of activated B cells–p65 subunit, TNF-α: Tumor necrosis factor alpha, ROS: Reactive oxygen species, iNOS: Inducible nitric oxide synthase, NO: Nitric oxide.

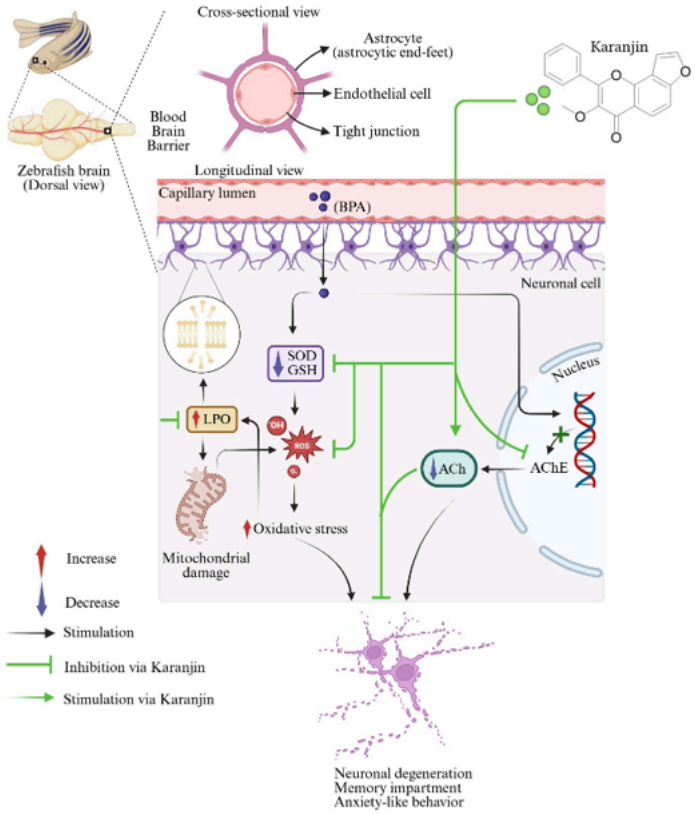

Figure 3

Proposed neuroprotective mechanism of karanjin against BPA-induced neurotoxicity in zebrafish. Because of highly lipophilic nature of BPA, it easily crossed the blood-brain barrier. Firstly, it causes a reduction in the antioxidant enzymes, such as SOD and GSH, leading an increase in the ROS production. Subsequently, increase the LPO and oxidative stress. Secondly, BPA causes the upregulation of acetylcholinesterase gene expression and exhibits increased acetylcholinesterase activity, resulting in decreased acetylcholine and reduced cholinergic and cognitive functions. These events collectively contribute to neuronal degeneration, memory deficits and anxiety-like behavior. However, karanjin facilitates to counteracts the BPA-induced neurotoxicity by improving antioxidant enzyme levels and inhibiting the activity of acetylcholinesterase. Therefore, decreased oxidative stress and neuronal degeneration, resulting in improved cognitive function, behavior and memory.

Abbreviations:BPA: Bisphenol A, SOD: Superoxide dismutase, GSH: Glutathione, ROS: Reactive oxygen species, LPO: Lipid peroxidation, AChE: acetylcholinesterase activity

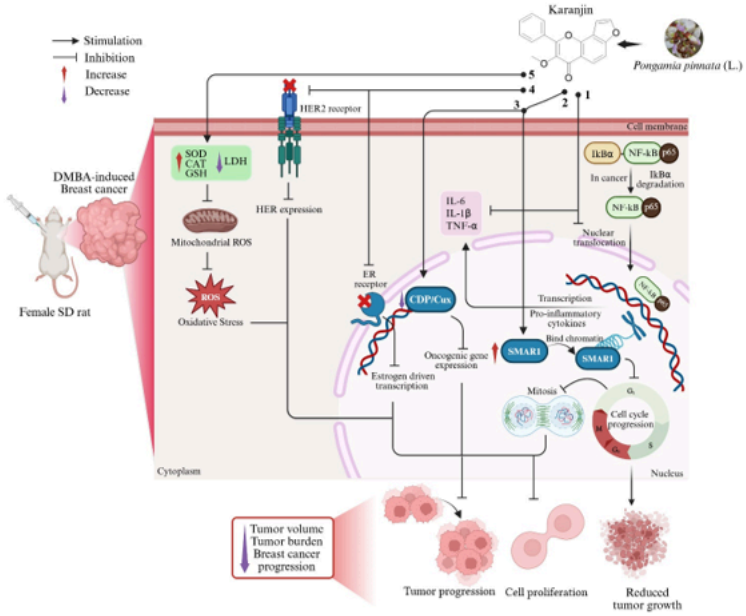

Figure 4

Proposed molecular mechanisms underlying the anticancer activity of karanjin against DMBA-induced breast cancer in female Sprague-Dawley rats. Karanjin demonstrated anticancer attributes via multiple pathways, which collectively contributed to exhibiting reduced tumor growth and its progression. [1] Karanjin facilitates to suppress the inflammatory cytokines (IL-6, IL-1β and TNF-α) for inhibiting the inflammation via controlling the nuclear translocation of NF-κB, [2] it aids in upregulating the SMAR1 which resulting the inhibition of cell cycle progression and mitosis to reduce tumor growth; [3] moreover, karanjin downregulates the CDP/Cux, leading the suppression of oncogenic gene expression and tumor progression. [4] Karanjin also integrated the hormonal pathway; herein, it reduced the expression of HER2 and ER receptors. [5] This treatment restored antioxidant defense systems by increasing SOD, CAT and GSH levels while reducing LDH and ROS generation. Collectively, these molecular alterations contributed to reduced tumor burden and inhibition of breast cancer progression.

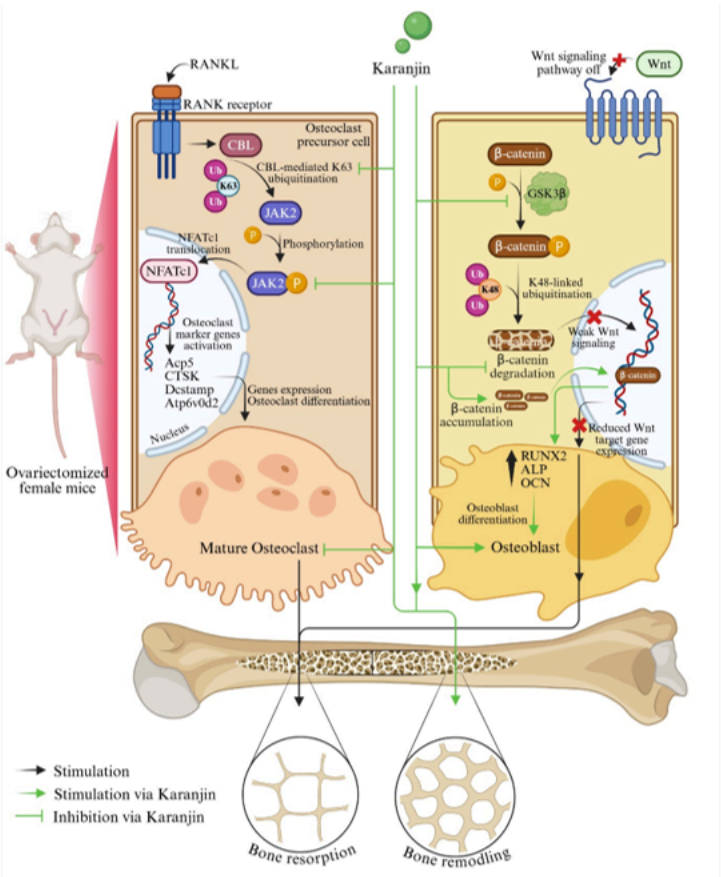

Figure 5

Proposed mechanism underlying the bone remodeling effects of karanjin in ovariectomy-induced osteoporosis. Karanjin restores bone remodeling homeostasis through dual regulation of osteoclast and osteoblast activities. In osteoclast precursors, karanjin disrupts CBL-mediated K63-linked ubiquitination of JAK2, suppressing JAK2 phosphorylation, NFATc1 activation and the expression of osteoclast-specific genes (Acp5, CTSK, Dcstamp and Atp6v0d2), thereby inhibiting osteoclast differentiation and bone resorption. In osteoblasts, karanjin targets GSK3β, reducing β-catenin phosphorylation and K48-linked ubiquitination, resulting in β-catenin stabilization and activation of canonical Wnt/β-catenin signalling. This enhances osteoblast differentiation and bone formation, ultimately restoring balanced bone remodeling and protecting against OVX-induced bone loss.

Abbreviations:RANKL: Receptor activator of nuclear factor-κB ligand, CBL: Casitas B-lineage lymphoma proto-oncogene, JAK2: Janus kinase 2, NFATc1: Nuclear factor of activated T cells 1, CTSK: Cathepsin K, Acp5 (TRAP): Tartrate-resistant acid phosphatase, Dcstamp: Dendritic cell-specific transmembrane protein, Atp6v0d2: Subunit of vacuolar H^+-ATPase, RUNX2: Runt-related transcription factor 2, ALP: Alkaline phosphatase, OCN: Osteocalcin.

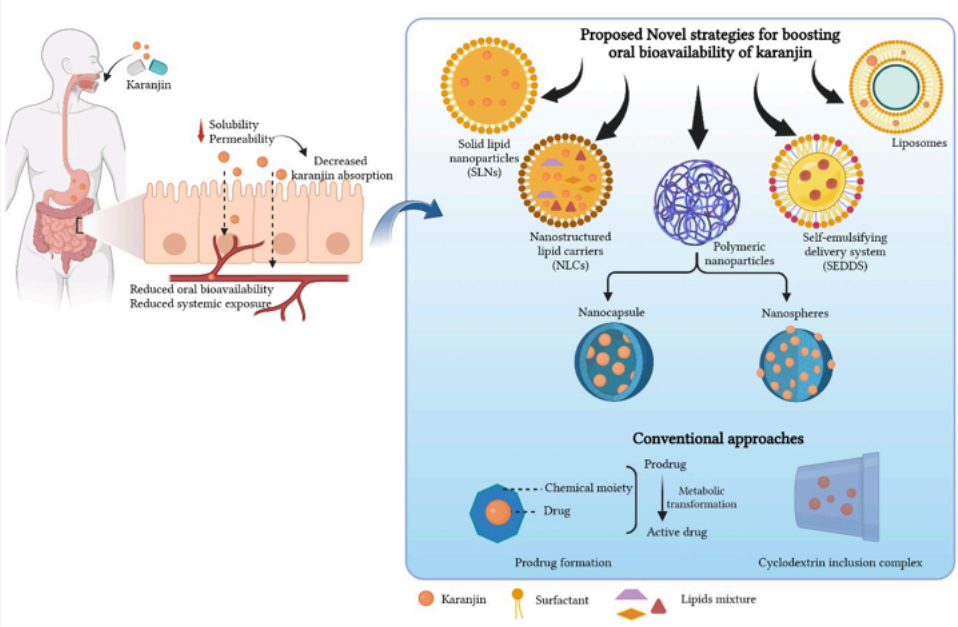

Figure 6

Proposed formulation strategies for enhancing the oral bioavailability of karanjin. Karanjin exhibits reduced solubility and permeability, resulting in low oral bioavailability and systemic exposure. Novel formulation strategies like solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), polymeric nanoparticles, self-emulsifying drug delivery systems (SEDDS) and liposomes; and conventional approaches like prodrug formation and cyclodextrin inclusion complexes can be utilised to overcome biopharmaceutical challenges and improve oral bioavailability.

Supplementary Files

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