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A SYSTEMATIC REVIEW OF THE EFFECTIVENESS OF TURMERIC IN THE TREATMENT OF BREAST CANCER

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Abstract

Breast cancer remains a significant global health concern, necessitating the exploration of innovative therapeutic strategies. Curcumin, a bioactive compound derived from turmeric, has garnered considerable attention due to its diverse pharmacological properties, including anticancer effects. However, the clinical translation of curcumin is hampered by its poor bioavailability. Nanoformulations of curcumin, particularly Cur-NPs, offer a promising solution to this challenge by enhancing drug solubility, stability, and targeted delivery. This in-depth abstract delves into the potential of Cur-NPs as a therapeutic approach for breast cancer treatment. The systematic review included in vivo studies investigating the efficacy and toxicity of Cur-NPs in breast cancer models. Notably, Cur-NPs demonstrated significant antitumoral activity across diverse breast cancer models, attributed to mechanisms such as apoptosis induction, inhibition of proliferation, and suppression of angiogenesis. Moreover, Cur-NPs exhibited minimal toxicity, with no or low adverse effects observed in terms of body weight, organ histopathology, and hematological/biochemical parameters. Characterization of Cur-NPs revealed a diverse array of nanoparticle types, including polymer NPs, micelles, lipid-based NPs, and metal NPs. Poly(ethylene glycol) chains were commonly incorporated into NP compositions, enhancing their biocompatibility and circulation time. Targeting moieties such as folic acid and hyaluronic acid further augmented the specificity of drug delivery to breast cancer cells. Experimental design varied among studies, encompassing different animal models, routes of administration, and treatment durations. Intravenous administration emerged as the predominant route, although alternative routes such as intraperitoneal and intratumoral administration were also explored. The onset of treatment protocols varied based on days after tumour induction or tumour volume, with curcumin doses ranging from 2 to 100 mg/kg and administered with varying frequencies. In conclusion, Cur-NPs represent a promising therapeutic approach for breast cancer treatment, offering enhanced efficacy and safety profiles compared to free curcumin. Further research is warranted to optimize Cur-NP formulations and elucidate their clinical potential in breast cancer patients. The review also identified a diverse array of nanoparticle formulations employed in breast cancer therapy, including liposomes, polymeric nanoparticles, micelles, and dendrimers. These nanoparticles were engineered to encapsulate various anticancer drugs, including chemotherapeutic agents, molecularly targeted therapies, and immunomodulators, with the aim of enhancing their efficacy and reducing systemic toxicity. Targeted drug delivery strategies were employed to selectively deliver therapeutic agents to tumour cells while sparing healthy tissues, thereby minimizing off-target effects and improving treatment outcomes. Preclinical studies provided compelling evidence of the efficacy of nanoparticle-based therapies in inhibiting tumour growth, suppressing metastasis, and overcoming drug resistance in breast cancer models. Nanoparticles demonstrated superior pharmacokinetic properties, enhanced bioavailability, and controlled drug release kinetics, leading to improved therapeutic efficacy compared to conventional formulations. Furthermore, nanoparticle-based therapies exhibited synergistic effects when combined with traditional chemotherapy agents, leading to enhanced antitumor activity and reduced treatment resistance. Clinical studies evaluating the safety and efficacy of nanoparticle-based therapies in breast cancer patients demonstrated promising results, with favourable tolerability profiles and encouraging preliminary efficacy outcomes. However, challenges remain in translating preclinical findings into clinical practice, including issues related to scalability, manufacturing, and regulatory approval. Additionally, further research is needed to optimize nanoparticle formulations, tailor treatment strategies to individual patient profiles, and elucidate long-term safety and efficacy outcomes.

Keywords: Curcumin, Breast cancer, Nanoparticles (NPs), Apoptosis, Bioavailability

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Introduction

Cancer is a complicated and intricate disease that is defined by aberrant cells within the body growing and spreading out of control (Hesketh, 2023). It affects almost every organ and tissue and is one of the main causes of death worldwide. It can take many different forms. Genetic changes that impair the regular control of cell growth and division are the usual cause of cancer, which usually develops gradually (Weinberg, 1996). Numerous causes, including exposure to carcinogens like tobacco smoke, UV radiation, certain chemicals, and infectious agents like the hepatitis B and C viruses and the human papillomavirus (HPV), can trigger these changes.

The ability of cancer to outlive the body's defences and multiply uncontrollably, producing tumours or infecting neighbouring tissues and organs, is its defining characteristic (Warburg, 1956) (Lazebnik, 2010). Cancer cells have the ability to metastasis when the disease worsens, traveling long distances through the lymphatic or circulatory systems and resulting in additional tumours in other body areas (Maman, 2018). Symptoms of cancer can vary greatly in severity, from minor alterations in body functioning to more severe indications including pain, exhaustion, and unexplained weight loss, depending on the kind, location, and stage of the disease (Whitaker, 2020) (Cleeland, 2000).

A variety of modalities are employed in cancer treatment approaches, such as immunotherapy, chemotherapy, radiation therapy, surgery, and targeted therapy (Nersesyan, 2007) (Candido, 2017). These modalities are often used in conjunction to enhance effectiveness and decrease adverse effects. Furthermore, developments in genomics and molecular biology have opened the door for personalized medicine techniques that adjust treatment plans in accordance with each tumour's distinct genetic composition (Chew, 2001). Even though research and treatment for cancer have advanced significantly over the years, there are still issues to be resolved, such as therapeutic resistance, toxicities associated with treatment, and the need for better early diagnosis and prevention techniques (Marine, 2020). The continued battle against this terrible illness depends on initiatives to better understand the biology underlying cancer, find new therapeutic targets, and improve access to care.

In this systematic review, we will be focusing on breast cancer. Breast cancer is a prevalent form of cancer that primarily affects the breast tissue. It occurs when cells in the breast grow uncontrollably, forming a tumour that can often be detected through screening mammograms, ultrasounds, or physical examination (Kelsey, 1988). While it predominantly affects women, men can also develop breast cancer, although it is much less common.

Breast cancer comes in a variety of forms, such as invasive ductal carcinoma, invasive lobular carcinoma, ductal carcinoma in situ (DCIS), and inflammatory breast cancer (Li C. U., 2005). Because each type is unique, specialized treatment methods are needed.

Early detection increases the likelihood of a successful course of treatment and survival, it is essential for managing breast cancer. A lump or thickening in the breast or underarm region,

alterations in the size or form of the breast, changes in the nipple, such as inversion, discharge, or redness, and skin dimpling or puckering, are typical signs of breast cancer (Pinto, 2011).

Depending on the kind and stage of the disease, treatment for breast cancer usually consists of a mix of surgery, chemotherapy, radiation therapy, hormone therapy, and targeted therapy (Irvin Jr, 2011) (Peart, 2015). The treatment plan may involve a heterogeneous team of healthcare specialists and is frequently tailored to the specific needs of the patient.

Breast cancer continues to be the primary cause of cancer-related mortality for women globally, despite notable breakthroughs in research and treatment approaches. Therefore, continuing awareness campaigns, early identification initiatives, and research are essential to the fight against this illness. Breast cancer prevention and management heavily rely on programs like routine breast cancer screenings, self-examinations, genetic testing for high-risk individuals, and lifestyle changes to lower risk factors (Britt, 2020). In order to successfully navigate the difficulties that come with receiving a breast cancer diagnosis, individuals and their families also need access to support networks and services.

In regards to breast cancer, we will be looking at the treatment options of it, in particular turmeric treatment in cancer. Curcuma longa, the scientific name for turmeric, is a flowering plant that is native to South Asia and is a member of the ginger family. It is well-known for its vivid yellow colour and unique flavour and has been utilized for ages in traditional medical and culinary techniques. Curcuminoids are the active ingredients that give turmeric its therapeutic qualities; curcumin is the most prevalent and well-researched of them (Kocaadam, 2017).

Strong anti-inflammatory, anti-cancer, and antioxidant characteristics characterize curcumin, a polyphenolic molecule. Through a variety of mechanisms, such as the control of inflammatory pathways, the reduction of angiogenesis (the development of new blood vessels to support tumour growth), the prevention of tumour cell proliferation, and the activation of apoptosis (programmed cell death), it achieves its effects. Additionally, curcumin has been demonstrated to strengthen the body's defences against free radicals and oxidative stress by scavenging them.

Turmeric has additional bioactive substances besides curcumin, namely demethoxycurcumin and bisdemethoxycurcumin, which enhance its medicinal value. These substances frequently interact favourably to improve human health (de Oliveira Filho, 2021).

Chemistry-wise, curcumin is a member of the polyphenols class of substances, which is distinguished by the presence of many phenolic rings and hydroxyl groups. It is a promising option for the treatment and prevention of a wide range of ailments, including cancer, neurological diseases, cardiovascular disorders, and metabolic syndromes. This is because of its molecular structure, which allows interactions with many molecular targets implicated in disease processes.

Curcumin's quick metabolism, poor bioavailability, and limited water solubility, however, limit its therapeutic value. In order to get around these obstacles, scientists have looked into a number of ways to improve the pharmacokinetic characteristics of curcumin, including co-administration of bioenhancers like piperine from black pepper, structural alterations, and nanoparticle formulations.

Turmeric in breast cancer

The key ingredient in turmeric, curcumin, has attracted a lot of interest in the field of breast cancer research because of its possible therapeutic benefits. Research has indicated that curcumin could potentially use a variety of methods to impact breast cancer-specific anti-cancer abilities.

Curcumin primarily affects breast cancer by preventing cancer cells from proliferating and causing breast cancer cell lines to undergo apoptosis, or programmed cell death. Furthermore, curcumin has demonstrated the ability to disrupt signalling pathways implicated in tumour development and metastasis, including the nuclear factor-kappa B (NF-κB) pathway, which is essential for inflammation and the advancement of cancer (Wang, 2016).

Furthermore, curcumin has encouraging anti-inflammatory properties, which are especially pertinent to breast cancer because the disease's onset and spread have been linked to chronic inflammation. Curcumin may contribute to the creation of an environment that is less favourable for tumour development and metastasis by regulating inflammatory cytokines and enzymes.

Additionally, curcumin's ability to improve the effectiveness of traditional breast cancer treatments including radiation and chemotherapy has been studied. According to some research, curcumin may both potentially lessen adverse effects associated with treatment and increase the sensitivity of cancer cells to its cytotoxic effects.

Curcumin also demonstrates anticancer properties in CSCs, or cancer stem-like cells. It has been demonstrated to significantly reduce CSC plasma membrane micro tentacles, which inhibits the cells from adhering to new locations. Additionally, it has been shown that curcumin allows breast CSCs to express the tumour suppressor gene E-cadherin again.

In a study that analysed the effects of curcumin on the expression of ER-alpha and p53 in the presence of estrogen, bisphenol-a (BPA) and anti-estrogens in T-47D breast cancer cells, the study showed that the p53 pathway has been linked to activation in CUR-induced apoptosis. It has been shown that CUR inhibits the transcription of the p53 gene, hence reducing the expression and function of p53 in colon cancer cells. These results are in line with our findings that CUR reduces the regulation of p53 in T-47D breast cancer cells. The T-47D breast cancer cell line possesses a p53 gene mutation, according to published research. The precise ratio of mutant to wild-type p53 in the T-47D cells is still unknown, though. Research has also demonstrated that the antiproliferative effects of CUR in breast cancer cells that express the estrogen receptor (ER) are reliant on the presence of estrogen.

According to reports, when compared to control cells, breast cancer cells treated with CUR exhibit a considerable downregulation of ERα expression. Previous research, including research conducted in the lab, has demonstrated that treating T-47D breast cancer cells with estrogen increases the cells' ability to proliferate and upregulates the expression of the tumour suppressor protein p53.

Turmeric Cur-Nps in Breast cancer

In recent years, there has been growing interest in harnessing the therapeutic potential of natural compounds for cancer prevention and treatment. Among these, curcumin, a

polyphenolic compound derived from the rhizome of Curcuma longa, commonly known as turmeric, has attracted considerable attention due to its diverse pharmacological properties and potential anticancer effects. Turmeric, a perennial herb native to South Asia, has been revered for centuries for its culinary, medicinal, and cultural significance. Central to turmeric's therapeutic properties is its bioactive component, curcumin, which imparts the characteristic yellow color to turmeric and exhibits a myriad of pharmacological activities (Zhao, 2015) (Thangapazham, 2008) (Ghosh, 2009).

Curcumin has demonstrated promising anticancer effects in a variety of cancer types, including breast cancer, through multiple mechanisms of action. These include inhibition of cell proliferation, induction of apoptosis, suppression of inflammation, modulation of signaling pathways, and inhibition of angiogenesis and metastasis. Preclinical studies have provided compelling evidence of curcumin's ability to inhibit breast cancer cell growth, induce cell cycle arrest, sensitize cancer cells to chemotherapy and radiotherapy, and suppress tumor progression and metastasis (Yallapu, 2012). (Duan, 2013) (Bansal, 2011)

However, despite its promising therapeutic potential, the clinical translation of curcumin has been hindered by several inherent limitations. Chief among these is curcumin's poor aqueous solubility, low bioavailability, and rapid metabolism, which collectively limit its systemic absorption and distribution to target tissues (Li L. B., 2005) (Anand, 2010). When administered orally, curcumin undergoes extensive metabolism in the gastrointestinal tract and liver, resulting in poor systemic absorption and limited therapeutic efficacy. Additionally, curcumin exhibits poor stability under physiological conditions, further compromising its therapeutic potential.

To overcome these challenges and enhance the therapeutic efficacy of curcumin, researchers have focused on developing innovative drug delivery systems, such as nanoparticle-based formulations. Nanoparticles offer several advantages over conventional drug delivery systems, including improved drug solubility, stability, bioavailability, and targeted delivery to tumor tissues. Among nanoparticle-based formulations, Cur-NPs have emerged as a promising approach for the delivery of curcumin in cancer therapy. Cur-NPs encapsulate curcumin within nano-sized carriers, such as liposomes, polymeric nanoparticles, micelles, and solid lipid nanoparticles, thereby enhancing its solubility, stability, and bioavailability.

In addition to improving drug delivery, Cur-NPs offer the potential for targeted therapy through the incorporation of targeting ligands, such as antibodies, peptides, or small molecules, which can selectively bind to receptors overexpressed on cancer cells, thereby enhancing drug accumulation and efficacy while minimizing off-target effects. Moreover, Cur-NPs can be engineered to enable controlled release of curcumin, allowing for sustained drug delivery and enhanced therapeutic outcomes.

Breast cancer presents a compelling indication for the use of Cur-NPs as a therapeutic intervention. The heterogeneity of breast cancer, characterized by diverse molecular subtypes and clinical behaviors, underscores the need for personalized and targeted treatment approaches. Cur-NPs offer a versatile platform for the delivery of curcumin to breast cancer cells, enabling precise modulation of molecular pathways implicated in tumour initiation, progression, and metastasis.

In this comprehensive review, we aim to provide an in-depth analysis of the current state of research on Cur-NPs in breast cancer therapy. We will examine the mechanisms of action, preclinical studies, clinical prospects, and future directions of Cur-NPs in breast cancer treatment. By elucidating the role of Cur-NPs in breast cancer therapy, we hope to contribute to the ongoing efforts to develop more effective and targeted therapies for this devastating disease.

Nanoparticles in Breast cancer

In recent years, nanotechnology has emerged as a promising frontier in cancer therapy, offering innovative solutions to overcome these challenges. Nanoparticles, engineered at the nanoscale, have shown remarkable potential in revolutionizing breast cancer treatment through targeted drug delivery, enhanced therapeutic efficacy, and reduced side effects (Maeda, 2013) (Wilhelm, 2016).

Targeted Drug Delivery:

Nanoparticles represent a versatile platform for targeted drug delivery in breast cancer therapy. By encapsulating anticancer drugs within nanoparticles, such as liposomes, polymeric nanoparticles, or dendrimers, drug molecules can be selectively delivered to cancer cells while sparing healthy tissues. This targeted approach minimizes off-target effects and systemic toxicity, thereby improving the therapeutic index of anticancer drugs. In breast cancer, nanoparticles can be functionalized with targeting ligands, such as antibodies or peptides, to specifically recognize and bind to receptors overexpressed on cancer cells. This allows for precise localization of therapeutic agents within the tumour microenvironment, enhancing their efficacy and reducing adverse effects on normal tissues.

Enhanced Bioavailability:

Poor aqueous solubility and low bioavailability are common challenges associated with many anticancer drugs, including chemotherapeutic agents and molecularly targeted therapies. Nanoparticle-based drug delivery systems address these limitations by improving the solubility, stability, and pharmacokinetic properties of drug molecules. By encapsulating drugs within nanoparticles, their systemic absorption and distribution are optimized, leading to increased plasma concentrations and enhanced therapeutic effects. In breast cancer therapy, nanoparticle formulations have been developed to improve the bioavailability of chemotherapeutic drugs, such as paclitaxel and doxorubicin, leading to improved treatment outcomes and patient survival rates.

Controlled Drug Release

Nanoparticles offer precise control over drug release kinetics, allowing for sustained and controlled delivery of therapeutic agents to cancer cells. By modulating the physicochemical properties of nanoparticles, such as particle size, surface charge, and composition, drug release profiles can be tailored to meet specific therapeutic requirements (Dreaden, 2012). In breast cancer treatment, sustained release formulations of anticancer drugs, such as tamoxifen and letrozole, have been developed using nanoparticle-based delivery systems. This prolonged

exposure of cancer cells to therapeutic agents results in sustained inhibition of tumor growth and metastasis, leading to improved patient outcomes.

Overcoming Drug Resistance

Drug resistance remains a major obstacle in breast cancer treatment, limiting the effectiveness of chemotherapy and targeted therapies. Nanoparticle-based strategies offer innovative solutions to overcome drug resistance mechanisms and enhance the efficacy of anticancer drugs (Blanco, 2015). By encapsulating multiple drugs within nanoparticles or incorporating drug efflux inhibitors, nanoparticles can circumvent resistance pathways and restore sensitivity to chemotherapy. Additionally, nanotechnology enables the development of combination therapies that synergistically target multiple pathways involved in tumor progression and metastasis, thereby overcoming resistance and improving treatment outcomes in breast cancer patients.

Imaging and Diagnosis

In addition to drug delivery, nanoparticles have applications in breast cancer imaging and diagnosis. Nanoparticle-based contrast agents, such as iron oxide nanoparticles and quantum dots, can be used for magnetic resonance imaging (MRI) and fluorescence imaging, respectively, to visualize tumors and monitor disease progression. Furthermore, nanoparticle-based biosensors and molecular probes offer sensitive and specific detection of biomarkers associated with breast cancer, enabling early diagnosis and personalized treatment strategies (Smith, 2020).

Nanoparticles hold immense potential in revolutionizing breast cancer treatment through targeted drug delivery, enhanced bioavailability, controlled drug release, overcoming drug resistance, and advanced imaging and diagnosis (Garcia, 2018). Continued research efforts in nanoparticle design, optimization, and clinical translation are essential for realizing the full therapeutic potential of nanoparticles in breast cancer therapy. By harnessing the unique properties of nanoparticles, we can pave the way for more effective and personalized treatment strategies that improve patient outcomes and quality of life.

Methods and Materials

Inclusion Criteria

The criteria for inclusion in this systematic review were meticulously crafted following the PICOS (Population, Intervention, Comparison, Outcome, and Study Design) framework (Specifically, the selection process encompassed studies examining the effectiveness and safety (Outcome) of Cur-NPs (Intervention), juxtaposed against free curcumin and/or a negative control (Comparison), within in vivo models of breast cancer in either mice or rats (Population).

Exclusion Criteria

The exclusion criteria were rigorously applied, resulting in the omission of studies for the following reasons: 1) literature reviews, letters, subjective opinions, book excerpts, and conference abstracts; 2) investigations limited to in vitro settings or clinical trials; 3) studies

exclusively focusing on free curcumin or its derivatives; 4) research involving cancer types other than breast cancer; 5) experiments involving Cur-NPs in combination with other anti-tumour agents; 6) unavailability of the complete manuscript; 7) studies deemed to have inadequate quality.

Sources and Search Strategy

Customized search strategies were meticulously crafted for each of the prominent bibliographic databases, including but not limited to MEDLINE, ProQuest, BSV regional portal, PubMed, ScienceDirect, Web of Science and Google Scholar. The search process was conducted over March 15th till May 7th 2024, without imposing any temporal constraints. Notably, no limitations were imposed regarding language or publication timeframe, fostering inclusivity across diverse linguistic and chronological spectrums.

Risk of Bias

While systematic reviews are designed to minimize bias through rigorous methodology, several potential sources of bias may still exist in this systematic review on Cur-NPs in breast cancer treatment. One potential bias could stem from the inclusion criteria applied during the study selection process. If the inclusion criteria were overly restrictive or ambiguous, certain relevant studies may have been inadvertently excluded, leading to selection bias. Additionally, the reliance on published literature in English language only may introduce language bias, as relevant studies published in other languages could have been overlooked. Furthermore, bias may arise from the heterogeneity of the included studies, particularly in terms of experimental design, tumour models, and treatment protocols. Variability in these factors could introduce performance bias, affecting the comparability and generalizability of the findings. Moreover, the reliance on preclinical studies may introduce publication bias, as studies with positive results are more likely to be published than those with negative or inconclusive findings. Another potential source of bias could arise from the assessment of study quality and risk of bias. If the quality assessment tools used were subjective or lacked transparency, it could lead to bias in the interpretation of study findings. Additionally, the involvement of multiple authors in data extraction and analysis could introduce observer bias if there were discrepancies in judgment or interpretation.

Results

Study Selection

Breast cancer research has increased significantly, biggest example of this is searching the scientific database using search strings such as (Breast-Cancer, Breast Neoplasm, Mammary cancer, Malignant neoplasm of breast, Mammary carcinoma). In this search key words such as (Mammary carcinoma, Breast cancer, Turmeric in breast cancer, Curcumin in breast cancer, Breast carcinoma, Nanoparticles, Nanomulsion, nanogels) were used.

Study	Population	Intervention			Outcomes	
Authors, year (reference)/country	Animal model[a]	Treatment regimen[b]	Dose; route	Nanostructure platform	Antitumor activity	Toxicity analysis
Shukla et al. (25)/India	Balb/c mice/n = 3/	Ten days from tumor inoculation;	100 mg/kg;	Lipid based CPC-SNEDDS NPs (Phospholipid, castor oil, Tween 80, PEG 400);	1) Cur-NP ↓TV (58.9%); free Cur ↓TV (29.5%); p<0.001	(ND)
	4T1/mouse/ (1 x 10^6 cells)/ subcutaneously on back skin	Daily for 28 days - Free Cur vehicle: gum acacia (1%, w/v).	Oral	HD: 83.27 nm/PDI: 0.151; ZP: −16 mV/EE: 29.1%		
Chen et al. (26)/China	Balb/c nude mice/n = 5/	TV of 200 mm^3	5 mg/kg;	Micelles NPs [POCA/C8 (phosphorylated calixarene) micelles – PM];	1) Cur-NP ↓TV (−60%) and ↓ TW (−80%); free Cur: ↓TV (−34%) and ↓ TW (−60%); p<0.05	No damage in major organs;
	BT-549/human/	Fourteen days at every 2 days	Intratumoral	HD: 3.86 nm/PDI: 0.125;	2) Cur-NP ↑TNC and ↑TAP	No WL; hematological indices: ~ control
	(2 x 10^6 cells)/	- Free Cur vehicle: (NM)		ZP: −25.18 mV/EE: 95.4%	3) Cur-NP ↓CD44+ CD133+ cancer stem cells	
	Subcutaneously on the upper right thigh					p<0.05
Mahalunkar et al. (27)/ India, Germany, and Norway	Balb/c mice/n = 6/	First day of treatment: (NM)	10 mg/kg;	Metal gold NPs (CurAu-PVP) with folic acid (FA) ($HAuCl_4$ and PVP polymer).	1) Cur-NP-FA ↓TV (−51%); free Cur: no ↓TV; p<0.006	(ND)
	4T1/mouse/	Twice a week for 2 weeks	Intratumoral	HD: 358.7 nm/PDI: 0.6	2) Cur-NP-FA ↓TW (−44%); free Cur: no TV↓; p<0.05	
	(1 x 10^6 cells)/ Mammary fat pad	- Free Cur vehicle: (NM)		ZP: −12.5 mV/EE: (NM)		
Akbarieh et al. (28)/Iran	Balb/c mice/n = 5/	14 days after tumor induction;	Dose: (NM);	Micelles/polymersomes NPs (PNP) [monomethoxy-PEG (mPEG 2000), oleic acid (OA)]	1) Cur-NP ↓TV (−80%); p<0.05	31.25 mg/Kg of PNP-CUR: no damage in major organs;
	Transplantation of spontaneous mouse mammary tumor/ Pieces < 0.3 cm^3/	Daily for 24 days	Intraperitoneal	HD: 99.44 nm/PDI: 0.182; ZP: −29.3 mV mV/EE: 64%	2) Cur-NP ↑TAP; ↓ANG (CD31); ↓PROL (Ki-67); p<0.05	Hematological and biochemical indices: ~ control p<0.05
	Subcutaneous on the left flank					
	Right flank					
Sahne et al. (34)/Iran	Balb/c mice/n = 4/	TV of 50–100 mm^3;	4 mg/kg;	Graphene oxide NPs (GO NPs with CMC, PVP, PEG, FA);	1) Cur-NP-FA↓TV (−86%); p<0.05	No damage in major organs;
	4T1/mouse/ (NM)/	Daily for a total 3 weeks	Intravenous	HD: ~60 nm/PDI: (NM) ZP: −48 mV/EE: 94%	2) Cur-NP-FA ↓TW (−76%); p<0.05 3) Cur-NP-FA ↑ ST and ↓ metastasis; p<0.05	No WL p<0.05
	Subcutaneous on the flank				4) Cur-NP-FA: ↑TNC; ↓ cell density; ↓ANG (CD31, CD34); ↑ pro-inflammatory response in the tumor microenvironment; p<0.05	
Ji et al. (35)/China	Balb/c mice/n = 5/	First day of treatment: (NM);	5 mg/kg;	Nanocrystals NPs with or without HA	1) Cur-NP-HA ↓TV (−86%); Cur-NP↓TV (−39%); free Cur: ↓TV (−21%); p<0.05	No damage in major organs; no WL;
	4T1/mouse/	Every 2 days for a total 10 days	Intravenous	Cur-NP and HA-Cur-NP: HD: 101.4 and 161.9 nm/ PDI: ~0.330 and 0.250, respectively	2) Cur-NP-HA ↓TW (−75%); Cur-NP↓TW (−37.5%); Free Cur: ↓TW (−25%); p<0.05	No hemolysis (<5%);
	(1 x 10^6 cells)/	- Free Cur vehicle: (NM)		HA-Cur-NP: ZP: −25.0 mV, respectively/EE: (NM)	3) ↑ ST: Cur-NP-HA > Cur-NP > Free Cur; p<0.05	Hematological and biochemical indices: ~ healthy control p<0.05
	Subcutaneous on the right flank					
He et al. (36)/China	Balb/c mice/n = 6/	TV of 100 mm^3	5mg/kg;	Polymeric micellar NPs [amphiphilic diblock copolymer— mPEG-b-PLG (Se)-TP];	1) Cur-NP ↓TV (−65%); Free Cur: ↓TV (−49%); p<0.05	No damage in major organs; no WL
	4T1/mouse/	Every 4 days, for 4 times; total 21 days	Intravenous	HD: 136 nm/PDI: 0.071	2) Cur-NP ↓TW (−82%); free Cur: ↓TW (−62%); p<0.05	p<0.05
	(1 x 10^6 cells)/	- Free Cur vehicle: (NM)		ZP: (NM)/EE: ~ 68%	3) Cur-NP ↑TNC and ↑TAP; ↓ANG (CD31); ↓PROL (Ki-67); p<0.05	
	Subcutaneous on the right back					
Jin et al. (37)/China and USA	Balb/c nude mice/n = 5/	7 days after tumor induction;	5 mg/kg;	Polymeric NPs with or without EGFR-targeting peptides (GE11) (PLGA-PEG);	1) Cur-NP-GE11 and Cur-NP ↓TV (−80%); free Cur: no TV↓; p<0.05	Inflammatory cytokine levels: ~ healthy mice
	MCF-7/human/	Every 24 h for 20 times	Intravenous	HD: 210 nm/PDI: 0.112;	2) Cur-NP-GE11↓TW (−68%); Cur-NP	p<0.05

					p<0.05	
	(1 × 10^7 cells)/ Subcutaneous on the dorsal flank	- Free Cur vehicle: (NM)		ZP: −22 mV/EE: 92.3	3) Cur-NP-GE11and Cur-NP ↑TAP	
Abd-Elstaf et al. (39)/ Italy and Egypt	Balb/c mice/n = 8/	TV of 50 mm^3;	5 mg/kg;	Solid lipid nanoparticles (SLN) with or without chitosan (CS) coating (cholesterol, trilaurin, butyl lactate, Epikuron®200, Cremophor®RH60, sodium taurocholate, Pluronic® F68);	1) Cur-NP-CS and Cur-NP ↓TV (−35%); Free Cur: no TV↓; p<0.01	Biochemical indices: ~ control
	JC/mouse/ (1 × 10^7 cells)/ Mammary fat pad	Thrice (on day 1st, 7th, 14th) - Free Cur vehicle: 10% v/v DMSO suspension	Intravenous	HD: < 200 nm/PDI: (NM) ZP: (NM)/EE: 70–75%		p<0.05
Li et al. (33)/China	Balb/c mice/n = 4/	Tumor diameter of 4 mm;	8 mg/kg;	Mesoporous silica nanoparticles with hyaluronan (MSN-HA) or polyethyleneimine-folic acid (MSN-PEI-FA);	1) Cur-NP-PEI-HA ↓TV (−50%); Free Cur: no TV↓; p<0.01	No damage in major organs; no WL;
	MDA-MB-231/human/ (1 × 10^7 cells)/ Subcutaneous	Every 3 days for a total of six times - Free Cur vehicle: (NM)	Intravenous	HD: < 300 nm/PDI: (NM)/ ZP: ~ −20 mV (MSN-HA); ~ +40 mV (MSN-PEI-FA)	2) Cur-NP-PB-HA ↓TW (−70%); free Cur: no TW↓; p<0.01	Hemolysis (<5%); Biochemical indices: ~ healthy control p<0.05
Kundu et al. (40)/India	Swiss albino mice/n = 6/	Ten days after induction;	10 mg/kg;	Metal NPs [Zinc oxide nanoparticles (ZnO) with PBA];	1) Cur-NP↓TV (−77%); free Cur: ↓TV (−66%); p<0.05	No damage in the liver and kidney;
	Ehrlich ascites carcinoma cells/	Alternate days for 14 days.	Intravenous	HD: 413.63 nm/PDI: (NM)	2) Cur-NP ↓TW (−72%); free Cur: ↓TW (−50%); p<0.05	Biochemical markers: ~control; ↓ tumor-induced splenomegaly
	(1.0 x 10^7/ml) Left flank	- Free Cur vehicle: (NM)		ZP: −15.4 mV/EE: 27%	3) Cur-NP and free Cur: ↑TAP; p<0.05	p<0.05
Lv et al. (41)/China	Kunming (mice)/n = 6/	TV of 300 mm^3;	10 mg/kg;	Polymeric NPs (PEG-PCDA) with or without biotin;	1) Cur-NP ↓TV (−69%); Cur-NP-biotin ↓TV (−79%); free Cur: ↓TV (−32%); p<0.05	no WL
	EMT6/mouse/	Daily for 9 days; total 14 days	Intravenous	PEG-PCDA and biotin-PEG-PCDA: HD: 94.2 and 125.1 nm/PDI: 0.170 and 0.08, respectively	2) Cur-NP ↓TW (−70%); Cur-NP-biotin ↓TW (−85%); free Cur: ↓TW (−25%);	p<0.05

Yang et al. (40)/China	Subcutaneously Balb/c nude mice/n = 5/ MCF-7/human/ (1 x 10^7 cells)/ Subcutaneous on the flank	TV of 200 mm^3/ Every other day for 5 times; total 20 days - Free Cur vehicle: (NM)	10 mg/kg; Intravenous	Micelle NPs (triblock copolymer PPBV); HD: 6.7 nm/PDI: 0.117 ZP: −1.42 mV/EE: 66.5%	1) Cur-NP ↓TV (−58.5%, day 12); ↓TV (−28.9%, day 20); p<0.05 2) Cur-NP ↓TW (−22%, day 20); p<0.05	No WL p<0.05
Yang et al. (42)/China	Balb/c nude mice/n = 5/ MCF-7/human/ (1 x 10^7 cells)/ Subcutaneous on the flank	TV of 200 mm^3/ Every other day for 5 times; total 20 days - Free Cur vehicle: (NM)	15 mg/kg; Intravenous	Hybrid NPs (PLGA NPs coated with a modified hialuronic acid (HA-hybrid)) HD: 350 nm/PDI: (NM) ZP: −22 mV/EE: 32%	1) Cur-NP-HA ↓TV (−43.8%, day 12); ↓TV (−24%, day 20); p<0.05 2) Cur-NP-HA ↓TW (−22%, day 20); p<0.05 3) Cur-NP-HA ↓tumor cell density; p<0.05	No WL p<0.05
Gresh et al. (44)/ Bahrain	Balb/c mice/n = 5/ 4T1/mouse/ (1 x 10^6 cells)/ Bilaterally on the flanks	TV of 100 mm^3; frequency of treatment: unclear; total 10 days	10 and 20 mg/kg; Intravenous	Micelles (curcumin-metal complex and SMA) HD: 248 nm/PDI: 0.274; ZP: −11 mV/EE: 80%	1) Cur-NP-10mg/Kg ↓TV (−61%); Cur-NP-20mg/Kg ↓TV (−92%); p<0.05	(ND)
Mukerjee et al. (45)/ USA	Balb/c nude mice/n = 8/ MCF10CA1a/human/ (3 x 10^6 cells)/ Flank	TV of 70 mm^3; Thrice week for a total 30 days	20 mg/kg; Intravenous	Polymeric NPs (PLGA/PVA with or without antibody targeting (AnxA2) Cur-NP and AnxA2-Cur-NP: HD: 150 and 157 nm/ PDI: ~0.240 and 0.200, respectively Cur-NP and AnxA2-Cur-NP: ZP: −27.5 and −28.6 mV, respectively/EE: 89.2%	1) Cur-NP- AnxA2 ↓TV (−44.0%); Cur-NP ↓TV (−33.5%); p<0.05 2) Cur-NP- AnxA2 ↓TW (−53.0%); Cur-NP ↓TW (−30%); p<0.05 3) Cur-NP- AnxA2 and Cur-NP: ↓ANG; ↓ NFkβ; ↓PROL (KI-67); p<0.05	No WL p<0.05
Mukhopadhyay et al. (46)/India	Balb/c nude mice/n = 5/ MDA-MB-231/human/ (5 x 10^6 cells)/ Right flank	8 days after induction; Thrice week for a total 21 days	20 mg/kg Route of administration: unclear	Polymeric NPs (PLGA/PVA with or without folate (F)) HD: 170 nm/PDI: 0.186; ZP: −28.2 mV/EE: 68.6%	1) Cur-NP-F ↓TV (−90%); Cur-NP↓TV (−75%); p<0.05 2) Cur-NP-F ↓TW (−92%); Cur-NP↓TW (−61.5%); p<0.05 3) Cur-NP-F and Cur-NP ↓ cell density	No damage in major organs p<0.05
Yu et al. (47)/China	Balb/c nude mice/n = 5/ MCF-7/human/ (3 x 10^6 cells)/ Subcutaneously right flank	TV of 100–400 mm^3; Every other day for 5 times for a total 24 days	40 mg/kg Intravenous	Micelles NPs (MPEG-PLA with or without PAE) HD: 128.4 nm to 171.0 nm/ PDI: 0.118 to 0.134 ZP: −2.0 to +4.0 mV/ EE: 96.5 to 98.8%	1) Cur-NP-PAE ↓TV (−65.8%); Cur-NP ↓TV (−47.1%); p<0.05 2) Cur-NP-PAE ↓TW (−76%); Cur-NP ↓TW (−53%); p<0.05	no WL p<0.05
Huang et al. (48)/China	Balb/c mice/n = 5/ 4T1/mouse/ (NM)/ Flank	TV of 40–50 mm^3/ Every 2 days for a total of 5 times	50 mg/kg Intravenous	Polymeric NPs (HA-CHEMS); pH-sensitive HD: 144 nm/PDI: (NM); ZP: −21.25 mV/EE: (NM)	1) Cur-NP ↓TV (−38%); p<0.05 2) Cur-NP ↑ ST; 3) Cur-NP ↑TNC and ↑TAP	↓ Damage in major organs no WL p<0.05
Shiri et al. (49)/Iran	Balb/c mice/n = 9/ 4T1/mouse/ (1 x 10^5 cells)/ left flank	Third day after tumor induction/ daily for 35 consecutive days	40 mg/kg or 80 mg/kg Route of administration: (NM)	Dendrosome NPs (DNC) [composition: not mentioned (patent number: 71753)] HD; PD; ZP; EE: (NM)	1) NP-40mg/Kg ↓TV (−72%); NP-80mg/Kg ↓TV (−76%); p<0.05 2) NP-40mg/Kg ↓TV (−61%); NP-80mg/Kg ↓TV (−64%); p<0.05 3) NP ↑ ratio of M1/M2 macrophages	no WL No change in food intake and behavior p<0.05
Lin et al. (50)/China	Balb/c nude mice/n = 6/ MCF-7/human/ (NM)/ Subcutaneous in the right armpit	First day of treatment: (NM)/ once every 3 days for 15 days - Free Cur vehicle: (NM)	Dose: (NM)/ Intravenous	Lipid based NPs (NLC) with or without folate (FA) coating (PEG-DSPE, soya lecithin, castor oil, Tween 80, Pracirol ATO-5); HD: 126.8 nm/PDI: 0.16 ZP: +12.6 mV/EE: 82.7%	1) Cur-NP-FA ↓TV (−83%); Cur-NP ↓TV (−66%); free Cur: ↓TV (−51%);	No WL p<0.05

Table 1,2,3,4,5,6: Descriptive characteristics of included studies.

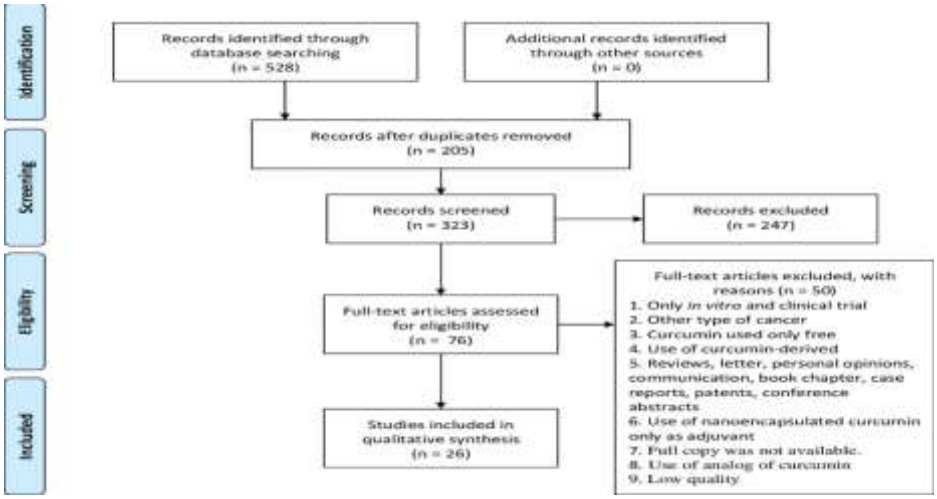

Figure 1: Flow diagram of literature search and selection criteria using PRISMA

Characteristics of the included studies

Each of the studies encompassed within this review comprises scholarly articles focusing on the assessment of the anti-tumour efficacy of Cur-NPs within in vivo breast cancer models. A detailed synopsis of the primary attributes of these studies can be found in Table 1.

The Cur-NPs featured in the studies under consideration were primarily characterized based on their hydrodynamic diameter (HD) (n=26), polydispersity index (PdI) (n=17), and zeta potential (n=21) utilizing techniques such as dynamic light scattering and electrophoretic mobility analysis. Furthermore, certain investigations employed transmission electron microscopy (TEM) and/or scanning electron microscopy (SEM) to assess the size and/or morphology of the nanoparticles. Evaluation of curcumin encapsulation efficiency (EE%) was predominantly conducted using high-performance liquid chromatography (HPLC) (n=18).

In all in vivo investigations, tumour volume progression throughout the experimental timeframe was meticulously monitored, typically by gauging tumour dimensions such as small/large diameters or width/length, with final volumes computed using mathematical equations. Additionally, studies undertook diverse assessments including tumour weight (n=16), survival duration (n=3), tumoral stem cell quantification via flow cytometry (n=2), M1/M2 macrophage ratio determination via RT-PCR (n=1), and scrutiny of apoptosis (n=8), necrosis (n=5), proliferation (n=4), angiogenesis (n=5), cell density (n=4), inflammatory response within the tumour microenvironment (n=1), and metastatic potential (n=1) through classical histological techniques (HE staining) and immunohistochemistry.

In terms of toxicity evaluation, 21 studies incorporated at least one parameter for assessment. These included monitoring of weight loss (n=15), examination of major organ integrity via classical histopathology (HE staining) (n=9), observation of food intake/behavioural changes (n=1), determination of inflammatory cytokine levels via enzyme-linked immunosorbent assay (ELISA) (n=1), assessment of haemolysis through absorbance measurements (n=2), and analysis of haematological (n=3) and biochemical parameters (n=6) via animal blood analysis using commercial kits and automated counters.

Synthesis

A diverse array of nanoparticle (NP) types were utilized across the included studies, showcasing the versatility in curcumin delivery strategies. Predominantly, polymer NPs emerged as the preferred choice (n = 9), closely followed by micelles (n = 6), lipid-based NPs (n=3), metal NPs (n = 2), hybrid NPs (n=2), dendrosomal NPs (n =1), nanocrystals (n=1), graphene oxide NPs (n=1), and mesoporous silica NPs (n=1). Notably, poly(ethylene glycol) chains (PEG) featured in the composition of NPs in 11 studies, underscoring their significance in NP design. Moreover, targeting moieties were incorporated into NPs in nine studies, including folic acid (n=5), hyaluronic acid (n=1), EGF peptides (n=2), and AnxA2 (n=1), augmenting the specificity of drug delivery. Characterization of these NPs revealed a hydrodynamic diameter (HD) spanning from 101.4 to 371.7 nm, with the majority exhibiting a negative Zeta potential (−48 to +40 mV) (n=22) and encapsulation efficiency percentages ranging from 32 to 98% (Table 1).

Regarding experimental design, considerable heterogeneity was observed across studies concerning the choice of animal model, route of administration, duration of the experiment,

and treatment dosage. Among the selected studies, ten opted for nude mice as the animal model, particularly when inducing tumours with human cells, while 16 studies utilized mice with intact immune systems for tumour induction with murine cells. Additionally, one study utilized rats with mammary tumours chemically induced by MNU (n=1).

The primary human cell lines employed in these studies included MCF-7 (n=6), MDA-MB-231 (n=3), MDA-MD-468 (n=1), MCF10CA1a (n=1), and BT-549 (n=1), while the murine cell lines encompassed 4T1 (n=9), EMT6 (n=1), JC (n=1), and Ehrlich ascites carcinoma cells (n=1). Notably, one study utilized the transplantation of spontaneous mouse mammary tumour pieces (n=1) to establish the breast cancer model.

Regarding tumour implantation techniques, three studies employed mammary fat implantation of tumour cells, while the remaining studies predominantly utilized subcutaneous implantation into the flank (n=14) or armpit (n=1).

The commencement of treatment protocols was delineated based on either days after induction (n=9), spanning from 3 days to 4 months, or tumour volume (n=13), ranging between 40 and 400 mm3. Curcumin dosages administered in the treatments exhibited variability, ranging from 2 to 100 mg/Kg, and were administered on a daily basis (n=8), every other day (every 2 days) (n=7), three times a week (n=6), or twice a week or less (n=4).

Intravenous administration emerged as the predominant route of drug delivery in the included studies (n=18). However, a few studies opted for alternative routes such as intraperitoneal (n=2), intratumoral (n=2), or oral administration (n=1), while one study did not clearly specify this information (n=1).

Potential Bias

After the investigation of bias potential, The majority of studies provided comprehensive details regarding the encapsulation methods employed for curcumin (n=24) and thoroughly investigated the characteristics of the nanoparticles (n=23). Notably, all studies ensured the adequacy of animal models utilized, with ethical committee approval clearly documented in 18 studies. However, a subset of six studies failed to distinctly report ethical approval, while two articles omitted this criterion altogether. Furthermore, the conditions under which animals were maintained during the experiments were ambiguously described in 13 studies, raising concerns regarding experimental reproducibility and reliability.

The study design pertaining to anticancer activity was robust in most instances, with several articles explicitly specifying the timing of treatment (n=25), route of administration (n=22), curcumin dosage (n=25), and inclusion of control groups (n=25). However, a mere 15 studies explored the anticancer activity of free curcumin for comparison with the Cur-NP effect, potentially limiting the comprehensive assessment of nanoparticle efficacy. Additionally, toxicity assays were conducted in 21 studies to evaluate the safety profile of Cur-NPs.

Notably, the statistical models employed across all studies were deemed unclear due to a lack of detailed information, posing challenges in assessing the reliability and validity of the reported findings. This discrepancy highlights the need for greater transparency and adherence to standardized statistical methodologies in future research endeavours.

Discussion

Curcumin's structure contains chemical groups that enable interactions with molecules implicated in the many routes of breast carcinogenesis of a variety of chemical natures (e.g., covalent, non-covalent, hydrophobic, and hydrogen bonds). Curcumin has been shown to impede angiogenesis, tumour invasion, and cell proliferation. Curcumin is an anti-proliferative drug that causes p53-dependent apoptosis and cell cycle arrest. Additionally, it modifies the expression of signalling proteins, including phosphatidylinositol-3-kinase (PI3K), protein kinase B (Akt), and Ras. Furthermore, curcumin has been suggested as a possible method of blocking EZH2, an enzyme-modifying protein component implicated in tumour growth, the ability for metastasis, and the control of drug resistance. Curcumin has been shown to suppress the growth of human breast cancer MDA-MB-435 cells in association with the downregulation of EZH2 expression in breast cancer (Mukhopadhyay R, 2020) (Huang C, 2020).

In MDA-MB-231 breast cancer cells, curcumin has shown anti-invasive properties by upregulating tissue inhibitor of metalloproteinase (TIMP-1) and downregulating matrix metalloproteinase (MMP-2). Curcumin's chemo preventive and chemotherapeutic properties are intriguingly linked to the modulation of miRNAs involved in signalling pathways related to tumorigenesis and metastasis, such as hedgehog, notch-1, PI3K/Akt/mTOR, Wnt/β-catenin, IGF, VEGF, and TGF-β/smad3 pathways (Mukerjee A, 2016). Gallardo et al. showed that curcumin inhibits miR-34a, a regulator of Rho-A and other genes implicated in the epithelial-mesenchymal transition, including Axl, Slug, and CD24, hence preventing the migration and invasion of breast cancer cells (MCF-10F and MDA-MB-231). Curcumin also suppresses the angiogenic cytokine interleukin-6 and inhibits vascular endothelial growth factor (VEGF) to prevent angiogenesis. Curcumin has a broad range of activities in regulating tumor growth by acting on various cancer hallmarks, as demonstrated by the methods listed above, as well as its anti-inflammatory properties and ability to inhibit cell growth factors (Lin M, 2016) (Hua WF, 2010).

Curcumin has fewer remarkable results in clinical trials due to its hydrophobic nature, which restricts its applicability. Furthermore, free curcumin can biomodify and in animal models, be primarily eliminated as bile or excrement (Simental-Mendía LE, 2017) (Saberi-Karimian M, 2019). In order to boost medication bioavailability, administer lower doses, lengthen the drug's half-life in circulation, and improve biological activity, another option is to use drug delivery systems using NP platforms. With regard to treating cancer, the field of natural product-based nanomedicine has grown and shown tremendous promise. The toxicity and antitumoral activity of Cur-NPs in in vivo models of breast cancer are reported in the current systematic review.

According to an analysis of the included research, curcumin has been loaded onto a wide range of NP platforms with varying compositions, sizes, and zeta potentials when examined in vivo models of breast cancer. Actually, all of the included studies that contrasted the reduction in tumour volume following Cur-NP treatment with treatments of curcumin in its free form and/or with negative controls showed benefits (Table 1) (Anemone A, 2019). For example, the tumours of animals treated with free curcumin showed a volume reduction of about 21%, whereas the tumours of animals treated with hyaluronic acid-coated curcumin nanocrystals showed a considerable reduction of approximately 86%.

NPs have the ability to build up into solid tumours, like breast cancer. According to the traditional theory, irregular angiogenic growth causes NPs to pass through gaps in the endothelial cells, which allows them to enter the tumour's vascular barrier. Because of inadequate local lymphatic drainage, the NPs are then retained in the tumour mass through a passive process called the enhanced permeability and retention (EPR) effect (Reuter S, 2008) (Zhang J, 2017). Updated research suggests that this pathway may not be the primary mechanism of NPs' extravasation into solid tumours, which has been a topic of controversy. The trans-endothelial pathway, a metabolically intensive procedure that necessitates endothelial cells to reorganize their structure in order to present vesicles that can ingest NPs and subsequently convey them to tumour cells nearby, is one of the other mechanisms of NPs' tumour accumulation that has been studied.

Enhancements in tumour NP accumulation and advantageous interactions between NPs and cancer cells can be achieved by modifying the NP surface to include moieties that provide longer blood circulation times (like PEG) and more targeted active targeting (like ligands that bind to molecules that are overexpressed in tumour cells). Hyaluronic acid (HA) and folic acid (FA) were the primary active targeted moieties identified in the included investigations. Folate receptors, tumour-associated proteins overexpressed in over 40% of human malignancies, including breast cancer, are affinities for FA. While non-modified NP accounted for ~44% of tumour volume decrease, a metal organic framework of FA-Cur-NPs greatly increased curcumin anticancer activity (~61%). The attachment of HA, a naturally occurring polysaccharide made up of repeating disaccharide units, to the surface of nanoparticles has also been studied because it binds to CD44, a surface protein that is frequently expressed in breast cancer. When combined with HA-mesoporous silica nanoparticles, curcumin significantly reduced tumour weight by approximately 70%; however, free curcumin had no discernible effect. It has also been investigated to modify the curcumin-NP surface in other ways using ligands particular to several tumour surface biomarkers for breast cancer.

When NPs reach the tumour location, tumour cells may internalize them or release their cargo into the surrounding tissue. Since the tumour microenvironment is known to be acidic, it is possible to leverage this pathological feature of cancer to your advantage by carefully releasing nanoparticles (NPs) that arepH-sensitive. This tactic helps to minimize potential negative effects and stops cargo from leaking into non-target organs. This method was used by Kundu and colleagues to develop their study, which used pH-sensitive NPs. They found that when the pH was lowered, the release of curcumin from the nanohybrid zinc oxide NPs enhanced, leading to a significant reduction in tumour volume (~77%) and an increase in curcumin accumulation in the tumour tissue. Furthermore, no structural damage or biochemical alterations were seen in the kidneys or liver. When curcumin was encapsulated in pH-sensitive polymeric NPs, Huang and colleagues observed a marked decrease in tumour volume and an extension of the survival period (Table 1). Depending on the mechanism that is triggered, internalization of NPs can be mediated or not by active targeting ligands. The primary mechanisms for internalization of NPs are endocytosis pathways, which include clathrin-mediated endocytosis, caveolae-mediated endocytosis (for NPs up to 200 nm), macropinocytosis, and other clathrin and caveola-independent endocytosis (for NPs between 250 nm and 3 μm). Once within the cell, NPs can engage in specialized interactions with

organelles and/or liberate their cargo to target possible locations, including those implicated in pathways leading to cell growth and death/survival.

Curcumin is able to alter several signalling pathways related to apoptosis. The major apoptotic mechanism varies depending on the kind of cell, stage of differentiation, or quantity of curcumin; it can be either intrinsic (mitochondrial) or extrinsic (mediated by receptors). Some of the apoptotic pathways that curcumin activates in different breast cancer cells include DNA fragmentation, induction of redox signalling, activation of caspase-3, suppression of telomerase, and increase of the Bax/Bcl-2 ratio. Free curcumin has also been reported to induce cell cycle arrest, which may be related to its antiproliferative properties. In vivo breast cancer models were demonstrated to exhibit at least tumour apoptosis, necrosis, and/or cell proliferation blocking in response to Cur-NPs, in accordance with the anticancer mechanisms outlined in the included research.

Angiogenesis, which is the process by which pre-existing blood vessels grow into new ones, is crucial to the growth, maintenance, and spread of tumours. In vitro and in vivo studies have shown that free curcumin has anti-angiogenesis actions through blocking or modifying numerous pro-angiogenesis factors, including vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), and basic fibroblast growth factor (bFGF) (Greish K, 2018). When curcumin was given in vivo breast cancer models in combination with micelles, graphene oxide, or polymeric NPs, comparable results were observed in the included investigations.

The tumour microenvironment is known to have a population of cancer stem cells (CSCs). These cells have the capacity to initiate processes that allow them to persist and replenish themselves, producing the diverse cancer cells that make up the tumour. Additionally, P-glycoprotein, a well-known protein implicated in multidrug resistance (MDR), is known to be highly expressed in CSCs, which renders them less amenable to anticancer therapy. It's interesting to note that free curcumin has been shown to impact CSCs without endangering healthy stem cells. The methods include P-glycoprotein regulation and reduction of cytokine release, including IL-6, IL-8, and IL-1, which encourage CSCs. Research using curcumin in conjunction with micelles and polymeric NPs have demonstrated a significant decrease in the percentage of CSCs present in in vivo breast cancer models (Table 1), suggesting that cur-NPs of the research reviewed herein appear to retain this feature.

The process through which cells from one tumour spread to other locations and develop into a second tumour is known as metastasis. Inoperable locally advanced breast cancer that has not progressed to distant organs is categorized as advanced breast cancer, as is stage (IV) metastatic breast cancer. The lungs, liver, bones, and axillary lymph nodes are the most often afflicted sites by breast cancer cells. In the in vivo breast cancer models examined in this research, Cur-NPs had notable effects on tumour spread. For example, curcumin in combination with graphene oxide nanoparticles decreased the metastatic regions in a triple negative breast cancer model.

Among the trials reviewed here, many administration routes were used. For medications and NPs, the oral delivery route is recommended over alternative methods because of benefits such pain avoidance, good patient compliance, and convenience of intake. The knowledge of the actual dose ingested is the primary constraint of this approach. The oral delivery of

curcumin encapsulated in a lipid-based nanoparticle was the subject of only one included investigation. Curiously, a noteworthy ~60% reduction in tumour volume was attained; however, the amount of curcumin administered—100 mg/Kg—was the highest of all the tests. This is likely because there are differences in NP absorption by this route.

To circumvent the gastrointestinal tract and possible degradation, other routes of administration can be utilized. An intriguing treatment approach for breast cancer is intratumoral injection. By administering NPs directly into the tumour site with a standard biopsy needle, for example, less invasive procedures can be performed while limiting the duration of medication in contact with cancerous target cells, minimizing side effects on healthy tissues, and avoiding liver metabolism. When micelle NPs were administered using this method to triple negative breast cancer models, two of the included studies demonstrated comparable tumour volume decrease (51–60%).

The intraperitoneal (IP) method, which involves injecting pharmaceutical medications into the peritoneal cavity, is frequently utilized with rodents. The IP approach is quicker, less stressful for the animals, and a safer way to administer high doses of medication when the intravenous route proves difficult. An entrance point for blood circulation via the capillary system is the IP route. According to two of the included trials, Cur-micelle NP delivery by IP route was successful, resulting in tumour volumes that were reduced by around 80 and 59.1%, respectively.

An administered medication can circulate quickly through the bloodstream when given via the intravenous (IV) method. While this mode of administration was used by about 70% of the included studies, differences in cell lines, the number of cells used for induction, the timing of the first treatment, and dose/treatment regimens limit the accuracy of efficacy comparisons regarding the composition and other characteristics (e.g., HD, PDI, PZ) of NPs. Analysing studies that assessed many experimental variables within the same experimental design allowed for the possibility of some comparisons including dosage concentrations and the existence of active targeting. All of the NP types that were employed generally produced better results in terms of tumour volume reduction in models of triple negative, chemically generated, and estrogen receptor (ER) positive breast cancer (Table 1).

One of the human cell lines that is most frequently utilized in breast cancer research is MCF-7 because it expresses significant amounts of estrogen receptor (ER), which is similar to the majority of breast cancers that are diagnosed these days. Through MCF-7 model analysis of the included research, it was found that the moiety employed determines how active targeting affects better efficacy outcomes. When FA is attached to lipid-based NPs as the targeting moiety, tumour volume reduction is significantly improved (83%) compared to non-targeted NPs (~66%), according to Lin and colleagues. Comparing the presence of folate as an active targeting moiety to non-targeted NPs (~75%), enhanced tumour volume reduction (~90%) was seen in MDA-MB-231 in vivo models (triple negative breast cancer). In contrast, when peptide moieties having affinity for EGFR were added to polymeric NPs, no appreciable improvement was seen in comparison to non-targeted NPs. The pH-sensitive NPs' design is an intriguing feature that appears to enhance efficacy results in MCF-7 models. Comparing animals treated with pH-sensitive micelles (mPEG-PLA with PAE) to those treated with non-pH-sensitive micelles (~47.1%), Yu and colleagues observed a greater reduction in tumor volume (~65.6%).

Since MCF-7 and MDA-MB-231 are human cell lines, they are both induced in immunocompromised mice for conventional in vivo models. These so-called xenograft models are irrelevant when the goal of the study is to assess the results and correlate them with a functioning immune system. In this instance, it is advised to employ syngeneic models, in which mice with a natural immune system are given cells from the same genetic background (murine). The 4T1 cell line is commonly utilized in synthetic breast cancer models as a representative model to replicate triple negative breast tumours. Tumour volume reduction results exhibited a dose-dependent response, according to an analysis of 4T1 models employed in the included research. Greish and colleagues demonstrated that, in comparison to a 10 mg/ml dose (~61%), a 20 mg/Kg treatment resulted in better tumour volume reduction (~92%). However, there were no appreciable differences between the high doses—40 and 80 mg/kg—when comparing them. Ji and colleagues as well as Laha and colleagues observed that the NPs' enhanced efficacy was due to the inclusion of the active targeting moieties, FA or HA.

Examining solely the research conducted by Sahne and associates, Ji and associates, and He and associates, all of whom induced breast cancer in Balb/c mice using 4T1 cells (10^6), and administering intravenous treatments at relatively similar dosages, 4 or 5 mg/kg, with variations in treatment plans (Table 1): Sahne and associates administered treatment daily for 21 days, Ji and associates administered treatment every two days for 10 days, and He and associates administered treatment every four days for a total of 21 days. Interestingly, it can be seen that both FA-GO-NP and HA-Cur-NP treatments with Cur-NPs led to a comparable percentage of tumour volume reduction (roughly 86%, Table 1). When evaluating NPs, a graphene oxide NP with a 60 nm HD is called FA-GO-NP, while a nanocrystal with a 162 nm HD is called HA-Cur-NP. When it comes to treatment, administering HA-Cur-NP every two days for a just ten days had an antitumor efficaciousness equivalent to administering FA-GO-NP everyday for a full twenty-one days. As a result, it is a less intensive and shorter treatment plan with comparable effectiveness.

Following intravenous administration, NPs are removed from organs and tissue systems by the renal and hepatic systems as well as the reticuloendothelial system (RES) or mononuclear phagocyte system (MPS). The characteristics of nanoparticles (NPs) such as their size, shape, degradability, surface charge, surface chemistry, and core type affect the clearance process. Degraded NPs are expelled into the bloodstream by the MPS, which is based on phagocytosis (mostly for NPs between 50 and 200 nm) or pinocytosis, which lowers the injected dose. The primary organs for the clearance of nanoparticles (NPs) smaller than 100 nm are the kidney and liver, via renal tubular secretion and glomerular filtration, respectively. Due to the abundance of Kupffer cells, which are able to sequester foreign substances, and the highly permeable sinusoidal endothelial cells, which improve liver uptake and retention of NPs, NPs that are not eliminated by the kidney can be processed in the liver.

None of the incorporated investigations exploring the toxicity effects of Cur-NPs elicited any indication of adverse impacts, as evidenced by the absence of perturbations in biochemical markers, haematological parameters, organ integrity, or weight dynamics (refer to Table 1). It is noteworthy that a substantial majority (~80%) of the inquiries delving into the efficacy of Cur-NPs concurrently scrutinized potential toxicity manifestations, reflecting a nuanced approach encompassing both therapeutic efficacy and safety considerations within the realm

of nanoparticle research. Nonetheless, approximately one-third of these inquiries relied exclusively on body weight fluctuations as a metric for toxicity assessment. Hence, it underscores the imperative for more exhaustive inquiries transcending these parameters to gain a comprehensive understanding of the safety profile of the intervention and facilitate its clinical translation.

Limitations

Several constraints surfaced during the development of this systematic review. Initially, notable heterogeneity was observed across various dimensions including nanoparticle types, NP characteristics, animal models utilized, duration of administration, and concentrations of interventions, rendering meta-analysis unviable. Additionally, a study was excluded during phase 2 due to unavailability of its complete manuscript. Furthermore, the majority of SYRCLE's Risk of Bias (RoB) criteria were inadequately reported across the included studies, thereby impeding a comprehensive assessment of study quality.

Conclusion

This systematic review underscores the promising potential of utilizing nanoparticles (NPs) as delivery vehicles for curcumin in breast cancer treatment. The findings reveal notable reductions in tumour volume across all breast cancer models, attributed to enhanced rates of apoptosis and necrosis, diminished tumour cell proliferation, suppression of angiogenesis, and even depletion of the cancer stem cell population, potentially extending survival times. Moreover, these favourable outcomes are coupled with minimal to no adverse effects, as evidenced by stable body weight, unremarkable histopathological profiles of major organs (such as the liver, kidneys, lungs, and spleen), and no significant alterations in haematological or biochemical parameters.

In-depth consideration of nanoparticle (NP) architecture is imperative, tailored to the specific characteristics of the breast tumour subtype, alongside meticulous attention to the route of administration and dosage regimen. Furthermore, the feasibility of cost-effective, large-scale production of these NP platforms is paramount. This facet holds significant weight in facilitating the practical implementation of these groundbreaking technologies, transitioning them from theoretical concepts in laboratory settings to tangible solutions at the patient's bedside.

While the present study does not delve into the integration of Cur-NPs with other therapeutic modalities, it is advised that comprehensive assessments be conducted to gauge both the efficacy and toxicity profiles of Cur-NPs when combined with other plant-derived compounds or conventional treatments such as chemotherapy and radiotherapy. This systematic review collectively advocates for the notion that Cur-NPs represent a promising and safe therapeutic avenue in breast cancer models, bolstering existing evidence that warrants their thorough examination in clinical trials as potential treatments for breast cancer.

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