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Review

Recent Developments in Luteolin-Loaded Nanoformulations for Enhanced Anti-Carcinogenic Activities: Insights from In Vitro and In Vivo Studies

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Abstract: With approximately 18 million people affected by cancer in 2020 globally, scientists are exploring innovative approaches to develop effective treatments for various types of cancer. Traditional chemotherapy drugs, although effective against cancer cells, often lead to significant side effects on healthy tissues, such as hair loss, anemia, and nausea. To discover safer alternatives, researchers are investigating natural bioactive compounds found abundantly in plants. Luteolin, a flavonoid found in celery and artichokes, stands out due to its diverse anti-carcinogenic properties, including inhibiting proliferation, inducing apoptosis, activating autophagy, and inhibiting angiogenesis and metastasis. However, the therapeutic potential of luteolin is hindered by challenges related to its bioavailability and solubility. This critical review explores the specific anti-carcinogenic properties of luteolin while analyzing the impact of its limited bioavailability and solubility on effectiveness. Additionally, it investigates the outcomes of encapsulating luteolin in nanoformulations, providing insights into potential strategies for enhancing its anti-carcinogenic effects. Finally, the review compares the efficacy of luteolin with and without nanoformulations. This review provides valuable insights into the potential of utilizing luteolin-loaded nanoformulations as a safer and more effective method for treating cancer, contributing to the ongoing efforts in improving cancer care and outcomes worldwide.

Keywords: luteolin; natural bioactive compounds; anti-carcinogenic activities; nanotechnology; nanoformulations

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1. Introduction

Cancer remains one of the leading causes of death worldwide due to its ability to evade early detection and develop resistance to treatment. Up to the year 2020, it has

affected approximately 18 million people worldwide, with 10 million succumbing to the disease [1]. Given the gravity of this issue, healthcare scientists are diligently exploring innovative treatment methods to combat this deadly disease, including surgeries, radiotherapies, phototherapies, and conventional chemotherapies [2–4]. However, many of these treatments are costly and carry significant side effects that can severely impact patients' quality of life.

While conventional chemotherapeutic drugs on the market typically target actively proliferating cells, a characteristic of cancer cells, it is important to note that tumor cells are not the only ones undergoing rapid proliferation. Other cells in the body, such as hair cells, gastric cells, and bone marrow cells, also proliferate rapidly. Consequently, these drugs not only exert their intended pharmacological action on cancer cells but also affect these healthy, rapidly dividing cells, leading to side effects like alopecia, nausea, vomiting, anemia, and neutropenia [5].

In light of these challenges, scientists worldwide are exploring novel alternatives for effective cancer treatments, shifting their focus from synthetic compounds to natural bioactive compounds abundant in plants and fruits. While some natural bioactive compounds like resveratrol and curcumin are undergoing clinical trials due to promising pre-clinical data, many more plant-based extracts with potential anti-cancer properties remain underutilized in cancer therapy [6,7]. One such example is luteolin, which has shown potent anti-tumor effects.

Luteolin, also known as 3′-4′, 5, 7-tetrahydroxyflavone, is a flavonoid abundantly found in vegetables and edible plants, as depicted in Table 1. It boasts a diverse array of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer properties [8,9]. Luteolin influences numerous molecular pathways involved in cancer development, such as inducing apoptosis, inhibiting proliferation, suppressing angiogenesis, and mitigating metastasis in various types of cancer cells [10]. However, like other flavonoids, fully harnessing the potential of luteolin is challenging due to limitations in its solubility, bioavailability, and stability. Therefore, the development of an effective drug delivery system capable of solubilizing and transporting luteolin is essential to enhance its bioavailability, efficacy, and safety for its intended therapeutic target.

Table 1. Total luteolin content in various edible plants.

Food Source	Scientific Name	Part of Plant	Total Luteolin Content (mg/100 g)	References
Radicchio	*Cichorium intybus* var. *foliosum*	Leaf	42.00	[11]
Celery	*Apium graveolens*	Seed	81.40	[12]
Beefsteak plant	*Perilla frustecens*	Leaf	3.20	[13]
Parsley	*Petroselinum crispum*	Not stated	19.75	[14]
Oregano	*Origanum vulgare*	Leaf	130.00	[15]
Thyme	*Thymus vulgaris*	Leaf	60.00	[16]
Broccoli	*Brassica oleracea botrytis cimosa*	Leaf	1.49	[17]
Cauliflower	*Brassica oleracea acephala*	Leaf	62.00	[17]
Artichokes	*Cyanara cardunculus*	Bract	93.00	[18]
Chinese pickled chili pepper	*Capsicum crustescens* L.	Fruit	2191.00	[19]
Sow thistles	*Sonchus arvensis* L.	Stem leaf	15.41	[20]

In this review, various aspects of luteolin's anti-cancer potential will be discussed. Firstly, the molecular mechanisms of luteolin on cancer cells will be examined, shedding light on its effects on cell cycle regulation, induction of cell death (apoptosis), and inhibition of key signaling pathways. Next, the focus will be on the challenges posed by luteolin's poor pharmacokinetic properties, which necessitates the development of advanced drug delivery systems. Lastly, recent advancements in drug delivery technologies designed to maximize the benefits of luteolin in cancer therapy, such as polymeric nanoparticle, micelle,

vesicles, and nanoemulsions will be dissected, examining how these technologies improve luteolin's efficacy both in vitro and in vivo.

2. Anti-Carcinogenic Activities of Luteolin

Luteolin exhibits anti-carcinogenic activities by affecting multiple processes, including cell cycle arrest, apoptosis, autophagy, and angiogenesis. Figure 1 provides a simplified illustration of these anti-carcinogenic activities of luteolin.

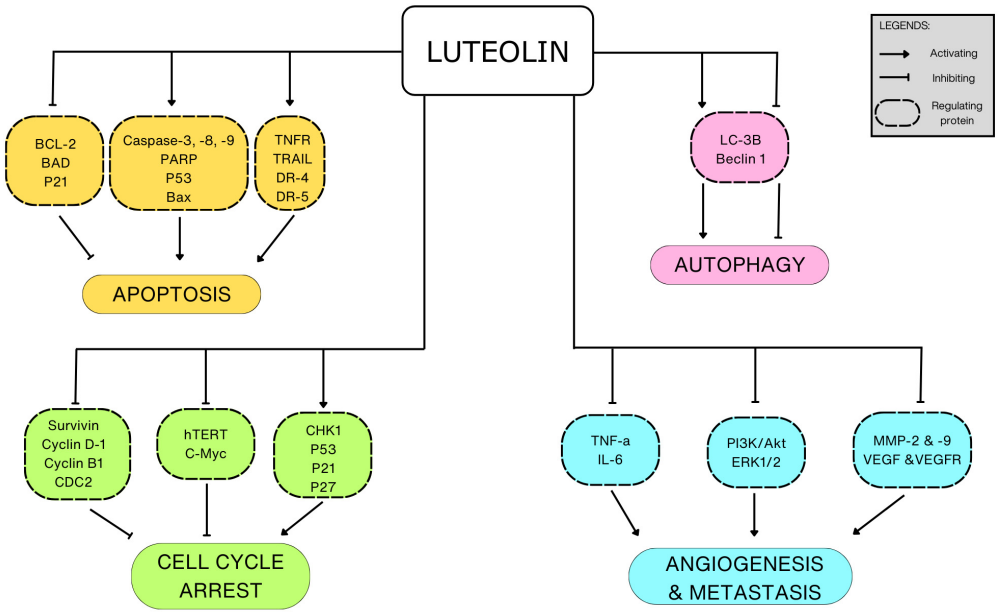

Figure 1. Overview of luteolin's anti-carcinogenic activities on various pathways.

2.1. Cell Cycle Arrest

The tumor suppressor gene p53 typically regulates the cell cycle by inducing cell cycle arrest, DNA repair, and apoptosis upon detecting any threats to the cells [21]. This protein also oversees other inhibitory proteins such as p21, which inhibits cell division cycle protein 2 (CDC2), a crucial factor for cell cycle progression through mitosis and meiosis. P21 enables the cell to transition from the S phase by regulating cyclin-dependent kinase 2 (CDK2), which is responsible for DNA synthesis. Mutations or damage to p53 can dysregulate these proteins and contribute to cancer [21,22].

Research indicates that luteolin can significantly upregulate the expression of p21 in various cancer cell lines. For instance, Kang et al. demonstrated that luteolin promotes the expression of p21 in a dose- and time-dependent manner, which is linked to its ability to enhance apoptotic cell death via the Nrf2 and p53 pathways [23]. Chen et al. found that luteolin treatment resulted in G2/M phase arrest in esophageal carcinoma cells, implicating p21 in this process [24]. This is consistent with the broader understanding that both p21 and p27 serve as critical regulators of the cell cycle, with p21 often acting as a more immediate response to cellular stress signals, while p27 plays a role in maintaining quiescence and regulating the transition between different cell cycle phases [25]. Niu et al. demonstrated that a knockdown of MSI1 led to increased levels of both p21 and p27, resulting in inhibited proliferation of osteosarcoma cells, underscoring the importance of these inhibitors in cancer cell growth regulation [25].

Luteolin disrupts cell proliferation by interfering with cell cycle progression, thereby impeding the uncontrolled replication of cancer cells and inhibiting tumor growth. Luteolin induces G1 cell cycle arrest by modulating various signaling pathways [26]. Studies by Huang et al. [27] showed that luteolin inhibited the cell cycle progression of MDA-MB-231 breast cancer cells by modulating regulatory proteins essential for the G1/S phase transition, including cyclin D1, survivin, and p21. Luteolin also induced G2/M phase

arrest by decreasing the levels of positive-regulating proteins such as cyclin B1 and CDC2 while increasing the levels of negative-regulating proteins like checkpoint kinase 1 (CHK1) in a dose-dependent manner [28]. Iida et al. [29] demonstrated that luteolin activated the tumor-suppressor protein p53, leading to G1 and G2/M phase arrest. Additionally, luteolin was found to reduce cell proliferation in NCI-H460 cells by inducing cell cycle arrest at the S phase [30].

The upregulation of human telomerase reverse transcriptase (hTERT) promotes breast cancer cell progression, proliferation, and tumor advancement. Huang et al. [27] showed that treating breast cancer cells with 10–30 μM of luteolin reduced the mRNA expression of the breast cancer cell line, resulting in reduced cell cycle progression. It was suggested that luteolin's inhibition of hTERT through the c-Myc pathway was due to the downregulation of c-Myc in a time- and dose-dependent manner.

2.2. Apoptosis

Cell death, or apoptosis, is integral to processes like normal cell turnover, immune function, and embryonic development. The dysregulation of apoptosis homeostasis is associated with various conditions such as neurodegenerative disease, ischemic disease, autoimmune disease, and cancer. This section will discuss two major pathways for cellular apoptosis: intrinsic and extrinsic pathways [31,32].

The intrinsic pathway, also known as the mitochondria pathway, initiates programmed cell death. It begins with mitochondrial outer membrane permeabilization (MOMP), triggered by pro-apoptotic proteins like Bax and Bak, leading to the release of apoptogenic factors into the cytosol. Cytochrome c, for instance, migrates to the cytosol and forms the apoptosome complex with Apaf-1 and procaspase-9, activating caspase-9. Caspase-9 then activates downstream effector caspases such as caspase-3, ultimately resulting in apoptosis execution [33]. The intrinsic pathway is regulated by Bcl-2 family proteins, with anti-apoptotic members like Bcl-2 and Bcl-xL inhibiting MOMP, while pro-apoptotic members like Bax and Bak promote MOMP and cytochrome c release, crucial for maintaining tissue homeostasis and eliminating damaged or unwanted cells [34].

Extracellular death ligands such as Fas ligand (FasL) or tumor necrosis factor (TNF) bind to their respective death receptors on the cell surface, initiating the extrinsic pathway of apoptosis. This interaction recruits and activates caspase-8, which triggers downstream effector caspases such as caspase-3, leading to apoptosis. Moreover, caspase-8 can crosstalk with the intrinsic pathway by cleaving and activating the pro-apoptotic protein Bid, inducing the release of cytochrome c from the mitochondria, thereby amplifying the apoptotic signal [32,35].

Luteolin regulates both extrinsic and intrinsic apoptotic pathways. It enhances the expression of death receptors and their downstream factors while suppressing other death receptor signaling pathways that promote cell survival [8]. A study by Cao et al. [36] demonstrated that luteolin regulated both apoptotic pathways by activating caspase-8 and reducing the expression of Bcl-2 in hepatocarcinoma cells.

In the intrinsic apoptosis pathway, luteolin modulates mitochondrial membrane potential, releases cytochrome c, and inhibits the expression of anti-apoptotic proteins like Bcl-2 in colon and gastric cancer [37,38]. Kang et al. [23] suggested that luteolin increased the expression of ten-eleven translocation methylcytosine dioxygenase 1 (TET1), which may activate nuclear factor-erythroid factor-2 (Nrf2) expression, enhancing apoptosis by upregulating the expression of Bax, caspase-3, and caspase-9, and downregulating Bcl-2 expression. Luteolin also enhances the expression of the Bax/Bcl ratio in a concentration-dependent manner but inhibits BAD expression at higher doses of 80 μM [30].

In the extrinsic apoptosis pathway, proteins like Fas-associated death domain protein (FADD), caspase-3, and caspase-8 are activated to increase the expression of poly ADP-ribose polymerase (PARP), with cleaved PARP often serving as a marker of apoptosis [39]. You et al. [39] found that luteolin increased the expression of caspase-3, caspase-8, and cleaved PARP in the LN229 glioma cell line. Furthermore, luteolin improved the expression

of FADD, Fas ligand, and DR5/TRAIL. Luteolin sensitized TNF-related apoptosis ligands (TRAILs) of liver cancer cells (HCCs) that were resistant to TRAIL-induced apoptosis, leading to cell apoptosis with a high expression of cleaved caspase-3 and caspase-8 in HCCs [40]. Another study showed similar results, with luteolin enhancing TRAIL sensitivity in non-small cell lung cancer cell lines by increasing the expression of death receptor-5 (DR-5) and subsequently inducing apoptosis [41].

2.3. Autophagy

Autophagy, a cellular process, involves the degradation and recycling of aged or damaged organelles and abnormal proteins, playing a crucial role in maintaining cell homeostasis during stress conditions [42]. It encompasses three types: macroautophagy, microautophagy, and chaperone-mediated autophagy, each with its own mechanism of delivering cargo to the lysosome for degradation [43].

The autophagy system comprises four major mechanisms: initiation, nucleation, elongation, and fusion [44,45]. Initiation involves the FIP200/ATG/ULK1 complex and PI3KC-C1 complex inducing phagophore formation. The mammalian target of rapamycin (mTOR) serves as an upstream regulator of the FIP200/ATG/ULK1 complex by effectively suppressing it. AMP-activated protein kinase (AMPK) modulates mTOR by inhibiting it, leading to phagophore activation. Nucleation involves Beclin 1 and Vps34 activating phagophore, modulated by Bcl2 through Beclin 1 inhibition. Elongation entails the formation of LC3-II from LC3-I, activating phagophore. Fusion involves lysosome and autophagosome fusion stimulated by syntax 17 (STX17), synaptosome-associated protein 29 (SNAP29), vesicle-associated membrane protein 8 (VAMP8), and Rab 7 [43–45].

Autophagy acts as a regulator of both tumor progression and tumor suppression [46]. However, luteolin exhibits complex effects on autophagy, both inhibiting and activating it. A study by Kwon et al. [47] demonstrated that luteolin induced apoptosis by upregulating Bax protein and downregulating Bcl-2 protein, concurrently downregulating Beclin-1 and LC3 expression, inhibiting autophagy in PC12 cells. This study revealed that luteolin inhibited autophagy while inducing apoptosis. Another study by Cao et al. [36] showed that luteolin induced apoptosis by upregulating caspase-8 expression and downregulating Bcl-2 expression. However, luteolin also stimulated autophagy activation by enhancing Beclin-1 expression. The inhibition of autophagy by chloroquine compromised the apoptosis efficacy of luteolin in HCCs. Therefore, further extensive studies are warranted at the molecular level to understand the precise mechanism of autophagy in cancer cells and luteolin's impact on autophagy mechanisms.

2.4. Angiogenesis

Angiogenesis, the formation of new blood vessels, involves several processes including vascular destabilization, extracellular matrix degradation, endothelial cell proliferation, migration, invasion, tube formation, and pericyte recruitment [48]. Various angiogenic factors regulate this process, including vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and angiopoietin 1 and 2 [49]. Normally, a balance between pro-angiogenic and anti-angiogenic factors ensures the establishment of a well-organized blood network and adequate oxygen supply to tissues. However, dysregulation can lead to pathological angiogenesis, such as in cancer [50,51].

Luteolin inhibits VEGF by directly and indirectly disrupting the pathways that lead to its expression. For example, luteolin downregulates hypoxia-inducible factor 1-alpha (HIF-1α), which is crucial for VEGF regulation under hypoxic conditions, to demote angiogenesis in an in vitro study [52]. Recent studies have demonstrated that luteolin downregulates VEGF expression in uveal melanoma C918 and OCM-1 cells [53] and inhibits VEGF by suppressing Notch-1 expression in gastric cancer, thereby inhibiting vasculogenic mimicry tubes and angiogenesis [54]. VEGF receptor-2 activates various downstream signal transduction proteins that promote the growth, migration, differentiation, and survival of

endothelial cells in pre-existing vasculature. Therefore, Pratheeshkumar et al. suppressed VEGFR-2 signaling to inhibit angiogenesis in tumor-bearing mice by treating the cells with luteolin [55].

TNF-α promotes angiogenesis by inducing the expression of various pro-angiogenic factors, including VEGF and matrix metalloproteinases (MMPs) [56,57]. Luteolin has been shown to inhibit the activity of MMPs, a group of enzymes that degrade the extracellular matrix, facilitating tumor invasion and metastasis [58]. For instance, a study demonstrated that luteolin reduces MMP-2 expression and activity, which is crucial for the invasion of various cancer types, including oral and pancreatic cancers [59]. Furthermore, Wu et al. signified the importance of regulating MMP-9 in inhibiting proliferation and metastasis on triple-negative breast cancer (TNBC) via the AKT/mTOR pathway [60]. This inhibition of MMPs not only curtails the invasive capabilities of cancer cells but also contributes to the overall suppression of angiogenesis, as MMPs play a role in the remodeling of the extracellular matrix necessary for new blood vessel formation [61].

Interleukins (ILs) are known to promote tumor angiogenesis, invasion, and metastasis. Luteolin suppresses the expression and secretion of these interleukins, inhibiting their downstream signaling pathways that lead to metastasis [58,62]. Luteolin suppresses IL-6, a cytokine known to promote tumor growth and metastasis, particularly in pancreatic cancer where IL-6 activates the STAT3 signaling pathway, facilitating cancer cell migration and invasion [63].

While most anti-angiogenic drugs target endothelial cell-dependent angiogenesis, they often fail to inhibit vasculogenic mimicry (VM), an alternative neovascularization pathway allowing tumor cells to form perfusable vasculogenic-like networks independent of endothelial cells [64]. Recent studies have found that luteolin inhibits non-endothelial cell recruitment, microvasculature density increment, and vascular surface area increment in uveal melanoma cells [65,66]. Luteolin has also been reported to inhibit endothelial cell interaction and VEGF secretion in C918 and HUVEC cells [65].

Luteolin targets EGF and EGF receptors to counteract angiogenesis and cell proliferation. Sui et al. revealed that luteolin inhibited EGF-induced activities of the EGFR signaling pathway in human breast cancer cell lines, with PI3K/Akt, MAPK/Erk1/2, and STAT3 signal pathways mediating the inhibitory effect of luteolin on EGFR signaling in MCF-7 cells [67]. Furthermore, luteolin attenuated the efficacy of erlotinib in inhibiting cell proliferation and angiogenesis in glioblastoma multiforme IV (GBM) cells [68].

3. Limitations of Luteolin

The therapeutic potential of luteolin has been extensively studied and emphasized in numerous research works. However, one of the primary challenges in fully harnessing the pharmacological benefits of luteolin lies in its limited bioavailability. Luteolin's absorption occurs across the gastrointestinal epithelium following oral administration. It traverses through various compartments such as the stomach, intestinal cells, hepatic portal vein, and the liver before reaching systemic circulation. This journey exposes luteolin to the first-pass effect, wherein its structure undergoes metabolism. These metabolites may either exhibit lower efficacy in pharmacological activity or lack therapeutic effects entirely, leading to their rapid elimination [69].

3.1. Solubility

Several factors contribute to the restricted bioavailability of luteolin, among which poor water solubility stands out. Luteolin's hydrophobic nature hampers its dissolution and absorption in the gastrointestinal tract, limiting its uptake into the bloodstream following conventional oral administration [70]. Consequently, this diminishes the amount of luteolin available for systemic circulation. A study by Sarawek et al. demonstrated that the peak concentration of orally administered luteolin in rats was 5.49 μg/mL, whereas via the intravenous route, it reached 23.42 μg/mL, indicating a mere 4.1% bioavailability of oral luteolin [71]. Another study reported an oral bioavailability of luteolin of up to 26% [72].

Despite luteolin's high lipophilicity posing a challenge, it also signifies its potential as a potent anti-cancer agent [73]. Hence, enhancing luteolin's solubility becomes imperative to bolster its anti-cancer effects.

Some studies have reported the rapid absorption of luteolin in rat models. When administered with a *Chrysanthemum morifolium* extract (200 mg/kg), rats rapidly absorbed luteolin, reaching a peak concentration of 4 μg/mL at 1.1 h post-dosing [74]. Yasuda et al. [75] observed a similar rapid absorption of luteolin in rats, with levels increasing rapidly at 0.5 h post-administration and peaking at 0.76 μmol/L after 1 h. However, despite rapid absorption, poor bioavailability was noted due to low luteolin levels in plasma. Furthermore, the bioavailability of luteolin varies depending on its administration form. Zhou et al. [76] found that luteolin in a peanut hull extract exhibited a better pharmacokinetic profile than free luteolin, possibly due to the presence of polysaccharides in the plant extract, which can enhance flavonoid solubility. Nonetheless, even with an improved maximum concentration, it remains low compared to the intravenous route.

3.2. Metabolic Stability

When passing through the intestinal mucosa, luteolin or its glycoside undergo conversion into glucuronides and sulphates, such as luteolin monoglucuronide, luteolin diglucuronide, and luteolin glucuronide sulphate. These metabolites can be detected in plasma samples of rat models following oral administration of Chrysanthemum morifolium [75]. Additionally, these metabolites may contribute to biological activity within the body, as they have been found in the liver, kidney, and small intestine. However, their pharmacological activity appears to be weaker than that of luteolin itself [77]. Luteolin undergoes phase II metabolism to yield methylated, sulphated, and glucuronidated metabolites, which may enter systemic circulation, undergo enterohepatic cycling, or be eliminated [78]. Furthermore, the methylation pathway is also involved in luteolin metabolism; for example, Chen et al. [79] discovered that luteolin can be methylated to diosmetin and chrysoeriol by catechol-O-methyltransferase.

A recent pharmacokinetic study of luteolin in rats conducted by Dang et al. suggested that the bioavailability of total luteolin was efficient (>50%), but it was hindered by extensive metabolism in the intestine and liver, with the bioavailability of free luteolin showing only 17.5%. This study, which administered luteolin orally and intravenously to rats, compared the pharmacokinetics of luteolin. The results revealed that luteolin was rapidly absorbed from the intestine, with a similar rate to intravenous administration (within 10 min), but with significantly different maximum concentrations. Moreover, when administered orally, the area under the curve (AUC) of free luteolin was much lower compared to its glucuronide conjugates at the 3′-OH position [80]. The rapid absorption and metabolism of luteolin may be influenced by intestinal microbiota, such as *E. ramulus*, *E. coli*, and *L. casei*, which degrade luteolin or convert it into easily absorbable conjugated forms [81,82].

From this evidence, it can be concluded that the solubility and extensive metabolism of luteolin play significant roles in limiting its pharmacological potential. Poor solubility may decrease the concentration of active luteolin, necessitating higher doses to compensate for the loss and potentially leading to adverse effects such as increased oxidative stress, disruption of metabolic pathways, and potential of neurotoxicity [83,84]. Furthermore, extensive metabolism, whether in the intestine or liver, further reduces the already limited amount of luteolin, resulting in lower plasma concentrations and bioavailability. Addressing these challenges requires extensive research efforts aimed at improving luteolin's bioavailability through the development of innovative drug delivery systems. These systems aim to enhance luteolin's solubility, protect it from rapid degradation, and improve its absorption, thereby increasing its bioavailability.

4. Nanoformulations

Nanoformulations offer a promising solution to address luteolin's poor water solubility, a significant obstacle in achieving adequate bioavailability. The smaller size of the carrier system provides a higher surface area-to-volume ratio, facilitating easy dispersion of luteolin in aqueous solutions and enhancing its dissolution rate. Additionally, nanoformulations serve to protect luteolin from aggregation and crystallization, common challenges faced by poorly water-soluble compounds such as quercetin, curcumin, and apigenin. Encapsulating luteolin within nanoparticles, including liposomes, micelles, or polymeric nanoparticles, results in the formation of a stable colloidal dispersion. This not only improves solubility but also extends circulation time and enhances bioavailability [85,86]. Figure 2 illustrates the impact of nanoformulations on luteolin's solubility and bioavailability.

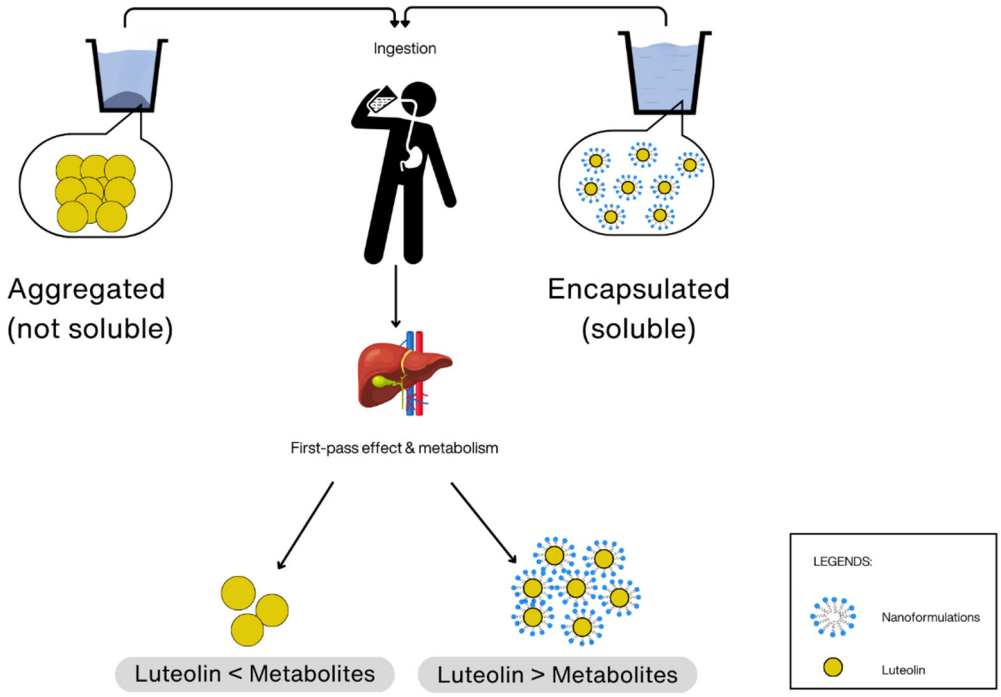

Figure 2. The effect of nanoformulations on luteolin towards solubility and metabolism.

In addition, nanoformulations provide a protective shield for luteolin against premature degradation, as it can be susceptible to degradation by various enzymes and microbiota within the intestinal tract [87]. Encapsulation within nanoparticles effectively isolates luteolin from the surrounding environment, reducing its exposure to enzymes and other degradative factors. This protective barrier helps maintain the structural integrity and bioactivity of luteolin during its journey through the digestive system. Additionally, nanoformulations release luteolin slowly at the targeted site, minimizing exposure to degrading factors and maximizing its bioavailability [88].

Moreover, nanoformulations enhance the efficacy of luteolin in several ways. Increasing the surface area facilitates efficient interaction with gut cells, enhancing absorption due to its nano sizing. Certain materials in nanoformulations contain P-glycoprotein (P-gp) inhibitors, which are responsible for drug efflux, and cell permeability enhancers. For example, vitamin E D-tocopheryl polyethylene glycol succinate (TPGS) not only acts as a cellular uptake enhancer by solubilizing poorly water-soluble drugs but also inhibits P-gp and reverses multidrug resistance (MDR) in cancer cells [89]. A study by Li et al. [90] compared the cellular uptake of A549 cells when treated with luteolin-loaded liposomes versus luteolin-loaded TPGS liposomes, with the latter demonstrating better cellular uptake via endocytosis compared to normal liposomes. However, research on luteolin-loaded

TPGS nanoformulations successfully inhibiting MDR remains to be reported, leaving room for further exploration.

Finally, nanoformulations can enhance the specificity of luteolin towards its targeted site through two mechanisms: passive and active targeting. Passive targeting leverages the enhanced permeability and retention (EPR) effect, allowing nanoparticles to accumulate in tumorous tissues due to leaky blood vessels and impaired lymphatic drainage. Active targeting aims to deliver nanoparticles to specific cells or tissues by attaching targeting ligands to the nanoparticle surface, such as folic acid, the HER2 antibody, and other ligands. This targeted delivery ensures that luteolin is absorbed at the desired site, increasing its therapeutic potential and reducing systemic side effects [91,92].

4.1. Types of Nanoformulations

Various types of nanoformulations designed to improve the effectiveness of delivering therapeutic benefits of luteolin are explored in this section. It also highlights the significance of nanoformulations in enhancing the solubility of luteolin in water, its bioavailability, and other important characteristics. Figure 3 offers a comprehensive view of luteolin-loaded nanoformulations, with a specific emphasis on their application in anti-carcinogenic activities. A summary of various nanoformulations developed in previous studies for the encapsulation of luteolin is presented in Table 2.

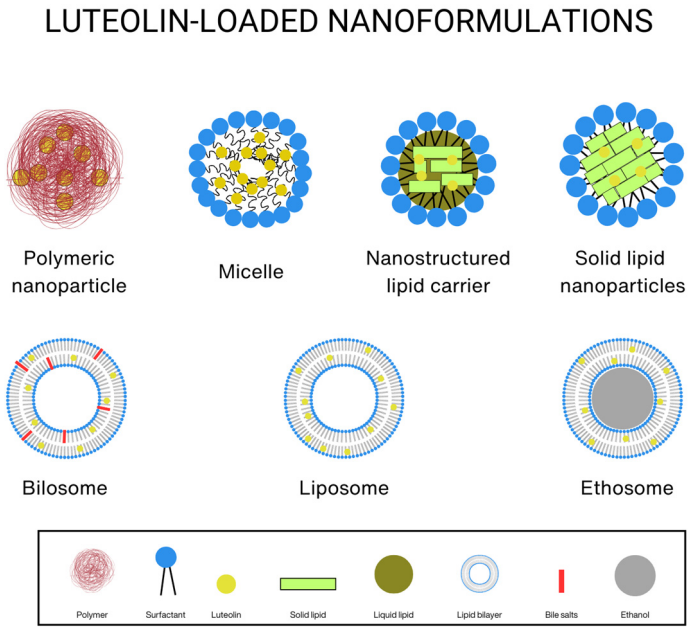

Figure 3. Diagram of nanoformulations used to carry luteolin for cancer treatment.

Table 2. Summary of various nanoformulations for luteolin encapsulation.

Nano Formulations	Components	Particle Size (nm)	Zeta Potential (mV)	Treatment	Outcome	References
Polymeric nanoparticle	Polypropylene sulfide (PPS) and polyethylene glycol (PEG)	169.77 ± 7.33	Not stated	Melanoma	• Efficiently internalized in SK-MEL-28 melanoma cells and showed low cytotoxicity against normal cells • Inhibit tumor 2-fold than control in rat subject	[93]
Polymeric nanoparticle	DSPE-PEG_{2000}, folic acid and Oxi-αCD	196.7 ± 1.8	−41.0 ± 0.01	Breast cancer	• Improved internalization and tumor cell inhibition in 4T1 cell line • Inhibit tumor growth 3 times than control group in animal study	[94]
Micelle	Poloxamer 407 and TPGS	24.57 ± 0.61	−30.97 ± 1.93	Breast cancer	• High encapsulation efficiency (90.7 ± 0.9%) • Sustained release profile with potential to release higher in microtumor environment	[95]
Micelle	MPEG and PLGA	30 ± 2	Not stated	Cerebral ischemia reperfusion (IR)	• Higher penetration and therapeutic effect in IR injury restoration • Strengthened protection of nerve cell by NF-κB and MAPK pathways • Inhibited inflammation and the oxidative stress response by the downregulation of TNF-α, IL-1β, and IL-6, and upregulation of SOD and GSH-px	[96]
Micelle	MPEG and PCL	36.5	−3.2	Glioblastoma	• Stronger cytotoxicity induced a higher percentage of apoptosis in C6 and U87 cells than free luteolin. • Induced more glioma cell apoptosis than free luteolin and inhibited neovascularization in tumor tissues. • drug accumulated much more in the nano-drug group than free drug group through imaging in vivo	[97]
Micelle	MPEG and PCL	38.6 ± 0.6	−3.54 ± 0.32	Breast and colon cancer	• Improve luteolin half-life, area under curve, and peak concentration when administered in rats • Improving IC50 of luteolin in 4T1 breast cancer cell line and C26 colon cancer cell	[98]

Table 2. *Cont.*

Nano Formulations	Components	Particle Size (nm)	Zeta Potential (mV)	Treatment	Outcome	References
Nanostructured lipid carrier	Glyceryl monostearate, Capryol 90, P-188, TPGS and chitosan	123.84 ± 3.72	+10.46 ± 1.84	Breast cancer	• Higher mucoadhesion, gastrointestinal stability, and intestinal permeation compared to free luteolin • Enhanced antioxidant potential and cytotoxicity against MDA-MB-231 and MCF-7	[99]
Liposome	Cholesterol and lecithin	105	0.12	Colorectal carcinoma	• Improved IC50 on colorectal carcinoma cell line CT26 • stronger tumor growth-inhibiting effects, suppressed angiogenesis, increased apoptosis than Free-Lut in vitro and in vivo • Improved luteolin concentration by 10 folds in plasma	[100]
Liposome	PLGA, cholesterol, DSPE-PEG and PD-L1 antibody	159.3 ± 7.45	−3.2 ± 0.44	Liver cancer	• Improved cellular uptake and higher toxicity compared to normal PLGA-liposome and free luteolin • Higher inhibition of HepG2 migration compared to normal liposome and free lutelin	[101]
Ethosome	Soy lecithin, cholesterol and propylene glycol	89.77 ± 0.86	−42.6 ± 3.01	Hepatocellular carcinoma	• Significantly improved hepatic biomarker, decreased expression of GPC3 gene, decreased hepatic tissue NO and MDA content. • Significantly reduced the number of hepatic adenomas and neoplastic hepatic lesions	[102]

4.1.1. Polymeric Nanoparticles

Polymeric nanoparticles (PNPs) serve as effective delivery vehicles for overcoming challenges in clinical applications. With sizes ranging from 10 to 100 nm, they load active compounds either by entrapping them within the core or by adsorbing them onto the surface of the polymeric core [103]. Researchers have identified various types of polymers suitable for efficiently delivering hydrophobic drugs, offering advantages such as improved bioavailability and circulation time, controlled drug release, and biodegradability [104]. Typically, PNPs consist of triblock copolymers with hydrophilic heads and hydrophobic cores. Hydrophobic drugs are loaded into the core, while the hydrophilic outer layer protects the drugs from the external environment. For instance, PVP-k30/cyclodextrin nanoparticles enhance luteolin solubility in water up to 132 times compared to free luteolin, consequently increasing luteolin's bioavailability in mice, as evidenced by peak concentration and AUC values [105].

In some cases, polymer blends are necessary to achieve the appropriate hydrophilic–lipophilic balance ratio. Hydrophilic polymers are more stable in physiological fluids but have limited capacity to encapsulate hydrophobic drugs due to their lack of hydrophobic structures, resulting in lower drug payloads. Conversely, hydrophobic polymers can encapsulate hydrophobic drugs more effectively but are prone to rapid excretion, leading to a lower half-life and necessitating frequent dosing. Combining hydrophilic and hydrophobic polymers mitigates these disadvantages and capitalizes on the advantages of both [106].

Additionally, polymer blends often possess unique properties that neither polymer possesses individually, enhancing the functionality of nanoparticles. For example, Fu et al. [93] developed luteolin-loaded nanoparticles comprising hydrophilic polyethylene glycol (PEG) and hydrophobic polypropylene sulphide (PPS), leveraging the stealth properties of PEG to evade the reticuloendothelial system (RES) and the ROS-responsive nature of PPS to release the hydrophobic drug in the presence of oxidizing environments like tumor cells. While this study demonstrated increased cytotoxicity against tumor cells compared to normal cells due to elevated ROS levels in tumor cells, it did not provide in vivo retention time comparisons between luteolin-loaded nanoparticles and free luteolin in mice, which could further support their claims.

One significant advantage of PNPs is their amenability to attachment or conjugation with various ligands such as folic acid, antibodies, and proteins to enhance nanoparticle specificity and efficacy at targeted sites. Tumor cells overexpress folate receptors on their surfaces to fulfill their folate requirements for active proliferation. Wang et al. [94] demonstrated the importance of folic acid conjugation to nanoparticles in improving luteolin's anti-tumor efficacy in breast cancer cells, showing that folic acid-bearing nanoparticles exhibited 2.5-fold higher cytotoxicity and prolonged retention compared to non-folic acid-bearing nanoparticles, facilitating cellular internalization. Xiao et al. [107] observed increased cellular uptake of SK-BR-3 breast cancer cells when luteolin was loaded into nanospheres conjugated with HER-2 antibodies, leading to cell proliferation suppression, downregulation of cell migration, and upregulation of FOXO1 expression, highlighting the potential of targeted nanoparticle delivery systems in cancer therapy.

4.1.2. Micelles

Micelles represent a nanoformulation composed of either amphiphilic surfactants or triblock co-polymers, known for their "self-assembly" property above the minimum micellar concentration (CMC) to form efficient spherical hydrophobic drug carriers. One of the key advantages of micelles is their capability to solubilize poorly water-soluble drugs, such as luteolin itself. An experiment investigating the development and optimization of luteolin-loaded TPGS/F127 micelles revealed that these micelles solubilized luteolin in water up to 350-fold compared to free luteolin in water, with the micellar storage system capable of loading up to 90% of 10 mM luteolin [95]. However, the encapsulation capacity of micelles is also limited; increasing the amount of luteolin in the micelle decreases the encapsulation efficiency, indicating an upper limit to micellar drug storage [96].

In addition to improving solubility, luteolin-carrying micelles also enhance the bioavailability of luteolin by encapsulating it within PEG-based micelles. MPEG-PCL micelles increase luteolin solubility, dissolving 98% of 6 mg (approximately 2 mM) of luteolin. Upon injection into male Sprague-Dawley rats, MPEG-PCL micelles were found to release luteolin in a controlled manner and prolong its circulation in the blood [97]. Further evidence supports these findings, as MPEG-PCL micelles increased luteolin's circulation time up to 21-fold and boosted its peak concentration from 7.73 mg/L to an impressive 92.7 mg/L [98].

4.1.3. Lipid-Based Nanoparticles

Lipid-based nanoparticles have garnered significant attention in drug delivery systems due to their ability to improve the bioavailability of hydrophobic drugs, control drug release, facile modification, biocompatibility, and scalability for large-scale manufacturing. Some researchers prefer lipid nanoparticles over polymeric nanoparticles due to their excellent cytotoxicity profile and suitability for large-scale production [108,109].

Nanostructured lipid carriers (NLCs), a type of lipid nanoparticle, enhance the bioavailability of hydrophobic drugs by improving their stability in the gastrointestinal tract and release profile [110]. Chitosan-coated NLCs, for instance, demonstrated a high encapsulation efficiency of up to 95.37% for luteolin and increased its permeation flux in the gastrointestinal tract by 4.5-fold compared to luteolin suspension [99]. Additionally, NLCs facilitated enhanced luteolin skin permeation, nearly 2.5 times more effectively than luteolin gel, thereby reducing inflammation and proliferation factors in skin lesions and blood [111].

Liposomes have garnered significant attention for their exceptional drug delivery capabilities, offering advantages such as improved pharmacokinetics, extended circulation time, and active and passive targeting options [112]. Wu et al. [100] developed a lecithin/cholesterol liposome to enhance the therapeutic effects of luteolin on colorectal cancer. Their study demonstrated that liposomal luteolin improved the solubility and bioavailability of luteolin, resulting in a more effective colorectal tumor-suppressing agent compared to free luteolin. This enhancement was evident in terms of apoptosis, angiogenesis, and cell proliferation. To further enhance the active targeting ability of liposomes, Cao and Wang [101] conjugated liposomes with programmed death ligand-1 (PD-L1), a receptor overexpressed on the surface of hepatocellular carcinoma (HCC) cells. This active targeting strategy increased the cellular uptake of the tumor cells compared to free and non-targeting liposomes.

Ethosomes, akin to liposomes, serve as nano-drug carriers composed of phospholipid, water, and ethanol. The key distinction lies in the inclusion of ethanol in ethosomes. This addition allows ethosomes to exhibit higher encapsulation efficiency compared to liposomes, owing to ethanol's ability to readily dissolve hydrophobic drugs. Elsayed et al. [102] demonstrated that increasing the ethanol concentration led to improved encapsulation efficiency; however, exceeding the optimal concentration resulted in a diminished solubility of luteolin in ethosomes. The study further revealed that luteolin ethosomes effectively decreased liver biomarkers that were elevated in hepatocellular carcinoma (HCC) cells, contrasting with luteolin suspension, which showed no such effect on liver biomarkers. This suggests that ethosomes enhanced the therapeutic potential of luteolin, potentially mitigating liver injury associated with liver carcinoma.

Bilosomes, a derivative of liposomes, involve the integration of naturally occurring lipids like lecithin with bile salts. Abbas et al. conducted a study showcasing the efficacy of bilosomes in dissolving and delivering luteolin across the skin barrier. Their research demonstrated that bilosomes significantly enhanced the permeation of luteolin through the skin layers. Furthermore, the study indicated a lower toxicity profile of the carrier along with superior antioxidant, anti-inflammatory, and anti-wrinkling effects compared to free luteolin [113]. While this study did not investigate the impact of luteolin on cancer cells, it presents an intriguing avenue for further exploration. The potential of bilosomal luteolin as a treatment for skin cancer remains to be thoroughly investigated by future researchers.

4.1.4. Other Nanoformulations

Liu et al. [114] introduced a surface-modified nanocrystal to enhance the bioavailability of luteolin further. Initially, the nanocrystal dissolved luteolin and improved its bioavailability by 1.9 times. However, after modification with sodium dodecyl sulphate (SDS), the bioavailability of luteolin increased even more, reaching 3.5 times the original level. SDS plays a crucial role in this enhancement by opening up the tight junctions of the intestinal caco-2 monolayer, facilitating the transport of luteolin across the intestinal membrane.

B-cyclodextrin has emerged as another notable nanoformulation for dissolving and delivering hydrophobic drugs to their targets. Zhu et al. [115] developed a B-cyclodextrin inclusion complex for luteolin, resulting in a remarkable increase in its solubility in water by up to 1600 times. As the amount of luteolin dissolved in water increased, so did its cytotoxic effect significantly compared to free luteolin.

5. In Vitro Study of Luteolin Nanoformulations

Nanoformulations of luteolin enhance the intake of luteolin into various cancer cell lines. For example, Yan et al. [116] conducted a study reporting that DSPE-PEG2000/TPGS micelles exhibited remarkable cellular intake in NSCLC A549 cells. When treated with free coumarin-6 fluorescence dye, cellular uptake increased in a time-dependent manner, reaching nearly 20% at 4 h. However, when the dye was encapsulated, cellular uptake significantly surged to almost 80% at the same time point. Majumdar et al. [117] explored the cellular uptake of luteolin nanoparticles in Tu212 cells using sulfo-rhodamine B acid chloride and observed under a confocal laser scanning microscope. The results indicated that Tu212 cells exhibited a time- and temperature-dependent uptake of PLA-PEG nanoparticles, with maximum uptake occurring at 37 °C after 4 h of incubation. Another recent study also demonstrated time-dependent cellular internalization. A recent study also showed that cellular internalization happens in a time-dependent manner. Sen et al. [118] labelled their active-targeting nanoparticle and non-targeting nanoparticle with FITC dye and analyzed cellular uptake via flow cytometry. The findings revealed superior intake of the active-targeting nanoparticle compared to the non-targeting nanoparticle, as well as a higher efficacy of the active-targeting nanoparticle containing luteolin in anti-cancer activity compared to other formulations and free luteolin, owing to increased cellular uptake and internalization.

Nanoformulations improve the cytotoxicity of luteolin on cancer cell lines. In glioblastoma cell lines C6 and U87, MPEG/PCL micelles reduced the half maximal inhibitory concentration (IC50) of luteolin by 73% and more than 70%, respectively [102]. Yan et al. [116] investigated the cytotoxicity difference between blank DSPE-PEG2000/TPGS micelles, luteolin-loaded micelles, and free luteolin. The study found that micelles reduced the IC50 of luteolin in A549 cells, indicating higher toxicity compared to free luteolin. Furthermore, the IC50 of luteolin-loaded micelles was almost the same as paclitaxel. Interestingly, blank micelles also exhibited cytotoxicity, but at a higher concentration (500 μg/mL), TPGS played a role in anti-cancer activity [116,119]. This underscores the importance of carefully selecting the polymer and its concentration when developing nanocarriers, as even blank micelles exhibit cytotoxicity.

On the other hand, Baz et al. [120] demonstrated the cytotoxicity of PLA-PEG nanoparticles loaded with luteolin on SCC25 cells compared to free luteolin and 5-fluorouracil. Results showed that nanoparticles significantly reduced the IC50 of luteolin by 88% in a 24 h viability test. In fact, the IC50 of luteolin nanoparticles was lower than that of 5-fluorouracil, albeit not significantly. Wu et al. [110] tested the cytocidal effect of luteolin-loaded liposomes and compared it with free luteolin. The results revealed that both formulations exhibited cytotoxicity towards colorectal cancer cell line CT26 in a time- and concentration-dependent manner, but luteolin liposomes had a significantly higher cytocidal effect on the cancer cells. Additionally, luteolin-loaded phytosomes attenuated the cytotoxicity of doxorubicin in MDA-MB-231 breast cancer cell lines. A significant improvement in the

cytocidal effect of doxorubicin was observed on the cells when treated together with phyto-luteolin compared to free doxorubicin, phyto-doxorubicin, and doxorubicin + luteolin [121].

In the case of surface-modified nanoparticles, better intake and cytotoxicity of luteolin towards various cancer cell lines were observed. A recent study showed the effect of the conjugation of ligands specific towards cancer cells on its cytotoxicity towards lung cancer cells. Luteolin nanoparticles required only 4.4 μg/mL to achieve IC50 compared to free luteolin at 7.8 μg/mL. However, the concentration further reduced when the nanoparticles were attached with Triticum agglutinin, a ligand whose receptor was overexpressed on the surface of cancer cells. This demonstrated that the more specific the targeting, the higher the cytocidal effects of luteolin towards cancer cells [118]. A study by Zhu et al. [115] modified the surface of the B-cyclodextrin inclusion complex with biotin, which is overexpressed in many cancer cells like leukemia, ovarian, and breast cancer cells. However, when the amount of biotin increased, the IC50 gradually increased, possibly due to receptor saturation, thereby reducing the targeting ability. This indicates that an increase in the concentration of the target ligand reduces the cytocidal effect.

Anti-cancer effects such as cellular apoptosis was also enhanced when luteolin was carried by nanoformulations. PLA-PEG nanoparticles have been found to enhance the expression of caspase-3 by luteolin, implying that nanoparticles improve luteolin's apoptosis properties by activating both intrinsic and extrinsic pathways [120]. Interestingly, caspase-3 expression was significantly higher when treated with luteolin-loaded nanoparticles compared to 5-fluorouracil. Luteolin induced apoptosis through the mitochondrial pathway by increasing the expression of pro-apoptotic Bax protein and reducing the expression of anti-apoptotic Bcl protein. In glioblastoma cells, luteolin micelles improved the expression of Bax and pro-caspase-9, and downregulated Bcl and p-MAPK expression in U87 glioma cells, indicating that luteolin micelles also inhibit cell proliferation by downregulating MAPK pathways [102].

Luteolin inhibits the tumor cell cycle by suppressing the clonal formation of cancer cells. Ding et al. [122] demonstrated that luteolin-loaded nanoparticles enhanced the ability of luteolin to inhibit tumor cell growth. When the nanoparticles were attached to HER-2 to increase the targeting ability of the carrier, the mRNA and protein expression of FOXO1 significantly increased up to 3.85- and 6.7-fold, respectively, compared to free luteolin, while the normal nanoparticles increased the expression up to 2.7- and 3.2-fold, respectively. Furthermore, luteolin inhibits nuclear factor erythroid 2-related factor 2 (Nrf-2), which is responsible for multidrug resistance. Sabzichi et al. [121] demonstrated the effectiveness of luteolin-loaded phytosomes in sensitizing MDA-MB-231 cells to anti-cancer agents. The results indicated that phyto-luteolin more effectively suppressed the Nrf-2 pathway by reducing the expression of HO1 and MDR1 compared to free luteolin. This suggests that luteolin enhances multidrug resistance sensitivity by inhibiting the Nrf-2 protein.

Additionally, luteolin nanoformulations amplify the ability of luteolin to impede metastasis. Naso et al. [123] proposed that the luteolin–oxidovanadium (IV) ion complex (VO-Lut) enhances luteolin's ability to suppress metastasis in breast and colorectal cancer. Their study demonstrated that VO-Lut improved luteolin's inhibition of invasion and migration in breast cancer cells by 64% and 54%, respectively. Additionally, in adhesion assays, VO-Lut reduced the adhesion ability of MDA-MB-231 cells to the extracellular matrix and basement membrane, whereas free luteolin did not show a significant effect compared to the control. These findings suggest an increased efficacy of luteolin in combating metastasis in breast cancer cells when in complex with oxidovanadium (IV) ions.

6. In Vivo Study of Luteolin-Nanoformulations

Luteolin nanoformulations improved biodistribution in pre-clinical studies. For example, Yan et al. [116] demonstrated that DSPE-PEG2000/TPGS micelles had better retention in tumor cells compared to a free fluorescence substance, DiR iodide, which represented luteolin. While this method was effective in determining the tissue distribution of the drug carrier, the free DiR iodide did not accurately represent the biodistribution of luteolin.

Therefore, alternative methods such as drug extraction from organs should be used to quantitatively determine the amount of free luteolin distributed to vital organs.

Another approach to assess biodistribution is by radiolabeling. Sen et al. [118] demonstrated an accurate radiolabeling technique to obtain better imaging of the luteolin biodistribution. In this study, free luteolin, luteolin-loaded nanoparticles, and Triticum agglutinin-conjugated nanoparticles were labelled to determine the specificity of the nanoparticles. The results showed that the surface-modified nanoparticles exhibited the highest accumulation in the target organ compared to non-targeted nanoparticles and free luteolin. Wu et al. [110] studied the pharmacokinetics of luteolin-loaded liposomes in mice and found that liposomes increased the bioavailability of luteolin in mice by reducing the elimination time from 5 min to 2 h. Similarly, Zheng et al. increased the half-life, Tmax, Cmax, and AUC of luteolin in rat models by encapsulating luteolin with MPEG-PCL micelles, thus improving the bioavailability and pharmacokinetics of luteolin [102]. Qiu et al. [103] obtained similar results using MPEG-PCL micelles.

Numerous studies have demonstrated the ability of luteolin to exhibit anti-tumor properties, not only in cell line studies but also in pre-clinical trials. Yan et al. claimed that luteolin-loaded micelles had a better anti-tumor effect compared to control and free luteolin based on the weight of Balb/C mice [116]. Luteolin-loaded micelles reduced the body weight of the mice, indicating a reduction in tumor mass. However, compared to paclitaxel, paclitaxel was more effective in reducing mouse weight than luteolin-loaded micelles. Luteolin reduced tumor weight in glioma-bearing mice by almost 50% compared to control mice. However, when researchers loaded luteolin into MPEG/PCL micelles, tumor mass was significantly reduced by up to 80% [102]. This indicates that nanoformulations significantly improve the anti-cancer effects of luteolin. However, Majumdar et al. used a different approach to measure the anti-tumor efficacy of luteolin, whereby tumor growth was measured using a Vernier calliper instead of measuring the weight of the rat models [117]. This was possible due to the clear visibility of the tumor on the neck of the rat models. Results showed that PEG-PLA nanoparticles enhanced the anti-tumor efficacy of luteolin, while free luteolin showed no significant difference with the control group in terms of tumor growth [117].

Additionally, a study reported the results from a TUNEL assay of luteolin liposomes on Balb/C mice, confirming that both free luteolin and luteolin in liposomes induced apoptosis in tumor cells [110]. Furthermore, both formulations showed less microvessel formation compared to the control group, indicating th einhibition of tumor vascularization, with liposomes further improving the inhibition of tumor vascularization by luteolin. Additionally, the expression of Ki-67, a protein associated with tumor cell proliferation, was further reduced when treated with liposomal luteolin. Zheng et al. supported this claim when luteolin was encapsulated into micelles. Luteolin decreased the expression of Ki-67 by 53%, while luteolin micelles dropped it to 22% of Ki-67 expression. Furthermore, the micelles improved the inhibition of microvessel formation by luteolin to almost 50%, signifying the anti-angiogenesis part of luteolin in vivo [102].

A study by Naso et al. [123] revealed that when luteolin was loaded into the oxidovanadium complex (VO-Lut), the efficacy of luteolin in suppressing the metastasis of colorectal cancer skyrocketed. In hematoxylin and eosin staining, VO-Lut eradicated metastatic nodules in the liver of mice, while the control group contained a high density of metastatic colorectal tumors. Luteolin alone only reduced 24% of metastatic nodules in liver cells. This highlights the importance of the drug delivery of luteolin in enhancing its efficacy against tumor cells.

Table 3 summarizes various types of luteolin nanoformulations utilized in previous in vitro and in vivo studies.

Table 3. In vitro and in vivo studies of luteolin nanoformulations.

Type of Nanoformulation	Type of Study	Summary of Outcome	References
Folic Acid-Conjugated Nanoparticles	Cytotoxicity Study	Increased cytotoxicity and retention in breast cancer cells	[96]
MPEG/PCL Micelles	Cytotoxicity Study	Reduced IC50 by 73% in glioblastoma cell lines C6 and U87	[102]
	Tumor Mass Reduction Study	Improved bioavailability, increased half-life, reduced tumor mass by 80%	
Luteolin-Encapsulated MPEG-PCL Micelles	Bioavailability Study	Enhanced bioavailability, prolonged circulation, reduced elimination time to 2 h	[103]
Liposomal Luteolin	Apoptosis and Angiogenesis Study	Improved solubility and bioavailability, suppressed colorectal tumors, induced apoptosis and angiogenesis	[110]
Luteolin-Encapsulated Nanocrystals	Bioavailability Study	Bioavailability improved by 3.5 times due to SDS surface modification	[114]
DSPE-PEG2000/TPGS Micelles	Cellular Uptake Study	Remarkable cellular intake in NSCLC A549 cells (80% cellular uptake) compared to free coumarin-6 dye	[116]
	Tumor Reduction Study	Enhanced retention in tumor cells, reduced tumor weight in glioma-bearing mice by 50%	
PLA-PEG Nanoparticles	Cellular Uptake Study	Time- and temperature-dependent uptake in Tu212 cells; maximum uptake at 37 °C after 4 h	[117]
	Tumor Reduction Study	Reduced tumor growth in rat lung cancer models	
Triticum Agglutinin-Conjugated Nanoparticles	Targeting and Accumulation Study	Highest accumulation in target organs, enhanced specificity over non-targeted nanoparticles	[118]
	Cellular Uptake Study	Superior intake and efficacy of active-targeting nanoparticle in lung cancer cells	
Luteolin-Loaded Phytosomes	Chemosensitization Study	Sensitized MDA-MB-231 breast cancer cells to doxorubicin by suppressing Nrf2 signaling	[120]
HER-2 Antibody-Conjugated Nanoparticles	Targeting and Proliferation Study	Upregulated FOXO1 expression, enhanced targeting, and suppression of breast cancer cell proliferation	[122]
Oxidovanadium (VO-Lut) Complex	Metastasis Inhibition Study	Inhibited metastasis in colorectal cancer, eradicated metastatic nodules in the liver	[123]

7. Challenges of Nanoformulations

The development, optimization, and application of nanoformulations face numerous challenges that can hinder their successful translation from laboratory to clinical settings. These challenges can be categorized into several key areas: physicochemical properties, toxicological concerns, regulatory hurdles, and manufacturing complexities.

One of the primary challenges in the development of nanoformulations is the physicochemical characterization of nanoparticles. The synthesis of nanoparticles often results in significant heterogeneity in size, shape, and surface properties, which complicates the manufacturing process and quality control [124,125]. This particle-to-particle variability can lead to inconsistent therapeutic outcomes and poses difficulties in meeting regulatory standards [125]. Furthermore, the stability of nanoformulations is a critical concern; engineered nanomaterials may exhibit instability in biological environments, leading to premature drug release or aggregation, which can diminish their therapeutic efficacy [126].

Toxicological issues also present significant challenges. The engineered nanoparticles may elicit unexpected immune responses or exhibit cytotoxicity, particularly when they interact with biological systems [127]. For instance, certain nanoformulations can disrupt

the blood–brain barrier (BBB), leading to neurotoxicity and inflammation [128]. Additionally, the potential for heavy metal ion release from certain formulations raises concerns regarding their safety [126]. Pre-clinical immunotoxicity studies are essential to assess these risks, yet they are often complicated by the unique properties of nanomaterials, which require specialized testing methods [127].

Regulatory hurdles further complicate the landscape for nanoformulations. The existing regulatory frameworks for pharmaceuticals may not adequately address the unique characteristics of nanomedicines, leading to uncertainties in approval processes [129]. Regulatory agencies are still in the process of defining critical quality attributes specific to nanoformulations, which can delay the development timeline [125]. Moreover, the need for comprehensive safety and efficacy data, including long-term studies, adds to the complexity of bringing nanoformulations to market [130].

Manufacturing complexities also play a significant role in the challenges faced by nanoformulations. The scale-up of production from laboratory to industrial levels often encounters issues related to the reproducibility and consistency of the nanoformulations [129]. The use of organic solvents and toxic compounds during synthesis can lead to environmental concerns and complicate the transition to large-scale manufacturing [129]. Additionally, the encapsulation efficiency of therapeutic agents within nanoparticles can vary, impacting the overall therapeutic potential and necessitating further optimization [126]. Therefore, addressing these challenges is crucial for the successful translation of nanoformulations into effective therapeutic agents.

8. Conclusions and Future Perspective

Luteolin's potential as a cancer treatment is indeed promising, given its ability to induce apoptosis, halt cell cycle and proliferation, inhibit angiogenesis, and activate phagocytosis in cancer cells. However, challenges such as poor bioavailability, low water solubility, and extensive liver metabolism have hindered its efficacy. Therefore, improving luteolin's bioavailability and protecting it in the bloodstream are crucial to maximize its anti-cancer effects. Current research on luteolin nanoformulations is still relatively limited compared to more established counterparts like quercetin and resveratrol. This suggests a significant opportunity for further exploration in this area, with the goal of advancing luteolin towards clinical trials. As of now, no clinical studies have been conducted on luteolin as a cancer treatment.

Future studies could focus on comparing the efficacy and toxicity profiles of luteolin with clinically tested counterparts like quercetin and resveratrol. Establishing the clinical use of luteolin in real-life scenarios would be an important milestone. Despite numerous molecular mechanism studies, the exact mechanisms of luteolin's action against cancer cells remain unclear. Therefore, further research is needed to elucidate the precise pharmacological mechanisms underlying luteolin's anti-cancer effects. While nanoformulations have significantly increased the bioavailability of luteolin, achieving specificity remains a challenge. Luteolin should ideally target cancer cells specifically to avoid cytotoxicity towards normal cells and minimize side effects. Active targeting or functionalized nanoformulations, such as ROS-sensitive or pH-sensitive formulations, could hold the key to achieving specific targeting of luteolin towards cancer cells.

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