Review

Multi-target mechanisms of hypericin: A systematic elucidation of pharmacological activities and therapeutic potential

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Abstract

Hypericin, a naturally occurring naphthodianthrone isolated from several species of the genus *Hypericum*, exhibits multiple pharmacological properties through its unique ability to interact with diverse molecular targets. As a multi-target agent, hypericin shows therapeutic potential in treating neurological disorders, viral infections, cancer, inflammatory-related diseases, and dermatological conditions. Despite these advances, challenges such as poor bioavailability, phototoxicity, and an incomplete mechanistic understanding of multi-target synergies hinder its clinical translation. Recent strategies, including nanoformulations to enhance solubility and combination therapies to optimize efficacy, demonstrate promise in overcoming these limitations. This review systematically consolidates the pharmacological actions, molecular mechanisms, and therapeutic applications of hypericin while highlighting future directions for precision multi-target drug development.

Keywords: hypericin; multi-target; pharmacological activities; clinical translation

1 Introduction

Hypericum perforatum L. (*H. perforatum*), also known as St. John's wort, is a perennial herb widely distributed around the world and belongs to the family Hypericaceae and the genus *Hypericum*. Hypericin is one of the major active components of *H. perforatum* and is also found in many other species of the genus *Hypericum*. In recent years, increasing research has revealed that hypericin exhibits several pharmacological activities, including antitumor, antiviral, and antibacterial effects. In addition, its metabolic pathways of hypericin have also been investigated in greater detail. This review summarizes recent advances in understanding both the pharmacological activities and metabolic mechanisms of hypericin.

2 Methodology

This review was conducted by searching major literature databases such as PubMed, ScienceDirect, Web of Science, CNKI (China National Knowledge

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Infrastructure), and Wanfang, using the keywords "hypericin", "photosensitizer", "pharmacological effects", and "metabolic mechanisms" to retrieve relevant publications. A total of 116 research articles and books related to hypericin were identified, covering both Chinese and English literature. During the literature screening and analysis process, all research data related to hypericin were summarized and categorized according to different research directions, including pharmacological effects, metabolic mechanisms, and clinical applications. These data were not only presented in a narrative form but also detailed in tables that summarize the experimental methods, results, and significance of each study, allowing readers to systematically understand the research progress on the mechanisms of action and application value of hypericin.

The inclusion criteria for this review were defined to include all relevant studies on the pharmacological effects, metabolic mechanisms and clinical applications of hypericin from 1995 to 2025, including *in vivo* and *in vitro* experimental studies, clinical studies and published literature. Studies were excluded if they were repetitive, presented only as abstracts or incomplete articles, lacked transparent and feasible research methods and objectives, or were reviews or research reports mainly discussing agriculture or engineering. These exclusion criteria ensured the scientific nature of the included literature and the relevance of the research content. The chemical structures of hypericin mentioned in this review were drawn based on published literature using ChemDraw Professional 22.0 software to ensure the accuracy and consistency of the structural formulas.

3 Origin and structure, physical and chemical properties

There are 460 species in the genus *Hypericum*, broadly distributed in tropical and temperate regions worldwide, which is the main source of hypericin [1]. Hypericin generally accumulates in the secretory cells of flowers, leaves, roots and stems of the plants, and the highest content of hypericin is found in flowers [2]. Its content can also be influenced by various natural factors such as the geographical location of plant growth, altitude, illumination and precipitation [3]. Moreover, scientific studies demonstrated that hypericin could be obtained not only from plants, but also from insects and protozoa [4].

As shown in Fig. 1, hypericin, 4,4′,5,5′,7,7′-hexahydroxy-2,2′-dimethyl-meso-naphthalene-dianthrone, is a six-hydroxyl substituted polycyclic quinone with a molecular formula of $C_{30}H_{16}O_6$ and molecular weight of 504.4 [5]. The large conjugated system in the structure is formed by the benzene ring and the exocyclic double bond, and two relatively large absorption peaks appear at 590 nm and 550 nm, which accounts for its photoreactivity.

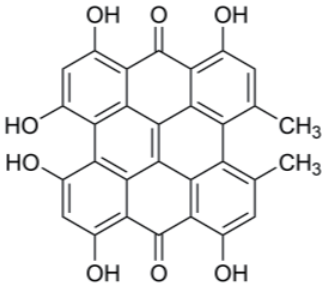


Fig. 1 The structure of hypericin

At room temperature, hypericin appears as purple-black needle-like crystals with a melting point above 320 °C. Recrystallized by pyridine or methanol solution containing hydrochloric acid, it became black powdery crystals with a faint scent [6]. The solubility of hypericin is closely related to its form [1-2]. the free state hypericin is insoluble in organic solvents, while its salts are soluble in alkaline aqueous solutions, pyridine, and organic amines. After dissolution, the solution exhibits a cherry-red color with red fluorescence. In addition, it is readily decomposed when exposed to light and should therefore be stored away from light [2].

Most of the pharmacological activities of hypericin are attributed to the naphthalene dianthrone moiety in the structure [5], which is an extensive electron-conjugated system showing great photosensitivity [4], making it suitable for use as a photosensitizer (PS) for photodynamic therapy (PDT). PDT consists of an optical source with appropriate wavelength, an amount of oxygen and the most critical part, a safe and effective PS. PS localizes to cytosolic organelles after administration [7], and is then irradiated with visible light of appropriate wavelengths to generate reactive oxygen species (ROS) in the presence of oxygen. These ROS cause oxidative damage to proteins, lipids and other intracellular biomolecules, leading to cell apoptosis or necrosis [8]. As a natural and powerful photosensitizer, hypericin contributes to the efficacy of PDT in anticancer, antibacterial and antiviral applications.

4 Pharmacological activities of hypericin

4.1 Antitumor activity

In recent decades, PDT has attracted extensive attention because it possesses many advantages over traditional cancer therapies, such as limited side effects and a low mutation rate [9]. Many researchers have demonstrated its powerful antitumor effect on different tumor cells both *in vivo* and *in vitro*, such as bladder cancer, colon cancer, breast cancer, glioma, leukemia, liver cancer, melanoma, and lymphoma.

The anti-cancer and anti-tumor effects of hypericin are mainly attributed to its excellent photosensitivity properties. Hypericin is a natural photosensitizer which is characterized by negligible dark toxicity, higher selectivity for tumor tissues than currently used PS [10]. The photo-activation behavior of hypericin can generate a large amount of singlet oxygen and O_2^-, thus greatly increasing the level of intracellular ROS [11]. Compared with normal cells, tumor cells exist in a high oxidative pressure environment [12], and excessive ROS may damage organelles such as DNA, protein and lipid membrane [13]. In tumor cells, the primary target of ROS attack is mitochondria, which will lead to the collapse of mitochondrial membrane potential and the oxidation of mitochondrial channels. These events may trigger the release of cytochrome C and the activation of caspase, thus initiating the apoptosis mechanism of cells [14] (Fig. 2). Hypericin, on the other hand, has a specific affinity for diseased tissues and can be used as a fluorescent imaging reagent in fluorescence-guided technology (FGT) [15]. FGT, as a new intraoperative technique that assists in resection, enables intraoperative visualization of some tumors, helping the surgeon distinguish tumors from normal brain tissue under the microscope, and greatly improving the resection rate, which in turn enhances patient prognosis [16]. Beyond its role as a photosensitizer in cancer treatment, hypericin also exerts cytotoxic effects on cancer cells. It eliminates cancer cells through a variety of signaling pathways and mechanisms, with studies investigating its therapeutic potential in various cancers.

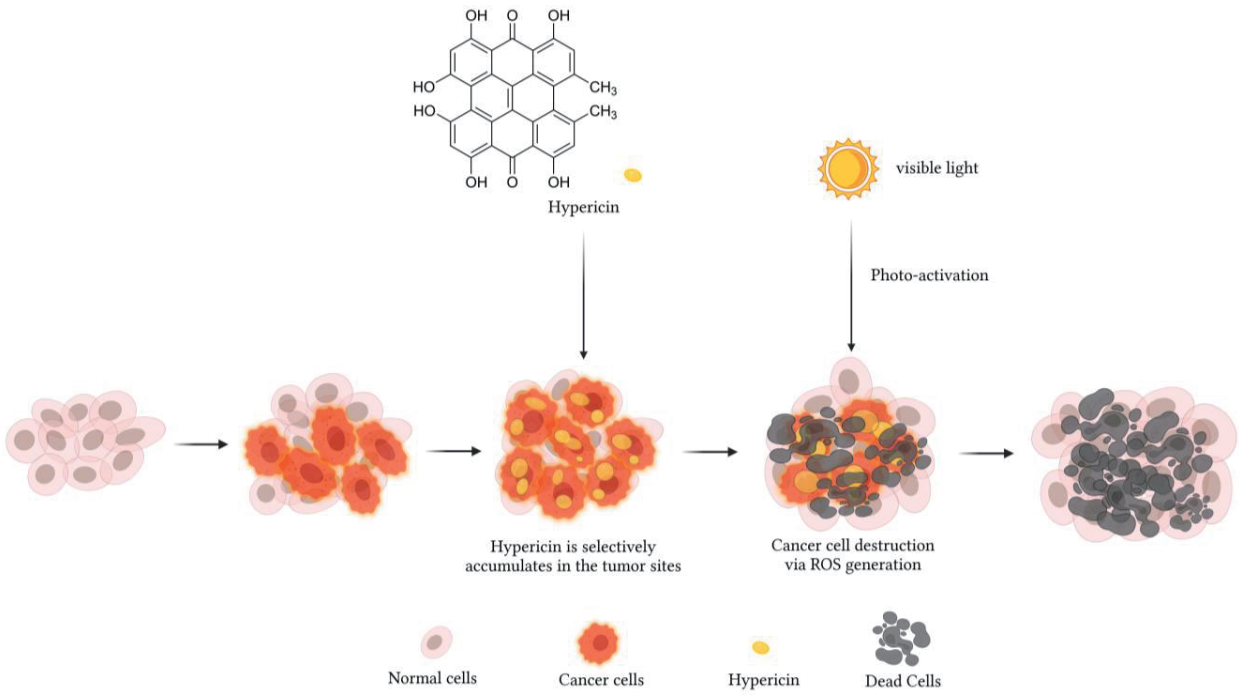


Fig. 2 Schematic representation of antitumor activity of hypericin-mediated photodynamic therapy (HYP-PDT)

4.1.1 Bladder cancer

The World Health Organization ranks bladder cancer as the ninth most common cancer. In the treatment of bladder cancer, resection of tumor fragments is most frequently performed. Photosensitive substances accumulate selectively in cancer cells at concentrations much higher than those in healthy cells, enabling clear visualization and identification of bladder cancer. It has been proved that hypericin as a photosensitive agent has higher sensitivity in urine fluorescence cytology examination of bladder cancer, representing a more effective approach. Exposure of tissues and cells to a laser with appropriate wavelength for a given photosensitizer produces ROS that indirectly destroy cancer cells and trigger apoptosis. HYP-PDT inhibits protein kinase C (PKC) and other growth factors, resulting in increased peroxidation of membrane lipids. This mechanism induces superoxide dismutase activity and leads to reduced cellular glutathione levels in the mitochondria. At the same time, hypericin accumulates on the endoplasmic reticulum membrane, leading to rapid loss of Ca^{2+} storage and cell death through caspase-dependent or autophagy-dependent apoptosis. HYP-PDT therapy clearly showed that the main mechanisms of bladder cancer cell death were apoptosis, autophagy and necrosis [17]. HYP-PDT can also induce apoptosis or necrosis of AY-27 bladder cancer cells by vascular effect, which disrupts the formation of vascular system around tumor cells, leading to obstruction nutrient supply and inhibiting cell growth without any damage to the nearby muscle layer [18].

4.1.2 Leukemia

In the treatment of leukemia, Zhang et al. tested the proliferation inhibition effect of HYP-PDT on a variety of leukemia cells using MTT

technology, and found that HYP-PDT could induce apoptosis of leukemia cells through the Caspase pathway, which was regulated by Bcl-2 family proteins [19]. Wang et al. observed the effect of HYP-PDT on the activity of chronic myeloid leukemia cells (K562 cell line), and proved its strong cytotoxic effect [20]. Previous studies have shown that moderate autophagy contributes to the survival of leukemia cells. Therefore, HYP-PDT may play a role in killing leukemia tumor cells by inhibiting Akt phosphorylation, reducing autophagy and promoting cell death. In addition, hypericin exhibits selective accumulation in spheroid cells, which is beneficial to kill leukocytes in the blood. Xu et al. investigated the effects of HYP-PDT on adult T-cell leukemia (ATL) cells and found that it inhibited the growth of ATL cells by inducing apoptosis and G2/M cell cycle arrest as well as inhibiting viral transcription [21]. They also revealed that HYP-PDT was highly effective against ATL cells by mitochondria dependent apoptotic pathway. These studies suggest that HYP-PDT is a promising drug in the treatment of leukemia.

4.1.3 Gliomas

Gliomas are the most common primary, malignant tumors of the central nervous system, characterized by poor prognosis, high morbidity and mortality [22]. The root cause of glioma recurrence is the residual tumor cells at the surgical margins. Hypericin can label the edge of glioma following intravenous injection, and serves as a fluorescent imaging agent to improve the intraoperative visualization of glioma [15].

The anti-glioblastoma activity of hypericin has been confirmed in the absence of light. But in the study of combining HYP-PDT with photobiological regulation (PBM), it was found that PBM-HYP-PDT significantly increased levels of caspase-3 and induced apoptosis [22]. In fact, not only mitochondria but also lysosomes and Golgi bodies are strongly affected by HYP-PDT, leading to more pronounced damage to glioblastoma cells.

Scientists have also found that ROS induced by HYP-PDT can cause a stress response in the endoplasmic reticulum of glioma cells, damaging their membrane structure or protein folding function, further triggering mechanisms such as cell autophagy and apoptosis. Sequences of FLIM images and fluorescence spectra analysis have demonstrated that cell membrane stability is destroyed, cell contents leak, and cell death occurs [23].

4.1.4 Thyroid cancer

Thyroid cancer is the most common type of endocrine-related cancer. Most thyroid tumors originate from follicular cells and develop into three major pathological types: papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC) and anaplastic thyroid carcinoma (ATC). PTC is the most common type of thyroid cancer [24-25].

Thyroid cancer is usually treated with surgery, radioiodine therapy, chemotherapy or thyroid hormone therapy. However, these approaches carry the risk of accidentally injuring healthy thyroid cells while failing to prevent cancer progression [26]. In order to find more effective therapeutic drugs, Piryaei et al. studied the effect of hypericin on thyroid B-CPAP cell line and thyroid cancer cell line TPC-1 with wild-type TP53, and found that hypericin had significant cytotoxic activity on B-CPAP [27]. In TPC-1 cell line, the dose required to induce cell death was lower and no cell death was observed in the normal cell group. Western blot analysis showed that hypericin induced exogenous caspase-dependent apoptosis in thyroid cancer cell lines. In this study, the researchers also measured four metastasis-related genes: LGALS3, CDH1, ITGA5 and ITGB3 in B-CPAP cells treated with hypericin for 24 h. The results showed that CDH1 expression was up-

regulated and LGALS3 expression was down-regulated. CDH1 is a transmembrane glycoprotein that mediates calcium-dependent intercellular adhesion and cell-cell adhesion in epithelial tissues. Downregulation of CDH1 promotes invasion and metastasis of many epithelial tumors [28]. In some cases, it is associated with the rate of tumor recurrence [29]. LGALS3 is an intracellular and extracellular lectin. Its expression plays a role in increasing angiogenesis and cell invasion in the process of metastasis, which has been proved to be related to the metastasis of some malignant tumors [30]. The effects of hypericin on the above two metastasis-related genes suggest that it can inhibit the metastasis of thyroid cancer cells and is a more promising compound for treatment.

Anaplastic thyroid carcinoma (ATC) is the most aggressive form of thyroid cancer, which invades surrounding tissues, lymph nodes, and blood, and is less responsive to treatment [31]. Although ATC accounts for only 2% of all thyroid cancers, the survival rate of patients is very low, and more effective targeted therapies have yet to be developed [32]. Kim et al. explored the therapeutic effect of HYP-PDT on ATC [33]. The photocytotoxic effect of HYP-PDT on FRO cells (ATC cells) was evaluated by MTT test, and it was found that HYP-PDT effectively induced the death of FRO cells. Mechanistically, HYP-PDT promotes the generation of intracellular ROS, significantly reduces the mitochondrial membrane potential of FRO cells, causes mitochondrial damage, and thus induces cell apoptosis. Mice transplanted with FRO cells and treated with HYP-PDT showed a significant reduction in tumor mass *in vivo*, which had almost completely disappeared on the 14th day of treatment. In contrast, tumors in control group, Hyp only group and laser only group gradually increased in size, and no therapeutic effect was observed. These results indicate that HYP-PDT is an effective treatment for FRO cell tumors in mice and is a promising approach [33].

4.1.5 Breast cancer

Hypericin has been used as an estrogen agonist in the systems affected by menopause and proved the dose-dependent cytotoxicity against MDA-MB-231 and MDA-MB-175VII cell lines, with higher cytotoxicity to the latter. The MDA-MB-231 and MDA-MB-175-VII cell lines are two examples of the breast cancer cell line P53 with different mutations. Hypericin also had dose-dependent cytotoxicity and apoptosis on breast cancer cell line (MCF-7) [11,34].

Previous studies investigated the anti-proliferation and pro-apoptotic effects of hypericin ethanol extract (HypE) on breast cancer cells [35]. When MCF-7 cells were cultured with HypE, it was found that HypE inhibited cell growth in a dose-dependent manner and induced apoptosis of MCF-7 cells through low phosphorylation of Akt. Akt plays an important role in the growth and survival of many cancer cells, and its phosphorylation inhibits the expression of pro-apoptotic proteins.

In addition, bone is the most common target organ for breast cancer metastasis, which occurs in about 80% of patients with advanced breast cancer. The increase of osteoclast activity is related to the growth of breast cancer in the bone microenvironment. Ouyang found that hypericin could inhibit upregulation of osteoclasts stimulated by breast cancer cells, which was related to the inhibition of NFATc1 signaling pathway and the weakening of Ca^{2+} oscillation [36]. In addition, hypericin can also inhibit the invasion and migration of breast cancer cells by directly reducing the osteoclast differentiation mediated by cancer cells and bone resorption, which is a promising natural drug for the treatment of breast cancer.

Hypericin as a photosensitizer in PDT also has a broad application prospect for the treatment of

breast cancer. In the experiment of Liu, hypericin could inhibit the proliferation and induce apoptosis of MDA-MB-231 cells at a high concentration under the condition of light avoidance [11]. But under the condition of light, the effect is more significant, even doubled. According to the experimental results, the killing effect of HYP-PDT on MDA-MB-231 cells was not the result of a single way. At low concentration, the main mechanism of action of HYP-PDT was to inhibit the proliferation of tumor cells and induce cell apoptosis. At higher concentrations, HYP-PDT releases large amounts of singlet oxygen and O_2^-, which greatly increases ROS levels and leads to tumor cell death [11].

Paclitaxel is one of the drugs used for clinical chemotherapy of early and advanced metastatic breast cancer. However, the effective response rate of paclitaxel to metastatic breast cancer is only 30% to 70%, which may be due to the development of resistance to paclitaxel [37]. Paclitaxel can be eliminated from cells by binding to reduced glutathione (GSH) catalyzed by glutathione S-transferase (GST) [38]. The large amount of intracellular ROS produced by HYP-PDT can oxidize GSH to glutathione oxidized (GSSG) and reduce GSH levels [39]. So the combination of HYP-PDT and paclitaxel could theoretically enhance the anti-cancer effect. Ren's experiment confirmed their conjecture. The apoptosis rate of metastatic breast cancer cell line MCF-7 and common breast cancer cell line Bcap-37 was increased by the combination of drugs, which provided a new research idea for the use of hypericin and the treatment of breast cancer [38].

In addition, HYP-PDT combined with cytokine-induced killer (CIK) cells also has a synergistic therapeutic effect on breast cancer [40]. Tumor cells generally lack strong immunogenic antigens, so that the immune system cannot effectively recognize tumor cells, resulting in CIK cells cannot maximize its anti-tumor effect [41]. PDT can improve the immunogenicity of tumor cells, and HYP-PDT can induce immunogenic cell death (ICD) of tumor cells, which provides experimental results for the combination of HYP-PDT and CIK *in vitro* [41].

4.1.6 Colon cancer

Yin et al. explored the anti-colon cancer effect of hypericin by the methylene blue method [42]. H_2S participated in a variety of biological processes in mammals. Moreover, cystathionine-β-synthase (CBS) catalyzed the synthesis of H_2S, and the increase of H_2S concentration promoted the proliferation and migration of colon cancer cells. Hypericin, as a selective and stronger inhibitor of CBS, expressed better efficacy compared with existing H_2S inhibitors such as aminoxyacetic acid (AOAA).

4.1.7 Liver cancer

Liver cancer is one of the most common types of cancer, and the current treatment is mainly a combination of surgical removal of the diseased liver, radiation and chemotherapy [43]. However, the treatment can still lead to complications, sometimes resulting in irreversible accidents [44]. Hypericin, as a valuable anticancer drug, has also been studied for its activity against liver cancer. Experiments showed that hypericin was cytotoxic to the hepatocellular carcinoma cell line HuH-7 and had no or minimal effect on control normal fibroblasts [45].

In addition, Shah et al. developed a hypericin lipopolyplexes (Hy-LPP) composed of hypericin, polyethylenimine (PEI) and pDNA [46]. Under light conditions, hypericin generated reactive oxygen species (ROS) that caused cell membrane damage in hepatocellular carcinoma cells (HepG2), enhanced the application of gene transfection in HepG2 cells, and induced HepG2 cell death through photocytotoxicity. The study showed that the

optimal photochemical dose (i.e., light intensity and hypericin concentration) enhanced the transfection effect while minimizing the damage to healthy cells.

Hypericin has toxic effects on many cancer cell lines. It can induce the expression of apoptosis-related genes, increase intracellular oxidative stress, cause the reduction of mitochondrial membrane potential, activate caspase-9 and caspase-3, and eventually lead to cell apoptosis. It has also been proposed that hypericin plays a key role in the induction of hypoxia-inducible factor-1α, thereby eliminating von Hippel-Lindau protein in cancer cells and preventing cancer cell growth [47]. Hypericin has significant anticancer effect, especially in combination with PDT or other drugs. However, the mechanism of action is complex and diverse, and the application of hypericin in clinical treatment still needs to be further studied.

Table 1 Summary of antitumor activity of hypericin

Cancer	Mode of action	Reported mechanism of action	References
Bladder cancer	HYP-PDT	HYP-PDT produces ROS that attack mitochondria and induce apoptosis. ROS can also disrupt the formation of vasculature around tumor cells and induce local inflammatory reaction to kill tumors.	[17-18]
Leukemia	HYP-PDT	HYP-PDT could induce apoptosis of leukemia cells through the Caspase pathway, which was regulated by Bcl-2 family proteins, and could inhibit Akt phosphorylation, reduce autophagy and promote cell death.	[19-21]
Gliomas	PBM-HYP-PDT	PBM-HYP-PDT significantly increased caspase-3 and apoptosis.	[22]
	Fluorescent imaging agent	Hypericin has the ability to label the edge of glioma by intravenous injection, and can be used as a fluorescent imaging agent to improve the intraoperative visualization of glioma.	[15-16]
	HYP-PDT	HYP-PDT induces ROS, which causes a stress response in the endoplasmic reticulum of glioma cells, damages their membrane structure, causes leakage of cell contents, and leads to cell death.	[23]
Thyroid cancer	Hypericin	Hypericin can induce exogenous caspase dependent apoptosis in thyroid cancer cell lines. Hypericin can up-regulate the expression of CDH1 and down-regulate the expression of LGALS3, and inhibit the metastasis of thyroid cancer cells.	[27-30]
	HYP-PDT	HYP-PDT promotes the generation of intracellular ROS, causes mitochondrial damage, and thus induces cell apoptosis.	[33]
Breast cancer	Hypericin	Hypericin induces apoptosis of MCF-7 cells through low phosphorylation of Akt. Hypericin also inhibits invasion and migration of breast cancer cells by directly reducing cancer cell-mediated osteoclast differentiation and bone resorption.	[11,34-36]
	HYP-PDT	HYP-PDT promotes the generation of intracellular ROS, causes mitochondrial damage, and thus induces cell apoptosis.	[11]
	Combination medication: paclitaxel, CIK	HYP-PDT reduces the resistance of lesions to paclitaxel and induces immunogenic cell death (ICD) of tumor cells.	[38,40]
Colon cancer	Hypericin	Cystathionine-β-synthase (CBS) can catalyze the synthesis of H_2S, and the increase of H_2S concentration can promote the proliferation and migration of colon cancer cells. Hypericin is a strong inhibitor of CBS.	[42]

(to be continued)

Continued Table 1

Cancer	Mode of action	Reported mechanism of action	References
Liver cancer	Hypericin	Hypericin was cytotoxic to the hepatocellular carcinoma cell line HuH-7.	[45]
	Hy-LPP	Hypericin generates ROS that damage the HepG2 cell membrane, enhances the application of gene transfection in HepG2 cells, and induces HepG2 cell death.	[46]

4.2 Anti-SARS-CoV-2 effects

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) posed a huge threat to international public health security. According to the World Health Organization, one of the important reasons for the continued spread of the disease worldwide is the lack of specific antiviral drugs. Natural products have gradually become a research hotspot against SARS-CoV-2 due to their abundant sources and few adverse reactions. To date, the number of natural products available for medicinal purposes is limited, indicating their broad potential for future application.

Recent studies have further highlighted the importance of hypericin as a representative bioactive constituent of *Hypericum perforatum* L. For instance, Mohamed et al. independently evaluated hypericin and pseudohypericin alongside the whole plant extract and demonstrated their distinct antiviral activities against SARS-CoV-2, supporting the view that hypericin is a key pharmacologically active component responsible for the antiviral effects of *H. perforatum* [48].

Studies have confirmed that the viral replication cycle goes through many distinct stages, and the viral proteins that operated on each stage constitute a single potential antiviral target. The SARS-CoV-2 genome encodes the structural protein of the virus, the spike protein [49]. SARS-CoV-2 binds to the angiotensin-converting enzyme 2 (ACE2) cellular receptor through the receptor-binding domain (RBD) of its trimeric spike protein, thereby initiating its entry into host cells and triggering fusion between the host and viral membrane [50]. Main protease (Mpro) found in SARS-CoV-2, proteolytically hydrolyzes transcribed viral polypeptides at 11 different sites to promote viral replication [51] (Fig. 3). Mpro inhibitors effectively prevent coronavirus replication and proliferation by interfering with post-translational processing of essential viral polypeptides [52]. Given the critical role of Mpro in SARS-CoV-2 infection, Mpro inhibitors can guide the design and development of anti-SARS-CoV-2 drugs.

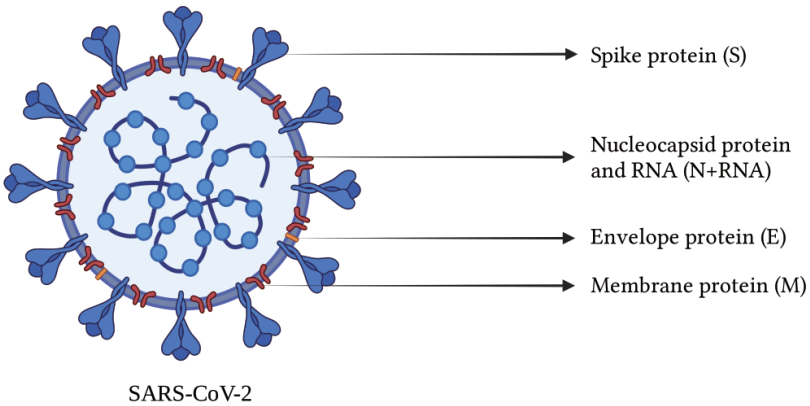


Fig. 3 Schematic representation of the structure of SARS-CoV-2 virus

Romeo used the SARS-CoV-2 trimer structure in the prefusion conformation to screen 8770 FDA drugs from the DrugBank database for virtual screening simulations [53], 10 high-affinity compounds including hypericin were screened. Due to the trimeric structure of the spike glycoprotein, at least 3 drug molecules of hypericin bind to the HR1 region of its lumen [54]. The hypericin molecule establishes 7 hydrophobic interactions and 4 hydrogen bonds with surrounding residues to bind to the HR1 region in alternating directions. Simultaneous binding of the three molecules creates a strong steric hindrance in the cavity that prevents conformational changes in the HR1 region, resulting in a 6-helix bundle that stabilizes the post-fusion conformation [55]. Next, the stability of the selected protein-drug complexes was tested using classical molecular dynamics (MD) simulations, and the results highlighted that, in addition to hydrophobic interactions, electrostatic interactions were also important for the stability of hypericin drugs. A refined assessment of the interaction free energy of hypericin was performed using molecular mechanics/Poisson-Boltzmann (Generalized Born) Surface Area (MM/GBSA) and residue energy decomposition analysis [56]. The results of the analysis showed that the drug could establish medium-to-high affinity contacts with 9-15 residues in the HR1 region inside the viral protein and other surrounding residues in the lumen. This evidence further supports the hypothesis that the drug may act as an anchor between surrounding internal regions. Based on the above results, it is speculated that hypericin blocks the HR1 region in the pocket, thereby blocking the spike conformational changes that allow SARS-CoV-2 to enter target cells.

After the SARS-CoV-2 virus enters the host cell, the single-stranded RNA genome is released into the cytoplasm, and the open reading frames ORF1a and ORF1b encode two overlapping polyproteins pp1a and pp1ab. The papain-like protease (PLpro) hydrolytically cleaves the peptide bond between pp1a and pp1ab to form the nonstructural protein nsp3 [57]. The nsp3 protein can cleave other proteins of the virus and remove tags that mark old proteins in healthy cells, thereby changing the protein balance and reducing the cell's ability to resist the virus. PLpro has deubiquitination activity affecting key signaling pathways, including inhibition of interferon and innate immune antagonism. Therefore, the inhibition of PLpro activity by small molecules is an important research direction. Researchers screened out hypericin as a potential lead compound to inhibit SARS-CoV-2 PLpro from a library of 300 ligands through molecular docking and protease analysis [58]. Using the non-covalent inhibitor GRL-0617 as a positive control, the *in vitro* inhibitory activity of PLpro protease was determined. The positive control drug at a concentration of 100 μM inhibited about 94% of the protease activity, and at the same concentration, the inhibitory activity of hypericin was 97%.

Researchers retrieved the three-dimensional structure of the main protein of SARS-CoV-2 from the protein database, and removed water molecules, inhibitors and other heteroatoms from the protein for molecular docking. The active site of the protease was predicted using the MetaPocket 2.0 online service software, and the 3 best results were selected from the potential ligand binding sites based on the z-score for docking. The binding conformation of hypericin to viral protease is great, with the binding energy of -11.17 kcal/mol, which is equal to that of the FDA-approved viral protease inhibitor. These experimental results suggest that hypericin is a potential inhibitor of the main protease of the virus.

Indian scholars Saravanan et al. obtained the three-dimensional structures of 41 natural products reported in the literature. These compounds exhibit anti-SARS-CoV-2 virus effects by removing natural ligands and heteroatoms from receptors, or tuning the preference and energy minimization of rotor

molecules. Molecular docking was performed using the Auto Dock 4.2 program to predict the affinity between antiviral compounds and the major protease of SARS-CoV-2. The binding energy of hypericin to protease is -8.4 kCal/mol. The interaction between hypericin and the major proteases was visualized using Discovery Studio Visualizer software. The interaction between the protein and hypericin was mainly van der Waals hydrophobic interaction and hydrogen bonding. During the 40-ns atomic molecular dynamics simulation, the main protease forms an average of 4 hydrogen bonds with hypericin, which is more stable than those of other compounds [59].

Viral RNA-dependent RNA polymerase (RdRp), another important protein of SARS-CoV-2, has a catalytic core with a differentiation domain. RdRp catalyzes the RNA replication of its RNA template. The RdRp active site is a deep, giant groove in which RNA polymerizes. This active site is the most accessible and conserved region, so targeting this region may be an effective therapeutic target for inhibiting viral replication. Molecular docking results show that the free binding energy of hypericin and RdRp is -9.1 kcal/mol, which is higher than that of antiviral drugs, such as favipiravir, hydroxychloroquine, tenofovir and sofosbuvir [60].

The N protein is the core component of the virion, which binds to viral genomic RNA and packages the RNA into a ribonucleoprotein (RNP) complex. In addition to assembly, N proteins play important roles in viral mRNA transcription and replication and are involved in immune regulation. Some studies have reported that N protein can bind to double-stranded RNA and exhibit RNAi inhibitory activity, which can antagonize host RNAi-mediated antiviral responses. At the same time, N protein can also induce humoral and cellular immune responses after infection, making it a key target for antiviral [61]. Molecular docking results showed that the free binding energy of hypericin to N protein was -7.1 kcal/mol, which was comparable to that of the N protein blocker balsamic resin (Fig. 4).

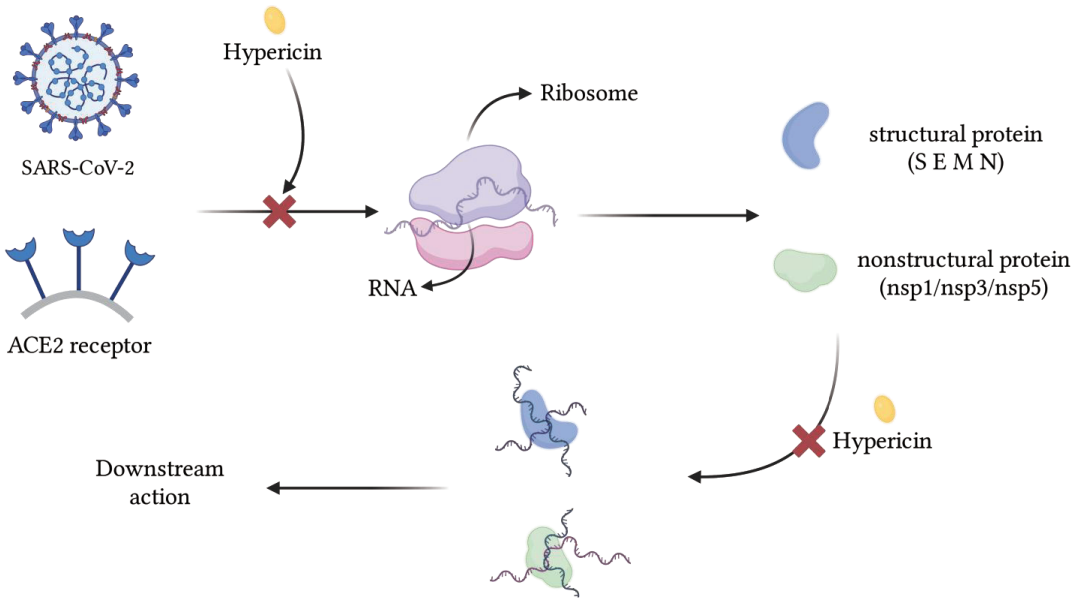


Fig. 4 Schematic representation of the mechanism of action of hypericin against SARS-CoV-2

Since the emergence of SARS-CoV-2, many researchers have tried to find small molecule anti-SARS-CoV-2 virus drugs in natural products. Among them, the activity of hypericin in anti-SARS-CoV-2 has been gradually developed. The efficacy, safety and stability of hypericin against SARS-CoV-2 virus live up to expectations. This information is valuable for the development of novel anti-SARS-CoV-2 drugs.

4.3 Treatment of skin diseases

The photosensitizing property of hypericin is the basis of PDT treatment for skin diseases, such as acne, psoriasis or port-wine stains (PWSs) on the skin.

As a congenital capillary malformation located in the upper dermis, PWSs are formed from excessively dilated and proliferative capillaries in the skin, often associated with systemic symptoms, leading to corresponding organ dysfunction, manifested as striking reddish patches on the skin, resulting in heavy burden on the physical, emotional and psychosocial wellbeing of the patient [62]. Li et al. proved that hypericin exhibited distinct therapeutic effect in the Roman chicken cockscomb model of PWSs. In the experiment, hypericin exhibited significant photocytotoxic activity on the cockscomb dermal capillary after intravenous injection and the growth of endothelial cells, indicating that HYP-PDT probably damaged excessively proliferative vasculature and eliminated malformed capillaries, represented a potential approach for the treatment of PWSs [63]. Furthermore, Li et al. intended to evaluate the influences of penetration enhancers on the skin penetrability of topically applied hypericin through the Franz diffusion cell, the outcomes of experiments in nude mice validated that the topical hypericin preparation containing 5% menthol could penetrate nude mice skin *in vitro* and *in vivo*. Under the 585 nm light illumination, HYP-PDT penetrated nude mice skin, diffused to the dermis, consequently causing substantial and recoverable photosensitivity damage on the upper dermal capillary vessels. Importantly, the skin lesions on nude mice gradually healed within 7 d until full recovery, suggesting that hypericin may play a potential part in the treatment of skin diseases involving excess capillary proliferation, such as PWS [62].

Psoriasis is a prevalent autoimmune, chronic and recurrent inflammatory skin disease, that may affect not only the skin but also other organs, such as liver, gastrointestinal tract and musculoskeletal system, resulting in corresponding injury. The transient receptor potential cation channel, subfamily V, member 3 (TRPV3) is a thermosensitive tryptophan channel to be activated by temperature changes and chemical substances, and it is considered a major channel involved in pruritus which is the common clinical symptom of psoriasis. To treat pruritus and other symptoms of psoriasis, researchers attempted to find suitable TRPV3 antagonists through molecular docking [64]. Results showed that hypericin exhibited good binding affinity in terms of TRPV3 channel and was consistent with the binding site, acting as an antagonist by inducing conformational changes required for desensitization. Moreover, Kamuhabwa et al. demonstrated that hypericin might adequately penetrate the region affected by psoriasis (in basal cell layer of the epidermis) when incorporated into a suitable vehicle [65]. Accordingly, hypericin has been incorporated into bioadhesive and thermoresponsive polymer mixtures to enable the localized application in different skin regions [66]. In addition, when topically applied to patients with psoriasis, hypericin was found to be effective, safe and well-tolerated for the treatment of plaque psoriasis, with the significant lesion improvement observed in most patients [65].

Acne vulgaris is a chronic inflammatory

disease associated with the sebaceous glands, primarily caused by infectious manifestation of Propionibacterium acnes, although other bacterial strains such as *Staphylococcus aureus* (*S. aureus*) may also be involved. This dermatologic disease affected many adolescents and a significant group of adults, often leading to severe embarrassment and anxiety in patients [67-68]. *In vitro* antibacterial studies have found that hypericin led to significant reduction of Propionibacterium acnes, supporting its potential application in PDT for the treatment of acne [67]. Furthermore, the use of *β*-lactoglobulin as a double payload carrier of hypericin and all-trans retinoic acid (an anti-inflammatory drug) for photoinactivation of acne-causing strains *in vitro* has been reported, providing a safe new therapeutic option for acne [68].

Table 3 Mechanisms of hypericin in the treatment of skin diseases

Treatment of skin diseases	Reported mechanism of action	References
Port wine stains (PWSs)	The topical hypericin formulation can penetrate the patient's skin, diffuse to the dermis and generate ROS under the light illumination, selectively damages the excessively proliferative, expanded capillary network to eliminate the abnormal capillaries.	[62-63]
Psoriasis	Hypericin employed in PDT can induce changes in the function of endothelial cells, promote dermal remodeling, modify the functional status of the skin immune system and resolve the chronic inflammation, which is a relevant treatment for psoriasis. Activation of TRPV3 channels is one of the major factors causing pruritus in psoriasis, and hypericin probably expresses antagonistic action by desensitizing TRPV3 channels to treat pruritus symptoms.	[64-66]
Acne vulgaris	Hypericin is topically applied to the infected part to irradiate the lesion area with a specific wavelength of light, kills the skin-colonizing strains such as *Propionibacterium acnes* (*P. acnes*) and *Staphylococcus aureus* (*S. aureus*), inhibits the secretion of sebaceous glands and enhances the healing of damaged skin, achieving the purpose of curing acne.	[67-68]

4.4 Anti-cardiovascular activity

In the treatment of cardiovascular diseases, Wei et al. have demonstrated that 20 main components of *Hypericum* have inhibitory effects on human thrombin [69]. Thrombin is the key enzyme responsible for the proteolysis of many endogenous peptides and proteins, such as soluble fibrinogen and factor XIII, which in turn controls the coagulation cascade and regulates the thrombosis process. Inhibition of thrombin is one of the most effective therapeutic strategies for the prevention and treatment of thrombotic diseases. Most naphthodianthrones and bisflavones, as well as some flavonoids from the genus *Hypericum*, show moderate or even strong inhibitory effects on human thrombin, of which hypericin is the most potent thrombin inhibitor and can serve as a lead compounds for the development of novel thrombin inhibitors with improved properties.

Viral myocarditis is a common disease of cardiovascular system that poses a serious threat to human health. Myocardial fibrosis is its characteristic pathological change [70]. Blocking myocardial fibrosis is an extremely important treatment to prevent chronic toxic myocarditis from transforming into dilated cardiomyopathy. In the study of Li et al. [71], hypericin was proved to have an obvious cardiomyocyte protective effect, inhibiting the apoptosis of normal cardiomyocytes in viral myocarditis [72]. Apoptosis mediated by Fas/FasL protein is involved in the pathogenesis of acute and chronic viral myocarditis, and hypericin can down-regulate the expression of Fas/FasL

protein and attenuate the signal transduction of apoptosis [73]. Later, they further investigated the mechanism of hypericin in the treatment of viral myocarditis and confirmed that hypericin increased serum levels of interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) in mice, which helped to clear the virus. The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway can promote collagen expression in the process of myocardial fibrosis. Hypericin can block this pathway and inhibit myocardial fibrosis in the chronic phase of viral myocarditis [70].

Hypericin still has certain value in the treatment of dilated cardiomyopathy (DCM). The main clinical manifestations of DCM are progressive congestive heart failure (CHF), arrhythmia, thrombosis and sudden death. Plasma BNP concentration and serum TNF level are important biochemical indexes in clinical diagnosis and treatment. Gap junction protein 43 (Cx43) is an important way of information and energy exchange between cardiomyocytes, which affects the physiological processes of cell metabolism, proliferation and differentiation [74]. Hypericin can prevent myocardial fibrosis and inhibit myocardial cell apoptosis in the chronic stage of viral myocarditis. Li et al. showed that hypericin (HYP) could improve cardiac function by reducing the levels of TNF-α and BNP, inducing the expression of Cx43 mRNA and protein in cardiomyocytes, and effectively alleviating the clinical manifestations of dilated cardiomyopathy [74].

Atherosclerosis (AS) is a chronic inflammatory vascular disease characterized by a massive accumulation of macrophages in the inner membrane. Macrophages play a complex role in the process of AS. They are functionally active cells in atherosclerotic plaques, which make plaques unstable and increase the possibility of plaque development in early AS lesions [75]. PDT has been recognized as the most effective treatment, but is limited to superficial lesions due to its poor tissue penetration. The efficacy of sonodynamic therapy (SDT), which can be used to penetrate tissue, was also investigated [76]. Hypericin, as a very promising photosensitizer, has also been studied as a sound sensitizer in this experiment. Under conditions of low HYP concentration and low intensity ultrasound, HYP enters macrophages through passive diffusion, hydrophobicity and phagocytosis, localizing mainly in mitochondria and Golgi, with a small amount in the nucleus. HYP-SDT can effectively induce apoptosis of THP-1 macrophages through mitochondria-caspase pathway and increase intracellular ROS levels.

Hypericin is also effective in the treatment of atherosclerosis. Endothelial cell damage has also been implicated in this condition, and methylglyoxal (MGO), a highly reactive glucose metabolite, causes endothelial cell damage and induces apoptosis. MGO rapidly reacts with proteins to form a group of chemically stable substances called advanced glycosylation end products (AGEs) [77]. Elevated levels of MGO and AGEs can lead to inflammation and oxidative stress, thereby damaging various organs [78]. The protective effect of hypericin on MGO-induced apoptosis of human umbilical vein endothelial cells (HUVECs) was investigated, demonstrating its anti-apoptotic activity. Hypericin inhibits the apoptosis of HUVECs by regulating MAPK signaling pathway, and also inhibits the formation of AGEs, which holds therapeutic potential for the prevention or treatment of vascular injury caused by MGO [79].

4.5 Anti-diabetic activity

Diabetes mellitus (DM) is a common chronic disease worldwide. Current treatments focus on optimizing metabolism and reducing the risk of vascular complications, without addressing the underlying mechanisms. In fact, targeting inflammation and its signaling pathways may

represent an effective way to prevent or treat diabetes and its complications [80].

Pancreatic β-cells are the only mammalian cells that synthesize and secrete insulin, which can be rapidly released to lower blood glucose concentrations. Type I diabetes is caused by autoimmune destruction of β-cells [81]. Type II diabetes is triggered by insulin resistance, which drives β-cells to release more insulin. This high demand leads to β-cells malfunction, de-differentiation and death [82]. In conclusion, the pathogenesis and development of both type I and type II diabetes mellitus are closely related to β-cell exhaustion. Protecting β-cells from loss and dysfunction is an important method to prevent or treat diabetes mellitus [83].

Pancreatic duodenal homeobox-1 (PDX1) is a key transcription factor for pancreatic development, maturation and β-cell function. PDX1 plays a pro-survival role in adult β-cells [84]. Glucotoxicity and lipotoxicity lead to oxidative stress, β-cell dysfunction and apoptosis and are therefore associated with diabetes [85]. Hypericin mitigated oxidative stress induced by high glucose or fat and restored PDX1 expression in glucotoxic and lipotoxic β-cells. This reduced β-cells apoptosis in the islets of high-fat/high-sucrose (HFHS)-fed mice and *in vitro* models of glycotoxicity and lipid toxicity, effectively protecting β-cells from death and loss.

The ERK signaling pathway is involved in hypericin-mediated restoration of PDX1 expression and protection of β-cells from apoptosis induced by glucotoxicity and lipotoxicity. Liang et al. also demonstrated that hypericin maintains PDX1 expression and protects β-cells by attenuating the decrease in ErK activity induced by high glucose or fat [84].

4.6 Treatment of rheumatoid arthritis

PDT has emerged as one of the most promising treatments for autoimmune diseases such as rheumatoid arthritis (RA) [86]. Hypericin, as a natural photosensitizer, also holds promise for the treatment of these diseases. Hypericin increases ROS production through PDT, leading to apoptosis and death of MH7A cells (RA fibroblast-like synovial cells). Mechanistic studies have shown that this therapy induces morphological changes (shrinkage and cytoplasmic vacuolation) in MH7A cells, thereby inhibiting their proliferation. Hypericin alone slightly reduced cell proliferation, but its performance was significantly improved when irradiated at its excitation wavelength. The therapeutic effect increased with the increase of PS concentration.

4.7 Antidepressant effect

As a common global disease, the incidence of depression is increasing annually. According to the World Health Organization (WHO) projections, depression is likely to become the second leading cause of disease burden after acquired immune deficiency syndrome (AIDS) by 2030 [87]. Current clinical antidepressant drugs are frequently associated with uncertain efficacy, various adverse reactions, poor tolerance and compliance of patients, and high recurrence rates [88]. Consequently, researchers are increasingly focusing on seeking natural products for the treatment of depression [89]. Numerous studies have shown that, *H. perforatum* L. is an effective and safe natural remedy for depression, and is widely utilized in clinical application due to its few side effects [90].

Depression is often characterized by symptoms such as low mood, loss of interest, slowed thinking, sleep disturbance and even suicide, and it may also co-occur with other mental illnesses. The incidence of depression is increasing annually, and it has now become a major mental illness in the world. Numerous studies have shown that monoamine neurotransmitters, such as norepinephrine,

serotonin, and dopamine, play important roles in pathophysiology and disease treatment. Antidepressants work by increasing monoamine levels at brain synapses [91]. Using behavioral assessment of stressor-induced depression rat model, hypericin was found to effectively improve the cognitive and memory ability of depressed rats [92]. In the sucrose preference experiment, the sucrose consumption of the depressed rats in the hypericin drug group was significantly higher than that in the control group, further supporting the potential of hypericin as an antidepressant. The mechanism of action of hypericin in chronic unpredictable mild stress (CUMS) model rats is similar to the bicyclic atypical antidepressant the atypical antidepressant venlafaxine. Analysis of stress hormones in rat urine shows that hypericin treatment group reduced the concentration of excitatory amino acid neurotransmitters glutamate and glutamine. Urinary tryptophan and tyrosine concentrations were increased compared with CUMS model rats. In addition, the hypericin group showed a significant increase of valine concentration in urine, which was even higher than that in the CUMS group, suggesting a potential target for the antidepressant effects of hypericin [93]. In the experimental process of improving maternal separation-induced cognitive dysfunction and hippocampal inflammation in rats, parental separation triggers the expression of pro-inflammatory cytokines in the cortex and hippocampus, activation of microglia and astrocytes during inflammation, and increased expression of TNF-α. Hypericin reduced hippocampal dopamine levels in maternally isolated rats compared to controls. Transcription of proinflammatory cytokine genes, TNF-α and interleukin-6 (IL-6), in rat hippocampus was significantly increased 9 d after maternal isolation. Hypericin significantly blocked pro-inflammatory transcription induced by maternal separation at both doses of 10 mg/kg and 50 mg/kg [94]. Hypericin significantly decreased corticosterone levels in hippocampus and plasma of bilateral ovariectomized rats in a dose-dependent manner. Prolonged elevation of corticosterone leads to reduced serotonin synthesis. Excessive corticosterone activates tryptophan 2,3-dioxygenase (TDO) enzymes, shunting the dietary amino acid precursor tryptophan from the serotonin pathway to the kynurenine-niacin pathway, thereby inhibiting serotonin production and desensitizing serotonin receptors [95].

5 Metabolic mechanism

Although hypericin holds significant potential for various applications, only a limited number of studies have reported on its metabolic characteristics. In this review, we aim to provide a comprehensive summary of the relevant literature reported from cell, rodent, and other animal studies, further elucidating the metabolic pathways of hypericin.

5.1 Cell experiments

Hypericin is a highly lipophilic compound which is virtually insoluble in aqueous solutions. The poor solubility inevitably impedes oral absorption, providing enormous challenges for drug developers working on oral delivery of drugs [96]. Scientists have investigated the effects of various polyphenols and flavonoids from St. John's Wort on the permeability of hypericin *in vitro* using the well-established Caco-2 cell model in experiments simulating intestinal absorption. The aim was to identify strategies to enhance hypericin absorption in the small intestine and improve its oral bioavailability.

Verjee et al. proved that the permeation capacity and intracellular amounts of hypericin with or without quercitrin (quercetin 3-*O*-α-L-

rhamnoside) were different [97]. Firstly, the water solubility of hypericin was significantly increased in the presence of quercitrin, improving from 0.07 μg/mL to 56.76 μg/mL when hypericin was combined with quercitrin at a ration of 1:4. These characteristics influenced the permeability of hypericin. Results from the subsequent experiments clearly indicated that the addition of quercitrin increased the amount of hypericin inside the cells or bound to the cell membrane. Therefore, the researchers reasonably speculated that the sugar moiety attached to C-3 of the aglycon influences solubility, enabling certain flavonoids to naturally solubilize hypericin. In the absence of serum proteins, the amount of hypericin in the cells after cell extraction ranged from 44% (hypericin alone) to 64% (hypericin and quercitrin). However, when 10% FBS (fetal bovine serum) was added to the transport buffer to enhance the solubility of hypericin in aqueous solution, approximately 68% of hypericin was bound to the serum proteins, resulting in lower intracellular accumulation of hypericin compared with protein-free conditions and the percentage of hypericin after cell extraction only ranged from 13% (hypericin) to 18% (hypericin and quercitrin). This finding supported the assumption that the binding of hypericin to plasma proteins and lipoproteins in FBS leads to a slow release of hypericin to cells. Using fluorescence imaging, researchers demonstrated that hypericin preferentially accumulated in the lipid membrane structures of cells. Notably, flavonoids may affect the physical properties of the lipid bilayer by modifying the arrangement of membrane proteins, thereby influencing membrane transport. That might explain why the permeation characteristics of hypericin improved in the presence of quercitrin.

Subsequently, Verjee et al. investigated the following questions through a series of experiments: (1) whether other flavonoids present in St. John's Wort could improve permeability characteristics for hypericin; (2) whether combinations of flavonoids had synergistic or additive effects; and (3) whether the transport characteristics of hypericin changed in the presence or absence of extract matrix. Including quercitrin, Verjee et al. examined the effects of quercetin, hyperoside, isoquercitrin and rutin on the transport of hypericin across the Caco-2 cell layer in transport buffer through the absorptive direction by the permeation experiment. They speculated that the transport characteristics did not observably change when hypericin was combined with various single flavonoids. As expected, the transport profile of hypericin looked almost identical to the transport characteristics of hypericin alone, and only quercitrin increased the basolateral amount of hypericin. Interestingly, the amount of hypericin detected after cell extraction was significantly increased when combined with various flavonoids compared with the absence of flavonoids, and the highest amounts of hypericin (approximately 52%) occurred in the presence of quercitrin. Overall, the amount of hypericin recovered after cell extraction depended on the sugar attached at C-3, and increased in the order aglycone < galactoside < glucose < rhamnoside. However, although rutin had a glucose-rhamnose moiety attached at C-3, it failed to further expand the intracellular amount of hypericin. In another set of experiments, the effects of dual flavonoid combinations on the transport characteristics of hypericin were tested, yielding results similar to those observed with single compounds. The most remarkable increase of the percentage of hypericin after cell extraction was observed for the combination quercetin + quercitrin (aglycone + quercetin-3-*O*-rham-noside), significantly rising to approximately 57%. In contrast to quercitrin, the presence of rutin in dual combination adversely decreased the percentage of hypericin after cell extraction. Researchers also tested potentially

synergistic effects of a quintuple combination, consisting of hypericin, quercetin, isoquercitrin, hyperoside, rutin and quercitrin. The experimental result showed that this combination only moderately increased the amount of hypericin measured after cell extraction, but was not superior to the dual or single combinations. Further studies confirmed that the amount of hypericin measured in the basolateral compartment (up to 6.7%) and attached to the cell membrane/intracellularly (up to 67%) dose-dependently increased in the presence of extract matrix, and approximately 65% of the hypericin were recovered after cell extraction when spiked with extract matrix concentration of 58 μg/mL. In summary, flavonoids can improve the solubility and permeability of hypericin. More hypericin was recovered after cell extraction in the presence of flavonoids, although the difference between single, dual or quintuple combinations was negligible. In addition, other constituents of the extract matrix (e.g. phenolic acids or proanthocyanidins) substantially improved the permeation characteristics of hypericin [98-99].

5.2 Rodent experiments

Hypericin is a herbal photomedicine capable of producing necrosis-inducing ROS moieties upon irradiation with visible light of suitable wavelength, which assists in PDT employed for cancer treatment. Its high tumor selectivity is attributed to slow uptake and rapid clearance from healthy tissues [100]. To optimize both efficacy and safety, researchers investigated the targetability and biodistribution of radioactive hypericin in tumor rodent models in order to establish the optimal therapeutic range.

Cona et al. utilized iodogen as oxidant to prepare the iodine-123-labeled hypericin (123I-Hyp) as a safer surrogate for 131I-Hyp, and intravenously injected 123I-Hyp in rats with re-perfused partial liver infarction (RPLI). Through macroscopical and microscopical examination, they determined biodistribution in rats and estimated dosimetry of 131I-Hyp in hepatic necrosis and in normal human organs. At 4 h after radioactivity injection, the maximal levels of radioactivity were detected in blood. In most normal organs, peaks of uptake were found at 4 h, which gradually declined since the 123I-Hyp was cleared from the blood over time. The highest 123I-Hyp percentage of injected dose (%ID) was measured in the liver followed by lungs, spleen, and intestines. Conversely, thyroid, brain and pancreas showed the lowest levels of radioactive uptake (Fig. 5b). Results of tissue gamma counting (TGC) showed that the uptake of 123I-Hyp and hypericin was at a low level in healthy liver, which selectively accumulated in hepatic infarction. The autoradiography and histology also confirmed that 131I-Hyp accumulated much more in the liver of rats with necrotic liver than in normal rats. High radioactivity concentrations (%ID/g) were also found in thyroid and lungs at 24 h, but 123I-Hyp was almost completely eliminated within 24 h from other normal organs (Fig. 5c). In addition, substantial levels of radioactivity were detected in intestines and feces. In contrast, the radioactivity concentrations in kidneys were lower, so that scientists speculated that hepatobiliary elimination was a major approach of 123I-Hyp generating high activity in the intestines. Dosimetry of 131I-Hyp in different organs and tissues of adult female (57 kg) and male (70 kg) humans was estimated according to distribution of RPLI rats. The longest retention time was found in the whole body, followed by the lungs, intestines and thyroid. The shortest residence time was found in the brain, pancreas, stomach wall and kidneys. In conclusion, necrotic tissues absorbed a higher radiation dose of 131I-Hyp compared with normal organs, whereas the lowest absorbed radiation dose and shortest residence time were found in the brain [101].

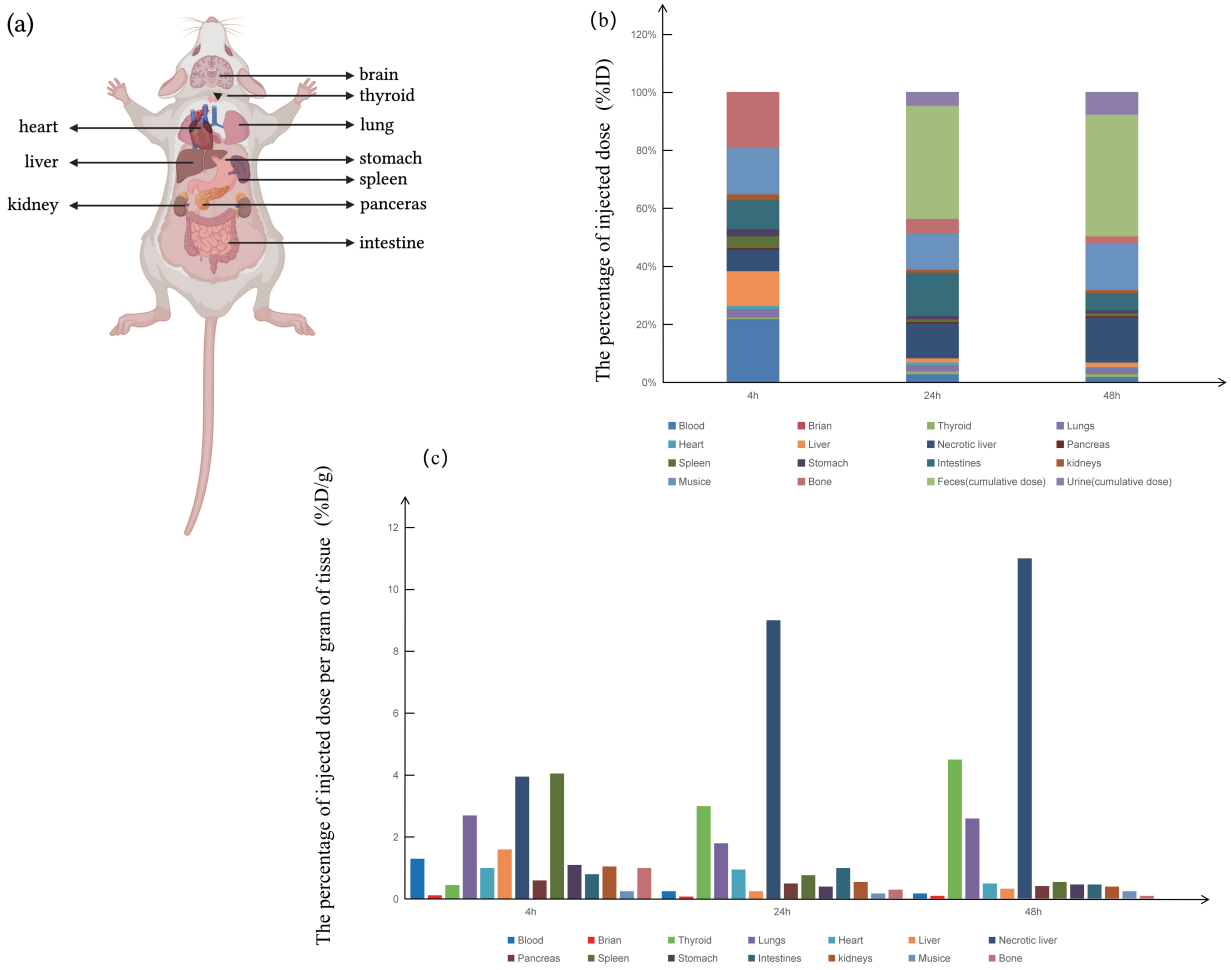


Fig. 5 Schematic representation of tissue distribution of 123I-Hyp/Hypericin in rats with RPLI: a: Schematic diagram of rat organs; b: The corresponding percentages of injected dose of 123I-Hyp from RPLI rat's tissue at 4, 24 and 48 h post-injection (%ID); c: The corresponding percentages of injected dose of 123I-Hyp from RPLI rat's per gram of tissue at 4, 24 and 48 h post-injection, as radioactivity concentrations of per gram of tissue (%ID/g)

Coincidentally, Cona et al. obtained some virtually unanimous results from similar studies. Using the well-established rat model with RPLI, they intended to examine the influence of co-injecting unlabeled hypericin on biodistribution, necrosis uptake and tumor retention of necrosis avid agent 123/131I-Hyp. The results of TGC, autoradiography and histology clearly revealed that there were no significant differences in organ biodistribution between RPLI-rats pre-injected with 123I-Hyp alone and receiving 123I-Hyp with hypericin co-injection in most normal tissues, except intestines, liver, lungs and stomach. Both experimental formulations showed 123I-Hyp occurred mainly via hepatobiliary route as the intestines accumulated the highest proportion of injected dose and retained the most radioactivity throughout the whole study period. In all groups of animals that received 123I-Hyp preparations, all 123I-Hyp exhibited strong selectivity and avidity for hepatic infarction, as radioactivity concentrations increased in hepatic necrosis but quickly cleared

from viable liver within 48 h. From the day after injection onward, RPLI-rats receiving 123I-Hyp with hypericin co-injection revealed greater target accumulation than those injected with 123I-Hyp alone, but the biodistribution of radioiodinated 123I-Hyp was largely unaffected. Furthermore, high levels of radioactivity accumulation were observed either partially or totally in the tumor necrosis of RPLI-rats that received 131I-Hyp with hypericin co-injection even over 40 d after administration, and the long-term high retention was considered to be a distinct character [101].

Previous biodistribution studies reported that Hyp and 131I-Hyp were mainly metabolized through the hepatobiliary pathway and excreted into the bile. Eventually, some metabolites were reabsorbed by the intestines into the enterohepatic circulation and blood circulation, while others were discharged with the feces, which led to alarming side effects. For instance, these radioactive metabolites became trapped in the intestines and evoked persistent radiation, causing radiation enteritis [102]. In order to reduce potential radioactive risk, researchers try to use a device for duodenal juice drainage to eliminate unwanted intestinal waste after intravenous injection of iodine-123/131-labeled hypericin. After 123I-Hyp administration, Cona and Feng collected duodenal juice via a catheter from groups of rats at intervals of 0–4, 4–8 or 20–24 h, and took blood and urine samples from rats at different intervals to study the biodistribution and pharmacokinetics of 123I-Hyp. In rats with intermittent catheterization, analysis of duodenal aspirate showed that the radioactivity excreted into the duodenum within 24 h represented about 46% of the total with two peaks at 3 h and 8 h, suggesting enterohepatic circulation. In contrast, rats with continuous catheterization for 9 h exhibited one single peak representing about 20% of the administered dose. As confirmed by TGC and HPLC-MS spectrometry, 123I-Hyp showed exponential elimination from plasma without significant dehalogenation, whether rats had a catheter or not. Regarding the dissimilarities, rats with a catheter exhibited lower urinary excretion and radioactivity in the small intestines than those without a catheter. Compared with catheterized group, non-catheterized group delayed excretion, showing a longer mean residence time, a higher maximum concentration (Cmax) and larger area under concentration-time curve [AUC(0-∞)]. There is no doubt that the use of duodenal catheters is effective in removing radioactive metabolites from the body and preventing damage to the intestine due to excessive radiation [103]. In addition to the aforementioned studies, Li and Jiang intubated rats with biliary and duodenal catheter, aiming to investigate two kinds of bile draining for reducing accumulation of unwanted radioactivity. During the experiment, bile and tissues were collected from catheterized rats, and TGC along with single-photon emission computerized tomography was used to compare the drainage efficiency between the common bile duct (CBD) catheterization and duodenum intubation. The results showed that hardly any intestinal radioactivity could be detected in those drainaged rats, whereas discernible radioactivity was present in undrainaged rats. Most of the radioactive 131I-Hyp was continuously excreted from the bile within the first 12 h, accounting for 92% of the excretion within 24 h, occupying 82% of the injected dose of 131I-Hyp. Following intravenous injection of 131I-Hyp, the radioactive metabolites entering the small and large intestines with bile peaked at 12 h and 18 h, respectively. This study demonstrated that drainage by either the CBD or duodenum was equally efficient, with no significant differences observed in two approaches of bile draining. Therefore, considering the roughly similar metabolism and excretion pattern of rats and humans, bile drainage during the first 12–24 h is strongly recommended to substantiallly reduce the bodily residence time of harmful radioactive

metabolites [102].

Researchers in Asia have also conducted studies on the metabolism of hypericin. Zang and Qi used the property of fluorescence to establish a HPLC-fluorescence detection method for determining the hypericin concentration of blood and tissue after intravenous or intragastric administration. According to results of pharmacokinetic analysis, the concentration-time curve of hypericin showed double peaks, suggesting that the blood drug concentration decreased rapidly at 20 min after intravenous administration and then tended to moderate. However, there was no obvious peak and valley phenomenon in the intragastric administration, and the curve was integrally flat. In general, given that hypericin was administered by 2 methods, both concentration-time curves showed a two-compartment model with a long half-life elimination. The concentration of hypericin in the liver was higher with a certain degree of accumulation, while concentrations in other tissues were lower with fast elimination [104]. Similarly, Wang et al. collected samples of heart, liver, spleen, lung, kidney, stomach and small intestine from healthy SD rats at 20, 45 and 90 min after a single tail vein administration of 50 mg/kg hypericin, and determined the concentrations by HPLC. The experimental results demonstrated that hypericin was mainly distributed in kidney and liver at 45 min and 90 min after administration, indicating that the elimination burden of liver and kidney was heavy at this dose [105]. In order to separate and identify the metabolites of hypericin in the bile and hepatic necrosis of rats, Zhao et al. employed high performance liquid chromatography coupled with electrospray tandem time of flight mass spectrometry (HPLC-TOF/MS). They found that the prototype and three glycosylation metabolites of hypericin existed in rat bile, whereas only the prototype of hypericin was detected in rat necrotic liver. This discovery indicated that prototype and glycosylation of hypericin were the major metabolic form in rat bile, and the original compound existed only in necrotic tissues [106]. On the basis of the clinical application of hypericin, Zhu et al. established a HPLC-MS/MS method to investigate whether the main active ingredients generated pharmacokinetic interaction when the combination of Acanthopanax senticosus extracts and *H. perforatum*. extracts was performed. Pharmacokinetic results showed that compared with single administration, hypericin's peak time (t_{max}) was advanced by 1 h, the half-life ($t_{1/2}$) was prolonged by about 2 times, and the area under concentration-time curve (AUC) was increased by approximately 30%, which possibly improved antidepressant activity [107]. In order to evaluate the intestinal and hepatic first pass effect of hypericin and explore the possible influence of intestinal cytochrome CYP3A4 on first pass effect, Yao administered hypericin into the intestinal and vascular access ported (IVAP) rats by intraduodenal instillation, intraportal infusion and intravenous injection. The HPLC analysis with fluorescence was used to quantify and distinguish the components of intestinal and hepatic first pass extraction of hypericin mediated by CYP3A4. Eventually, the experimental results indicated that oral hypericin in rats undergoes significant intestinal first-pass metablism, with the degree of this effect being significantly higher than that in the liver. Although intestinal cytochrome CYP3A4 is involved in intestinal first pass metabolism, it may not play a major role in this process [108].

5.3 Other animal experiments

According to the previous results, hypericin tended to congregate in necrosis while normal tissues merely contained small amounts of hypericin in animal models of hepatic infarction. However, when using other animal models to conduct research, scientists found that was not in accordance with the

results in the former rat models. For instance, Cona and Feng utilized rabbit models to reveal the target selectivity and organ distribution of 123I-Hyp in rabbits with acute myocardial infarction. Through the findings of TGC, autoradiography and histology, 123I-Hyp retention was preserved in infarcted myocardium, but not in normal myocardium. Unlike rats, although 123I-Hyp also undergoes hepatobiliary elimination in rabbits, the research group found a mass of 123I-Hyp could be measured in the normal rabbit livers, which proved that the speed of early uptake and hepatic clearance of radioiodinated hypericin was slower in rabbits, in contrast to quick early hepatic uptake and clearance of 123I-Hyp in rats. This was probably because the rabbit's herbivore digestive system may contain receptors for nutrient absorption and retention on plant-derived substances such as hypericin. Furthermore, the liver/heart radioactivity ratio detected in rabbits was consistently over 6-fold higher than those in small rodents [109]. To explain this phenomenon, scientists speculated that the tissue distribution of hypericin might be species-dependent across different animals. Besides, Qi and Shao intended to assess the tissue distribution characteristics of 131I-HYP in a large animal models such as coagulation necrosis induced by radiofrequency ablation (RFA) of canine liver. Based on the statistical quantification and analysis of the experimental data, 131I-HYP was only momentarily absorbed by most tissues and organs, principally metabolized in the liver and subsequently excreted into the bile. In summary, 131I-HYP demonstrated persistent high affinity to RFA-induced hepatic thermo-coagulative necrosis in dogs, similar to the findings in rodents but different from rabbits, further proving that the uptake levels of hypericin in tissues and organs exhibited apparent species differences [110]. In general, hypericin is used in combination with vascular disrupting agents in clinical anticancer therapy. The study of Abma and Peremans firstly reported the biodistribution of a single dose intravenous administration of 131I-Hyp in dogs without the concomitant use of a vascular disrupting agent. After dosing, the highest radioactivity was detected in canine colon and liver within the first 24 h, indicating the accumulation of 131I-Hyp in the colon and delayed retention in the liver and gallbladder. Moreover, the experiments clearly showed that the elimination of 131I-Hyp from every organ was efficient within 7 d and the effective half-life was established at 80 h [111].

6 Formulation strategies and delivery systems of hypericin

Despite the fact that hypericin has various pharmacological activities, its application in PDT is limited due to its high hydrophobicity and low bioavailability. In order to overcome this challenge, a variety of different drug delivery carriers have been attempted, aiming to improve the bioavailability of hypericin without compromising its photodynamic properties. As described above, 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC) liposomes have been used as a delivery system to solubilize hydrophobic hypericin. As a member of the phosphatidylcholine class of lipids, DPPC is one of the most abundant saturated phospholipids in the mammalian membranes, exhibiting desirable biological acceptability and no cytotoxic effect. Cell biomimetic experiments also proved that hypericin wrapped in DPPC liposomes was intensively absorbed by melanoma B16-F10 cells with its photodynamic effects lasting longer after application. Therefore, researchers considered that hypericin-DPPC formulations may extend the circulation time of hypericin *in vivo* in patients, with higher bioavailability for tumor lesions [112]. The configuration of cyclodextrin (CD) possesses a hydrophilic outside surface and hydrophobic cavity, which enables cyclodextrin to envelop the hydrophobic molecules, consequently improving its

poor solubility. Hence, scientists prepared a soluble inclusion complex by complexing the molecules of hypericin and *β*-cyclodextrin, and found that the photochemical properties of included hypericin did not change, and *β*-CD-hypericin showed better phototoxicity in breast cancer MCF-7 cells than free hypericin, indicating better uptake of hypericin by cancer cells after inclusion, which is more beneficial to clinical application in PDT [113]. In addition, some attempts have been made to modify the surface of zein (the main prolamin protein obtained from maize) through pegylation or physical coating with biocompatible polymers for hypericin encapsulation. Experiments revealed that surface-tailored zein improved the water solubility and stability of hypericin and enhanced the delivery of hypericin in PDT. According to the outcomes on hepatocellular carcinoma HepG2 cells *in vitro*, hypericin formulated with pegylated zein exhibited higher phototoxic effects on HepG2 cells than other physically-coated zein formulations, which was attributed to its relatively higher accumulation rate in cancer cells [114]. Single-walled carbon nanohorns (SWNHs) are two-dimensional carbon nanomaterials with a large and easily accessible π-conjugate structure, which have many advantages in biological applications, such as strong permeability and retention, high biocompatibility and low cytotoxicity. Thus, SWNH-hypericin nanohybrids were fabricated by loading hypericin on the surface of SWNHs relying on π-π interactions in a simple one-step sonication. As expected, SWNH-hypericin could simultaneously generate local hyperthermia and sufficient reactive oxygen species (ROS) to significantly kill tumor cells both *in vitro* and *in vivo* under the laser irradiation, and the anticancer effect was superior to that of HYP alone [115]. Moreover, out of the urgent need to develop novel antiviral drugs against Novel duck reovirus (NDRV), researchers tried to load hypericin onto graphene oxide (GO) with high drug-loading capacity and low cytotoxicity, and investigated the antiviral activity of GO/HY complex in ducklings infected with NDRV TH11 strain. The results demonstrated that GO/HY extended the survival time of ducklings by reducing the pathological damage caused by the infection and inhibiting the replication of NDRV in infected ducklings thereby demonstrating its good anti-NDRV activity, which paved the way for the development of new applications of hypericin in the future [116].

7 Conclusion and future prospects

Hypericin is recognized as one of the major bioactive constituents extracted from *Hypericum perforatum* L. (*H. perforatum*), as supported by phytochemical analyses indicating its high abundance in *H. perforatum* and exhibits well-defined pharmacological activities. Although extensive studies have investigated the pharmacological effects and metabolic pathways of hypericin, many questions remain to be further clarified. By systematically summarizing existing experimental evidence, this review aims to provide useful references for future network pharmacology studies as well as subsequent *in vitro* and *in vivo* investigations.

In addition, hypericin has demonstrated a broad range of pharmacological activities, particularly in photodynamic therapy (PDT). Nevertheless, its clinical application may be limited by phototoxicity to skin and ocular tissues, poor water solubility, and sensitivity to light, heat, and pH. With continuous advances in drug delivery systems and formulation strategies, these limitations are expected to be gradually addressed. Overall, the significant therapeutic potential of hypericin warrants further systematic investigation.

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