Research progress on the chemical and pharmacological effects of *Semen Strychni* (Review)

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Abstract. *Semen Strychni* (family *Loganiaceae*) has a long-standing history of cultivation in South Asia. The medicinal value of *Semen Strychni* has attracted extensive attention due to its potential efficacy in the intervention of nervous system diseases, anti-arrhythmia, analgesia, anti-inflammation and antitumor fields. However, research on its pharmacological effects are predominantly limited to *in vitro* experiments, and a systematic pharmacological exploration of different extracts and monomer compounds from *Semen Strychni* is lacking. Additionally, notable safety risks exist both before and after the processing of *Semen Strychni*, thus its clinical application and toxicity mechanisms require further in-depth analysis. The present review comprehensively evaluated the latest research progress on the traditional medicinal history, chemical composition analysis and pharmacological activity of *Semen Strychni*, and aimed to provide a systematic theoretical reference for the follow-up basic research of *Semen Strychni* and its industrial application in the pharmaceutical field.

Contents

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1. Introduction

Semen Strychni refers to the dried seeds of the *Strychnos nux-vomica* tree of the Loganiaceae family. There are 190 species of the genus *Strychnos* worldwide. It is widely distributed in tropical and subtropical regions. Phytochemical studies have found that the main compounds of *Strychnos nux-vomica* are iridoid glycosides (1), polysaccharides (2), phenolic glycosides (3) and alkaloids (4). Modern pharmacological studies have shown that the compounds and extracts of *Semen Strychni* have a variety of biological activities, such as nervous system (5), orthopedic (6), antibacterial (7) and antitumor (8,9) activities. However, pharmacological studies on the traditional uses of these compounds and extracts are currently limited; further studies are required to investigate the clinical application and toxicity of *Semen Strychni*. The present review summarized the latest research progress on the chemical components, pharmacological activities and toxicity of *Semen Strychni*, and aimed to provide theoretical basis for further research on *Semen Strychni* and its application in the pharmaceutical industry.

The present review comprehensively evaluated the latest research progress on the traditional medicinal history, chemical composition analysis and pharmacological activity of *Semen Strychni*. For this purpose, the VIP, Wanfang, CNKI, ACS, Science Direct, Web of Science, Cochrane library, PubMed and CNKI databases were searched for relevant literature. The search terms included '*Semen Strychni*', 'isolation', 'phytochemistry', 'pharmacology', 'toxicology' and 'mechanism of action'. In total, >120 articles describing the phytochemistry, pharmacology and toxicology of *Semen Strychni* were reviewed.

2. Phytochemistry

Alkaloids. *Semen Strychni* contains a variety of chemical components, including alkaloids, glycosides and terpenoids (Fig. 1). Among the chemical components of *Semen Strychni*, alkaloids (10-15) are the key components, among which monoterpene indole alkaloids have the highest content; their structure is novel, and they are highly similar. Strychnine and brucine are the main active and toxic components of *Semen Strychni*, which is also a representative indole alkaloid in this genus.

Non-alkaloids. There have been numerous literature reports on the isolation of alkaloids from *Semen Strychni*; however, reports on the extraction and separation of non-alkaloids from *Semen Strychni* are limited (10-15). The present review introduces the non-alkaloid chemical composition of *Semen Strychni*.

Glycosides. Six glycosides have been found in *Semen Strychni* which are: Loganin, sinoside A, sinoside B, icajine, lanatoside and daucosterol. Loganin is a common iridoid glycoside extracted from *Semen Strychni* (16). It is a key intermediate in the synthesis of indole alkaloids (17).

Steroids and terpenoids. At present, the steroids found from *Semen Strychni* include β-sitosterol. Terpenoids identified include α-balsamol and 5,6-isoprenol (18).

3. Pharmacology

To date, researchers have revealed the biological activities of extracts or compounds from *Semen Strychni (*19), among which the anti-inflammatory and analgesic effects are similar to those of traditional uses (20). *Semen Strychni* is often used for fall injury, fracture swelling and pain, rheumatic diseases and stubborn arthralgia in the application of traditional Chinese medicine (TCM) (21). In addition, further novel pharmacological activities have been identified, such as immunomodulatory, antiarrhythmic and nervous system effects (Table I).

Nervous system effects. *Semen Strychni* and its active components (brucine and strychnine) has been reported to promote functional recovery following spinal nerve root injury (22). Previous transcriptome demonstrated that *Semen Strychni* and its active components initiated transcriptional reprogramming that affected cell morphology and extracellular matrix remodeling of dorsal root ganglia after spinal nerve root injury, suggesting its potential role in promoting axon regeneration. The imaging data further confirmed that *Semen Strychni* and its active components promoted axon regeneration in mice with spinal nerve root injury. By integrating protein-protein interaction prediction, ultrastructural protein detection and molecular docking analysis, myeloperoxidase was identified as the key factor in the axonal regeneration effect conferred by strychnine and its active components (23).

The γ-maze test, passive avoidance test and Morris water maze test were adopted to explore the influences of active components of *Semen Strychni* on learning and memory dysfunction in mice. In terms of latency, *Semen Strychni* significantly inhibited acetylcholinesterase activity in the hippocampus and frontal cortex, memory impairment was significantly improved in the *Semen Strychni* treatment group, compared with control mice (24).

Abirritation. Brucine is the active ingredient of *Semen Strychni* for its analgesic properties; its advantage is that it has a notable analgesic effect without drug dependence. It was previously demonstrated that treatment with brucine treatment significantly inhibited pain responses elicited by thermal and mechanical stimuli, which was achieved by establishing acute and chronic pain models in mice. In addition, brucine was shown to reduce thermal hypersensitivity and mechanical allodynia in a mouse model of chronic contractile injury (25). Extracts of *Semen Strychni* have been shown to exert considerable analgesic effects in writhing and hot plate tests in mice and tail bath test in rats (20). A previous study found that brucine effectively relieved pain caused by related stimuli and combat lipid peroxidation in a variety of artificial animal pain and inflammation models (26).

Anti-inflammatory effects. *Semen Strychni* exerts potent anti-inflammatory and analgesic effects. The anti-inflammatory effect of brucine has been demonstrated by using it in combination with liposome emulsion gel as a potential nanocarrier (27). Furthermore, a previous study reported that a nano-emulsion based on cinnamon oil may be a promising drug delivery carrier to enhance the anti-inflammatory and analgesic effects of brucine (28).

Brucine faces several challenges that impede its clinical translation, including inherent hydrophobicity, poor permeability, a narrow therapeutic window, a short half-life and high toxicity. To address these issues, another research effort focused on developing and evaluating a novel hydrogel formulation: β-cyclodextrin (β-CD) nano sponges (BRUNs) hydrogel, loaded with brucine and integrated with rosemary essential oil (RO) (29). This optimized formulation was designed to achieve sustained drug release, improve skin permeability, mitigate irritation and preserve the antioxidant and anti-inflammatory bioactivities of the active components. First, BRUNs were prepared by a melting technique and comprehensively characterized. The *in vitro* results revealed that brucine in BRUNs achieved delayed release within 24 h by a molecular diffusion mechanism. In addition, the anti-inflammatory and antioxidant potential of the bioactivity observed in BRUNs was preserved. The results demonstrated that the nano sponges hydrogel with RO further delayed the release of brucine under the Fickian mechanism. The significant enhancement of skin permeability and preservation of anti-inflammatory activity were observed in BRUNs hydrolysate containing RO. It was found that the irritation caused by brucine was reduced by half when it was coated with nano sponges (29).

High performance liquid chromatography (HPLC)-tandem mass spectrometry (MS/MS) analysis was previously used to confirm whether the combination of *Semen Strychni* and *Rhizoma Atractylodis macrocephalae* could reduce the toxicity content of *Semen Strychni (*26). Arthritis fibroblasts (MH7A cells) were stimulated with interleukin (IL)-1β to investigate the effects of *Rhizoma Atractylodis macrocephalae* and *Semen Strychni* on the Toll-like receptor 4 (TLR4)/NF-κB/NLR family pyraline-containing domain containing 3 (NLRP3) pathway, to verify its role in the treatment of rheumatoid arthritis. The results revealed that the combined application of the extracts from *Semen Strychni* and *Rhizoma Atractylodis macrocephalae* detected by HPLC-MS/MS were able to reduce the toxicity content of *Semen Strychni*. The combined use of *Semen Strychni* and *Rhizoma Atractylodis macrocephalae* extract promoted the apoptosis of synovial cells and inhibited the expression of TLR4, NF-κB and NLRP3 (30).

Semen Strychni was preivously shown to exert a significant inhibitory effect on foot swelling in rats with adjuvant arthritis (AA). Its total alkaloids, administered at doses of

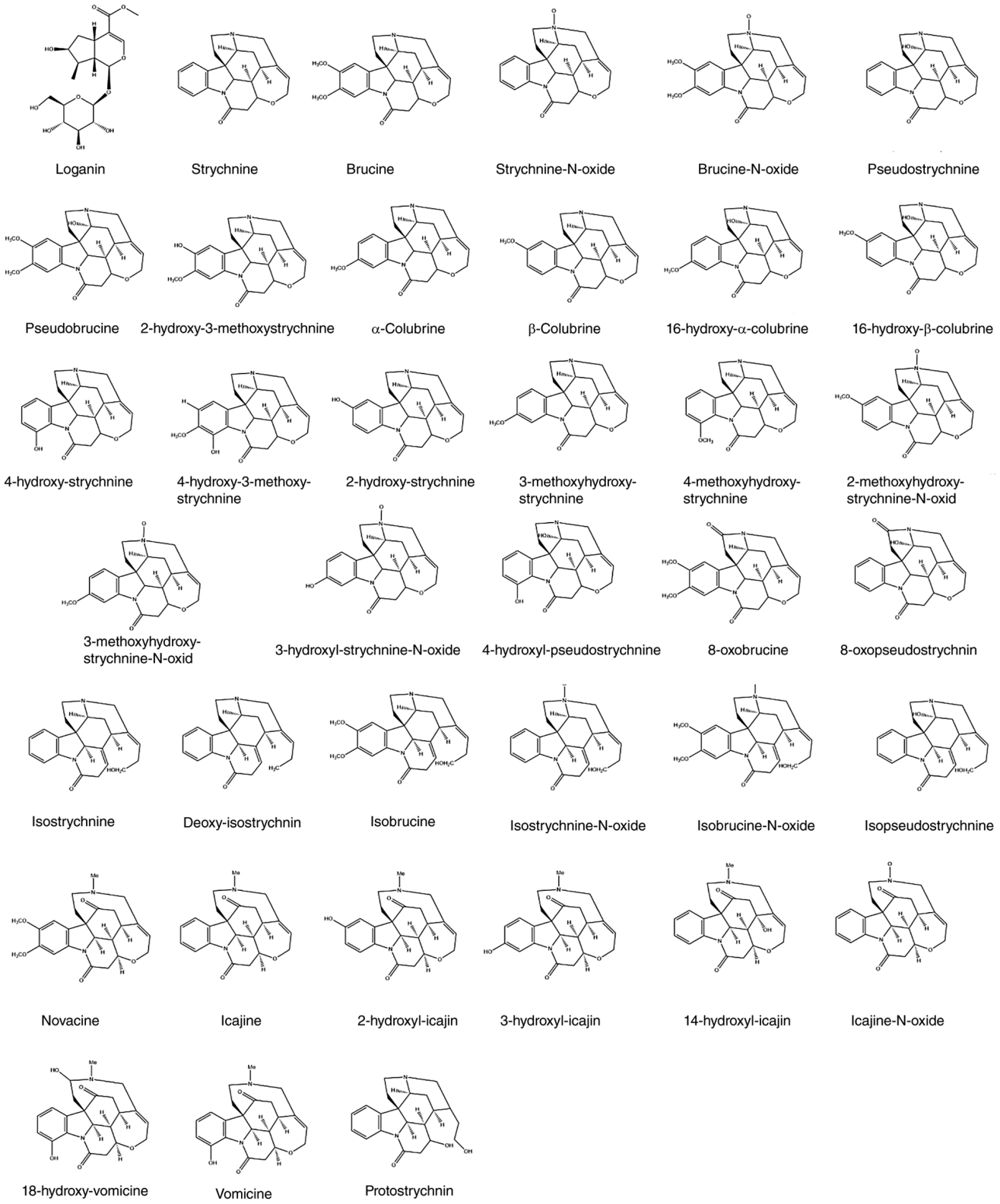

Figure 1. Chemical structure of the compounds from *Semen Strychni*.

6.25-25 mg/kg, exert an anticholinergic effect during AA progression in the rats, which was manifested by a marked reduction in foot swelling degree and polyarthritis index levels, as well as alleviation of joint pathological damage. Mechanistically, the therapeutic effect of *Semen Strychni* may be associated with the suppression of inflammatory mediator release, evidenced by decreased levels of IL-1, prostaglandin E2 (PGE2), IL-6 and tumor necrosis factor α (TNF-α) in rats with AA (Fig. 2) (31-33). The analgesic and anti-inflammatory mechanisms of brucine and brucine N-oxide share some similarities; both can act on central and peripheral nerves, inhibit the release of PGE2 in inflammatory tissues, reduce vascular permeability and the content of 5-hydroxytryptamine in plasma of arthritis rats and increase the content of

Table I. Pharmacological effects of *Semen Strychni*.

Pharmacological action	Diseases	Mechanism of action (Refs.)[a]	Achievement (Refs.)[a]
Nervous system	1. Spinal nerve root 2. Learning and memory disorder	1. Myeloperoxidase may be a key factor in the axonal regeneration effect conferred by Strychnine and its active components for functional recovery after spinal nerve root injury (23). 2. Significantly inhibits acetylcholinesterase activity in hippocampus and frontal cortex (24).	
Abirritation	Acute chronic pain	Significantly inhibits the pain response caused by thermal and mechanical stimulation, reduce the heat allergic reaction and mechanical allodynia in chronic contractile injury; effectively relieves the pain caused by related stimuli, and at the same time, it can resist the lipid peroxidation of the body (20,25,26).	
Anti-inflammatory	1. Rheumatic arthritis 2. Adjuvant arthritis	1. The combination of extracts of Strychnine and *Atractylodes* can promote the apoptosis of synovial cells and inhibit the expression of TLR4, NF-κB and NLRP3 (30). 2. The release of mediators that inhibit inflammation reduces levels of IL-1, PGE2, IL-6 and TNF-α (31-33).	Liposome emulsion gels wereused in combination as potential nanocarriers (29).
Antineoplastic	1. MM 2. Liver cancer 3. Lung cancer 4. Intestinal cancer 5. Breast cancer 6. Cervical cancer 7. Gastric cancer 8. Skin cancer 9. Others	1. Inhibits osteoclast differentiation and promotes osteoclast apoptosis; Phosphorylation of c-Jun; downregulating the mRNA expression of stat3 and stat5 and inhibiting the JAK-STAT signaling pathway (37-41). 2. Inhibition of transcription of HIF-1 targeted genes involved in metastasis of HCC cells. Cell contraction, vesicle formation, and apoptosis were induced (43,44). 3. Leads to cell lysis and induces apoptosis of lung cancer cells. Arrest the cell cycle (46,47). 4. Inducing the apoptosis of colon cancer cells, this effect is concentration- and time-dependent within a certain dose range. The phosphorylation signaling pathways of KDR, PKCα, PLCγ-1 and Rafl are downregulated. Results in cell atrophy and membrane depression, resulting in changes in cell morphology and DNA. By up-regulating Bax and down-regulating Bcl-2, it affects the cell cycle and induces apoptosis, thereby inhibiting the growth of colon cancer (48-53). 5. Reducing the expression of VEGF and COX-2 proteins; suppressing the activities of MMP-2 and MMP-9; suppressing parathyroid hormone-related protein/ mRNA expression (56-61,64-66). 6. It negatively regulates the PI3K/Akt/mTOR pathway (72).	2. Brucine and strychnine transdermal transporter (BSTE), which can be taken up by hepatocellular carcinoma cells *in vitro* and inhibit their proliferation (45). 5. The synthesized BRU-AuNPs have the potential to be an efficient targeted delivery system for breast cancer therapy (63). 8. Preparation of a strychnine loaded transporter liposome (STCN-TL) for use in skin delivery to treat skin cancer agents (77).

Table I. Continued.

Pharmacological action	Diseases	Mechanism of action (Refs.)[a]	Achievement (Refs.)[a]
		7. The effect of brucine on the behavior and ferroptosis of gastric cancer cells is related to the inhibition of NF-κB signaling pathway (73). 8. The upregulation of Bax protein expression and downregulation of Bcl-2 protein expression regulated by the pathway inhibited the growth of melanoma cells in a concentration-dependent manner (74-76). 9. Inhibition of mitochondrial apoptosis signaling pathway leads to apoptosis of prostate cancer cells. The up-regulation of ATF3 promotes intracellular H_2O_2 accumulation and induces TFR-regulated iron overload leading to glioma cell death (83,84).	
Immunoregulation	1. Delayed type hypersensitivity 2. Myasthenia gravis	1. Brucine can regulate the nonspecific immune function of mice in a dose-dependent and functionally dependent manner when the effective dose of brucine is within the range of analgesia (85). 2. Reduced the level of AChRAb and regulated the content of TGF-β1, thereby maintaining the dynamic balance between immune activation and immune suppression (86).	
Cardiovascular system	1. Arhythmia 2. Myocardial preservation	1. The action potential has a significant effect and has a certain antagonist effect on arrhythmia (47). 2. The protective effect on myocardium may be related to the activation of TNF-α and IL-6 signaling molecules (87).	
Other applications	1. Anti-malaria 2. Blood system 3. Bacteriostatic action 4. Antidiarrheal effect 5. Pharmacodynamics	1. Exhibited IC_{50} values in the high nanomolar/low micromolar range (89). 2. Regulates the balance of bax/bcl-2, releases cyb-c, and activates caspase-9 and caspase-3, thereby inhibiting the proliferation of human chronic myeloid leukemia kcl-22 cells.The inhibition of Bcl-2 is related to the activation of Bax (91,92). 3. different concentrations of Strychnine were active against all tested microorganisms. (93,94) 5. Uptake and intestinal permeability in intestinal models, and showing a clear concentration dependent transport, Strychnine may be an inhibitor of P-glycoprotein. Due to its high brain permeability, it is important to strictly control the clinical dosage and production quality of biotracheal capsules to avoid side effects and obtain good therapeutic effects (98).	4. Preventive effects of *Strychnos nux-vomica* homeopathic preparation on diarrhea in foals (95).

Table I. Continued.

Pharmacological action	Diseases	Mechanism of action (Refs.)[a]	Achievement (Refs.)[a]
Clinical test	1. Ankylosing spondylitis 2. Bortezomib induced peripheral neuropathy	1. *Semen Strychni* alone is effective, safe and reliable in the treatment of ankylosing spondylitis (99). 2. Roasted *Semen Strychni* capsule may be related to regulating IncRNAXIST, promoting miR-96-5P/FN1 expression and inhibiting p-FAK-mediated neuroinflammation (100).	

[a]The numbered text in the columns pertains to the condition in the column 'Diseases' allocated the same number. TLR4, Toll-like receptor 4; HCC, hepatocellular carcinoma; BRU-AuNPs, brucine-loaded gold nanoparticles; ATF3, activating transcription factor 3.

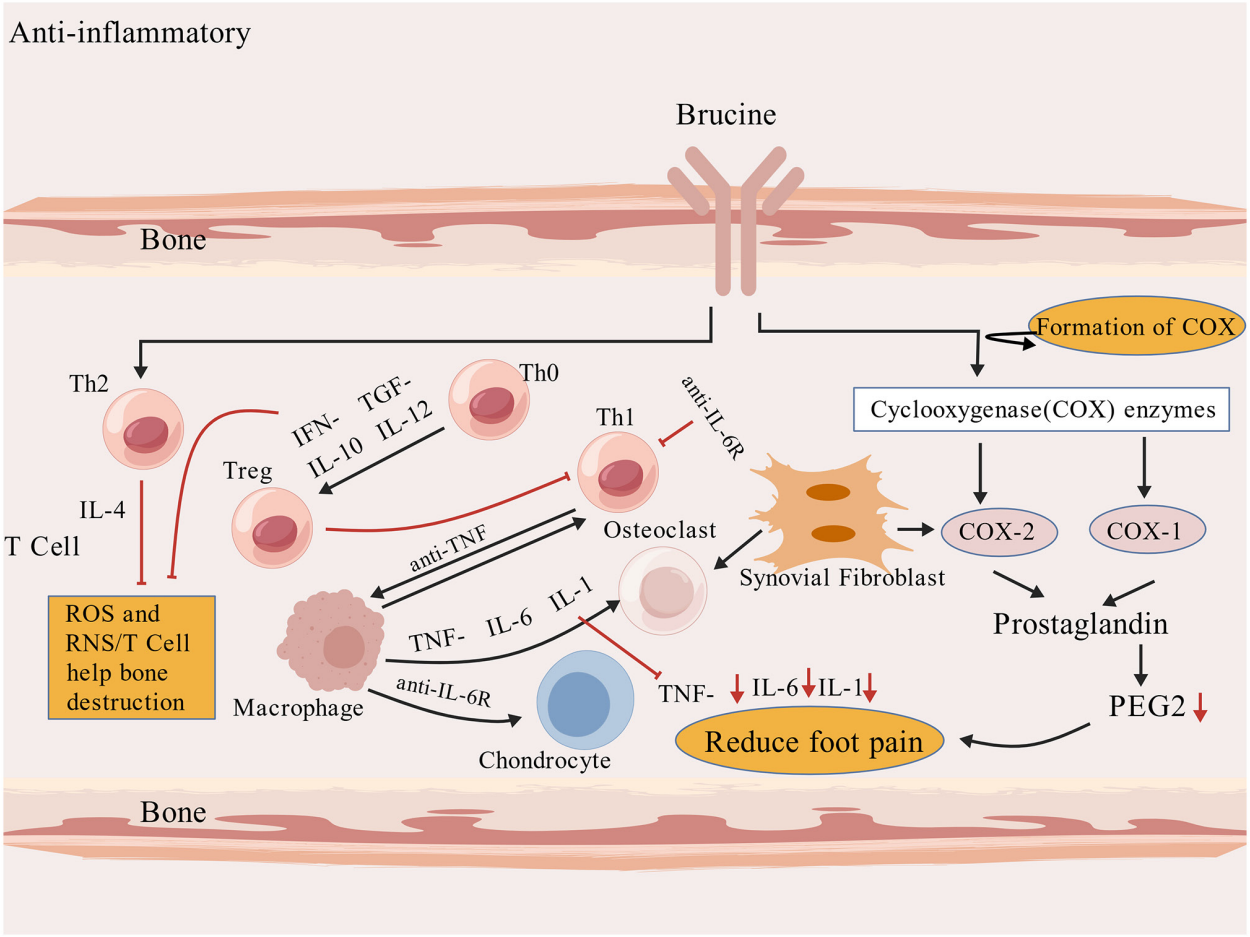

Figure 2. Schematic diagram illustrating the mechanistic pathway of brucine in the treatment of adjuvant arthritis. The figure was created using BioGDP (https://biogdp.com/).

5-hydroxyindole-3-acetic acid to exert anti-inflammatory functions (34). However, it has been reported that brucine N-oxide is more effective than brucine in inhibiting the foot swelling induced by diagonal carrageenan in rats (35).

Allergic rhinitis is a type 2 inflammatory disease caused by airborne allergens, which is mediated by immunoglobulin E in the nasal mucosa and forms an inflammatory infiltrate containing eosinophils and T cells. These cells secrete granular proteins, cytokines and chemokines, which trigger clinical symptoms. However, the effect of brucine on allergic rhinitis has not been clearly defined. Allergic rhinitis was previously induced in mice by injecting them with ovalbumin, which was preceded by administration of brucine and dexamethasone; the mechanism by which brucine exerts its key defensive role was thus discovered. It was found that malondialdehyde (MDA) levels were decreased, and the activation of

cytoplasmic signal transducer and activator of transcription 3 (Stat3) and NF-κBp65 pathways were inhibited by regulating anti-inflammatory cytokines. Brucine alleviated symptoms, such as enlarged goblet cells, basal vascular congestion, increased cilia shedding and improved eosinophil filtration in a mouse model (36).

Antitumor effects. Due to increasing pharmacological research interest, *Semen Strychni* has been found to exert potent inhibitory effects on liver, lung, bowel and breast cancer in recent years.

Effects on bone metabolism. Multiple myeloma (MM) is a cell line malignant tumor characterized by a large number of malignant plasma cells in the bone marrow accompanied by extensive osteolytic lesions. Bone destruction is one of the prominent clinical manifestations.

The efficacy of brucine against MM has been investigated, using the U266 MM cell line, which demonstrated that brucine exerts a pro-apoptotic effect on these cells (37,38). To evaluate the impact of brucine on MM cell proliferation, researchers employed the MTT assay, a common method to assess cell viability, on cultured U266 cells. The results indicated that the inhibitory rate of brucine on MM cell proliferation, across varying concentrations, exhibited a certain time-dependent association. Furthermore, mechanistic analyses revealed that brucine induced U266 cell apoptosis via the c-Jun N-terminal kinase (JNK) signaling pathway and the phosphorylation of c-Jun, a subunit of the activator protein-1 (AP-1) transcription factor (39,40). Another study demonstrated that brucine inhibited the proliferation of multiple myeloma U266 cells by downregulating the mRNA expression of Stat3 and Stat5 and inhibiting the Janus Kinase (JAK)-STAT signaling pathway (39).

Additionally, accumulating evidence suggests that the anti-myeloma mechanism of brucine may be associated with osteoclasts. Studies focusing on the osteogenic precursor cell line, MC3T3-E1, and the MM cell line, U266B1, have further confirmed that brucine can markedly suppress osteoclast differentiation and induce osteoclast apoptosis (40,41).

The mechanism of *Semen Strychni* in the treatment of MM may involve its antiproliferative and cytotoxic effects; *Semen Strychni* was previously used to treate the human MM cell line RPMI 8226; *Semen Strychni* inhibited cell proliferation in a concentration- and time-dependent manner. In addition, the disruption of mitochondrial membrane potential and subsequent leakage of mitochondrial cytochrome *c* were observed in MM (42).

Liver cancer. In HepG2 and SMMC-7721 hepatocellular carcinoma (HCC) cell lines, brucine was found to suppress the transcription of hypoxia-inducible factor 1 target genes associated with HCC cell metastasis. This inhibitory effect was accompanied by reduced levels of fibronectin, matrix metallopeptidase (MMP) 2, lysyl oxidase and cathepsin D, thereby impeding cancer metastasis (43). Another study demonstrated that brucine also inhibited the proliferation of HepG2 cells by inducing cell shrinkage, vesicle formation and apoptotic body formation (44). Moreover, brucine significantly decreased the expression of cyclooxygenase-2 (COX-2), but increased the expression of caspase-3 and the activity of caspase-3-like protease in HepG2 cells (44).

A separate research effort aimed to develop a brucine-strychnine transdermal delivery system (BSTE) capable of being internalized by HCC cells and inhibiting their proliferation *in vitro* (45). The central composite design-response surface methodology was adopted to optimize the BSTE formulation. Dynamic dialysis and Franz diffusion cell methods were used to investigate the *in vitro* release and percutaneous permeability of BSTE, while fluorescence microscopy combined with flow cytometry was applied to analyze the *in vitro* cellular uptake of the delivery system. Cytotoxicity was evaluated via MTT assay. The results indicated that BSTE exhibited superior transdermal performance compared with free brucine and strychnine. From the *in vitro* experiments, it was found that the optimized BSTE formulation was internalized by HCC cells, enabling sustained release of active ingredients and providing long-term effective inhibition of cell proliferation. That study provided novel insight for the development and clinical translation of brucine and strychnine-based formulations (45).

Lung cancer. Brucine has been shown to exerts effects on the map kinase kinase 7 (MKK7) gene in lung cancer cells. Specifically, MKK7 kinase activates the JNK gene, which in turn triggers the production of the transcription factor AP-1. Subsequently, the Fas gene (a member of the tumor necrosis factor receptor superfamily) and the Fas-associated death domain protein gene undergo alterations, driven by the activation of the Fas-mediated death receptor pathway. These changes induce intracellular Ca^{2+} fluctuations, ultimately resulting in lung cancer cell lysis and apoptosis induction (Fig. 3) (46). Furthermore, a previous study also found that the mechanism of brucine in the treatment of lung cancer may cell cycle arrest and the expression of related genes. Brucine can significantly inhibit the proliferation of human lung cancer cell line PC-9, and the mechanism is mainly related to cell cycle arrest by downregulation of cyclin D1 and cyclin E expression levels (47).

Intestinal cancer. *Semen Strychni* was previously shown to exert a potent inhibitory effect on HT-29 human colon cancer cells; the inhibitory capacity was enhanced in a concentration-dependent manner with the increasing brucine concentrations (48). At a concentration of 250 μmol/l, brucine induced cell cycle arrest at the $G_1/S/G_2$ phases and impaired the activity of HT-29 cells in the G_1 phase. When administered at concentrations of 125, 250 and 1,000 μmol/l, brucine downregulated the expression of the B-cell lymphoma/leukemia-2 (Bcl-2) gene, while upregulating the expression of the tumor suppressor p53, as well as caspase-3, poly ADP-ribose polymerase (PARP) and caspase-9. These findings indicate that brucine can hinder cell proliferation and the cell cycle progression of the HT-29 cell line, promote the loss of extracellular MMPs, and induce apoptosis by regulating the expression of tumor suppressor (48).

In another study, in rats treated with dimethyl-hydroxyanthracene (DMH), elevated levels of MDA and reactive oxygen species (ROS), increased the activity of cytochrome P4500-2E1 and higher levels of the serum marker enzyme carcinoembryonic antigen (CEA) were observed (49). Additionally, the expression of inflammatory and proliferative proteins was upregulated. DMH treatment also downregulated the expression of nuclear factor erythroid 2-related factor 2 and NF-κB. Notably, brucine treatment restored the activities of CEA and cytochrome P450-2E1, blocked the expression of

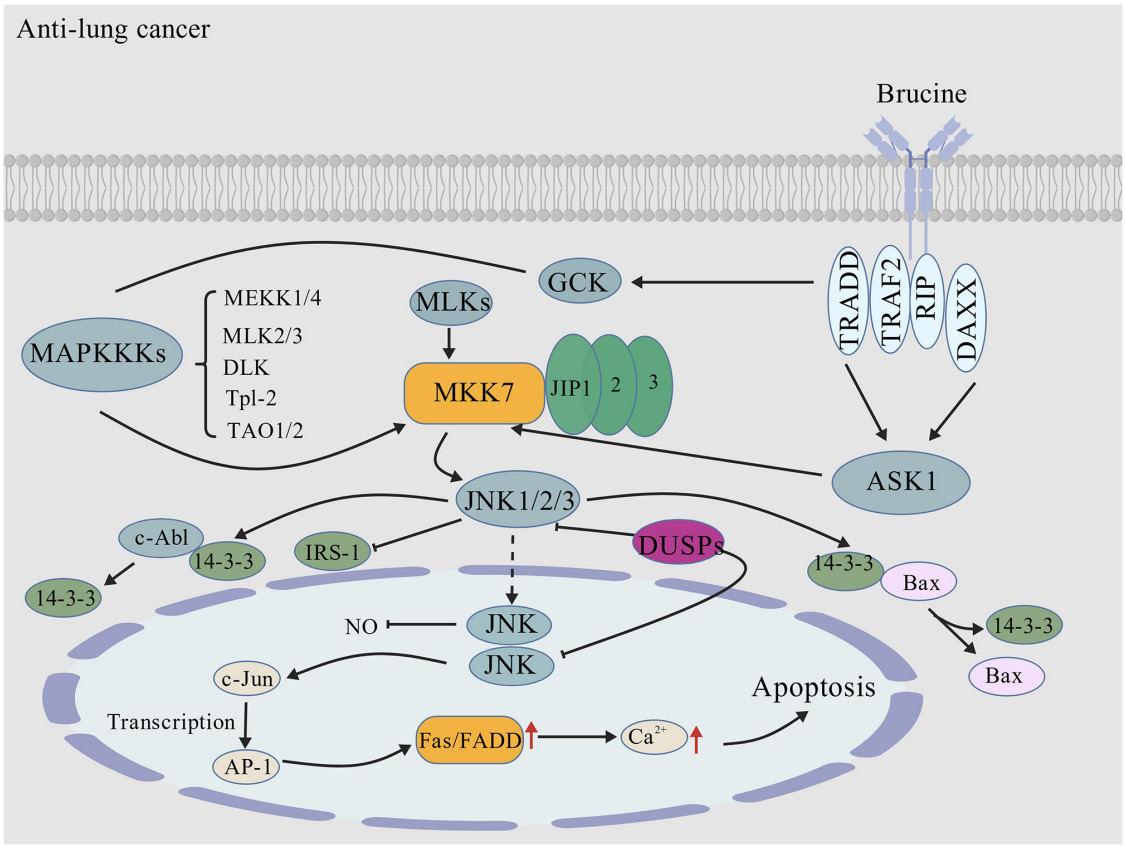

Figure 3. Schematic diagram illustrating the mechanistic pathway of brucine in the treatment of lung cancer cells. The figure was created using BioGDP (https://biogdp.com/).

inflammatory and proliferation markers, and prevented the development of DMH-induced colon cancer in rats (49). In the regulation of cell proliferation and apoptosis, an essential feature of tumor cells is the blockade of the apoptotic pathway, whereby the cell cycle dysregulated and uncontrolled proliferation is achieved (50). Brucine can effectively interfere with this pathological process, by breaking the molecular pathological balance of tumor apoptosis escape and restarting the endogenous apoptosis pathway of cancer cells. Brucine can block the abnormal proliferation of cancer cells at the molecular level and reverse the pathological state of cell cycle disorder.

Semen Strychni can inhibit the growth of SW480 colon cancer cells. Specifically, in SW480 colon cancer cells, brucine exerts its antitumor effect by mediating apoptosis through the IL-6/Stat3 pathway: It inhibits the phosphorylation of Stat3, upregulates Bcl-2 associated X protein (Bax), downregulates Bcl-2 and increases the expression of cleaved DNA repair enzyme PARP (Fig. 4) (51).

Angiogenesis plays a critical role in colon cancer development. As previously demonstrated, brucine significantly reduced the angiogenesis of chicken chorioallantoic membrane and tube formation, inhibited the vascular endothelial growth factor (VEGF) secretion and mammalian target of rapamycin (mTOR) expression in LoVo cells and downregulated the mRNA and phosphorylation protein expression of kinase insertion domain receptor (KDR), protein kinase c (PKC), phospholipase C (PLC) and raf protein kinase(Raf1). These results suggest that brucine can inhibit the growth of LoVo cells by inhibiting angiogenesis (52).

Investigations into the impact of brucine on cell viability, the cell cycle and apoptosis have revealed that it exerts a potent growth-inhibitory effect on LoVo colorectal cancer cells. The underlying mechanism is hypothesized to involve brucine-induced cell atrophy and membrane depression, which further trigger alterations in cell morphology and DNA (53). Specifically, brucine significantly impairs LoVo cell viability, suppresses colony formation and induces apoptosis. It also inhibits LoVo cell migration in a dose-dependent manner; western blot analysis confirmed that this migration-inhibitory effect is linked to reduced expression of MMPs, including MMP2, MMP3 and MMP9. Additionally, brucine treatment was shown to downregulate the expression of frizzled homolog 8, ingless type MMTV integration site family member 5A (Wnt5a) and antigen-presenting cell, while upregulating the expression of axis inhibition protein 1 (AXIN1). Simultaneously, brucine reduced the phosphorylation levels of low-density lipoprotein receptor-related protein 5 and 6, as well as glycogen synthase kinase 3β, and elevated the phosphorylation level of β-catenin. In a nude mouse xenograft model study, the oral administration of brucine was found to inhibit the growth and migration of LoVo cells by activating AXIN1 and promoting β-catenin phosphorylation. Collectively, these findings indicate that brucine can suppress colorectal cancer migration both *in vitro* and *in vivo*, with its efficacy associated with the inhibition of the Wnt/β-catenin signaling pathway (54). Furthermore, it has been verified that both brucine and strychnine exert targeted inhibitory effects on colon cancer proliferation *in vitro* and *in vivo*, which holds

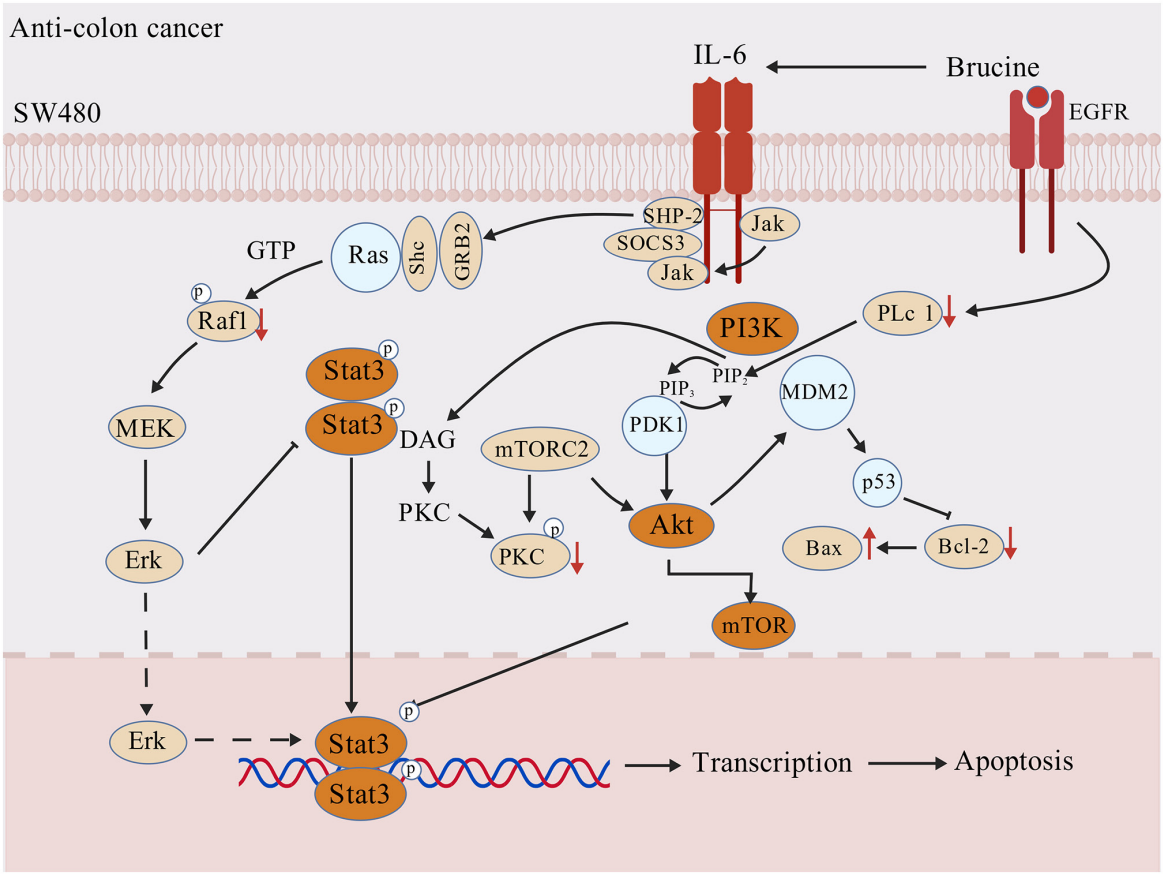

Figure 4. Schematic diagram illustrating the mechanistic pathway of brucine in the treatment of colon cancer cells. The figure was created using BioGDP (https://biogdp.com/).

significant value for their future development and application as antitumor drugs for colon cancer (55).

Breast cancer. Breast cancer is the most prevalent malignant tumor among women. Relevant studies have indicated that the inhibitory mechanism of brucine against breast cancer is associated with protein regulation. Previously, utilizing methods such as MTT assay, researchers evaluated the inhibitory effects of brucine on the proliferation of the human breast cancer cell line, MDA-MB-231, and the experimental results suggested that brucine may effectively suppress angiogenesis in breast cancer cells *in vitro* (56) Specifically, it downregulates the expression of proteins including VEGF, VE-cadherin, erythropoietin-producing hepatocellular A2, MMP9 and MMP2, thereby inducing apoptosis (56). Another study proposed that brucine exerts therapeutic effects on breast cancer by inhibiting vasculogenic mimicry (57), while a separate investigation found that the combination of brucine and gemcitabine yields enhanced efficacy in treating MCF-7 breast cancer (58).

A further mechanism of brucine in inhibiting the growth of breast cancer xenografts and tumor neovascularization may involve reducing the expression of VEGF and COX-2 proteins, which was demonstrated by immunohistochemical staining with the streptavidin-peroxidase (59). Another study aimed to explore the impact of brucine on VEGF expression and microvessel density (MVD) in nude mice with breast cancer bone metastasis. It was observed that VEGF expression in the brucine-treated groups (both high and low doses) and the thalidomide-treated group was significantly lower compared with that of the model group, with no significant difference between the high-dose brucine group and the thalidomide group, but notable differences between the high and low-dose brucine groups. Additionally, VEGF expression levels in the low and medium-dose brucine groups were significantly higher compared with the thalidomide group. Regarding MVD, values in all three treatment groups (low, medium, high-dose brucine) were significantly lower compared with the model group; no significant difference was found between the medium/high-dose brucine groups and the thalidomide group, while the low-dose brucine group exhibited significantly higher MVD than the thalidomide group. These findings suggest that brucine can inhibit the growth of breast cancer bone metastases, potentially through suppressing tumor angiogenesis (60).

Researchers have also confirmed via microscopy and other techniques that brucine exerts a dose-dependent inhibitory effect on the growth of human triple-negative breast cancer MDA-MB-231 cells (61). Furthermore, brucine can trigger a cellular stress response in MDA-MB-231 tumor cells and act as an inducer of immunogenic cell death, thereby enhancing tumor treatment efficacy (62). Gold nanoparticles (AuNPs) serve as ideal carriers for targeted therapy, given their excellent optical and physical properties, which enable precise delivery of drugs to cancer cells while minimizing adverse side effects. A previous study focused on synthesizing and characterizing brucine-loaded gold nanoparticles (BRU-AuNPs) to evaluate

their antioxidant and apoptotic mechanisms for targeted breast cancer therapy demonstrated that BRU-AuNPs reduced MCF-7 cell viability in a concentration-dependent manner with an IC_{50} value of 11.47 μg/ml. Treatment with BRU-AuNPs disrupted the antioxidant balance, increased ROS production, depolarized mitochondrial membranes and induced apoptosis. Therefore, these synthesized BRU-AuNPs hold potential as an efficient targeted delivery system for breast cancer treatment, capable of delivering brucine directly to tumor cells while reducing side-effects and improving therapeutic outcomes (63).

It has also been demonstrated that strychnine inhibits the metastasis of triple-negative breast cancer cells by reversing epithelial-mesenchymal transition and suppressing the activities of MMP-2 and MMP-9 (64). In another investigation, Transwell assays, *in vitro* bone resorption assays and enzyme-linked immunosorbent assays were used to evaluate an established bone resorption cell model. The results indicated that strychnine can reduce the migration of MDA-MB-231 cells and inhibit the receptor activator of nuclear factor-κB ligand (RANKL)-induced bone resorption process in RAW264.7 cells in a dose-dependent manner (65). Furthermore, the transdermal administration of strychnine was found to effectively inhibit breast cancer xenografts; its mechanism may involve preventing osteoclast differentiation by suppressing parathyroid hormone-related protein/mRNA expression, ultimately inhibiting the growth of breast cancer bone metastases (66).

Bevacizumab (Avastin®) was the first anti-VEGF monoclonal antibody developed to inhibit angiogenesis, and it was quickly approved by the US Food and Drug Administration in 2008 for the treatment of patients with metastatic breast cancer; however, it was not as effective as in preclinical studies (67). Clinical studies suggest that the expression level of VEGF in serum of patients with breast cancer is significantly higher than that of healthy people, and it is closely related to tumor stage, lymph node metastasis and prognosis, which is clinically recognized as a potential target for breast cancer treatment (68).

VEGF is a key target of *Semen Strychni* in its mechanism of action against breast cancer. In the regulation of tumor invasion and metastasis, the process of tumor growth, invasion and metastasis requires VEGF to induce angiogenesis *in vivo*; reducing the expression of VEGF can achieve the purpose of tumor inhibition (69). *Semen Strychni* and its active components can directly downregulate the transcription and expression level of VEGF in breast cancer cells, inhibit the proliferation, migration and tube formation of vascular endothelial cells, block the blood supply of breast cancer tissue, reduce the nutrient uptake and metabolism of tumor cells and then inhibit the growth of primary breast cancer and distant metastasis. This effect has been verified in breast cancer cell lines and animal models of transplanted tumors (70).

Cervical cancer. Cervical cancer is the most prevalent malignant tumor in gynecology, and its incidence has been on the rise among young populations in recent years. Using a combination of experimental methods including CCK-8, Transwell invasion assay, reverse transcription-qPCR and western blotting, it previously was demonstrated that brucine exerts anti-proliferative, anti-migratory and anti-invasive effects on cervical cancer cells by upregulating the expression of microRNA-34a-5p, thereby achieving its antitumor activity (71).

Additionally, it has been found that brucine can inhibit inflammatory responses, suppress cell proliferation, reduce mitochondrial membrane potential and induce apoptotic cell death. This series of biological effects is hypothesized to be mediated by the downregulation of the PI3K/AKT/mTOR signaling pathway (72), which further supports that brucine possesses certain therapeutic potential for cervical cancer.

Gastric cancer. Gastric cancer is the third leading cause of cancer-related mortality worldwide, and its symptoms are often cryptic, resulting in an often late diagnosis, highlighting the urgent need to develop innovative diagnostic and therapeutic strategies. One such approach is to investigate ferroptosis, a form of cell death associated with a variety of pathological conditions and malignancies. In a previous study, by use of the ferroptosis assay, brucine enhanced the effect of ferroptosis inducer elastine, increased the intracellular iron content, MDA level and ROS content, and decreased the content of glutathione (73). In addition, the results showed that the effects of brucine on Gastric cancer cell line HGC-27 behavior and ferroptosis were related to the inhibition of NF-κB signaling, suggesting an indirect regulatory effect on this pathway (73).

Skin cancer. The anticancer potential of a brucine-loaded ethanol gel derived from brucine seeds was previously evaluated using a series of experimental approaches, and the results demonstrated that this gel exerted significant anticancer effects on treated melanoma cells (74). Additionally, findings from that study indicated that the developed tomato lectin drug carrier holds potential for transdermal delivery of brucine, which could facilitate the treatment of skin cancer (74). Another study revealed that under ultrasound irradiation, the skin permeability of both fluorescent markers and brucine was higher compared with that under passive diffusion conditions (75). *In vitro* photodynamic therapy experiments revealed that two porphyrin-brucine quaternary ammonium salts immobilized on gold nanoparticles were more effective in reducing the volume of basal-like carcinoma PE/CA-PJ34 cells *in vivo*, with complete tumor regression observed in the experimental group (76).

Strychnine is highly effective; however, its clinical application has been hampered by its low water solubility, narrow therapeutic window, short half-life and high toxicity. To design and optimize formulations of strychnine loaded transport liposome (STCN-TL) for dermal delivery for the treatment of skin cancer, formulations of STCN-TL were evaluated in terms of vesicle size, polydispersity index, encapsulation efficiency and *in vitro* release (77). In *in vitro* permeation experiments, the permeability of the prepared STCN-TL agent was increased by 2.5-foldd compared with that of the STCN solution. Confocal laser scanning microscopy (CLSM) imaging of the skin (rat) revealed deeper penetration of the transport liposome preparation loaded with rhodamine B than the rhodamine B hydro-alcohol mixture. In addition, the skin of rats treated with STCN-TL nanogels was significantly higher than that of rats treated with conventional STCN gels. These results suggest that the transport liposome preparation may be a suitable nanocarrier for improving the distribution of STCN in the skin for skin cancer treatment (77).

Other types of cancer. Previous studies have demonstrated that brucine markedly suppresses angiogenesis in both the nude mouse sponge implantation model and the breast

cancer-induced bone metastasis model by lowering VEGF levels, and this inhibitory effect is associated with a reduction in MVD (78). Further investigations have revealed that brucine decreases VEGF production by inhibiting VEGFR2 signaling pathways, both *in vitro* and *in vivo* (79). Additionally, brucine-induced VEGF downregulation has been shown to inhibit inflammatory angiogenesis in the mouse sponge implantation model (80).

Loganin, at concentrations of 2-12 μg/ml, has shown significant cytotoxic activity against a variety of human cancer cell lines, human liver (WRL-68), colon (COLO-320 and CaCo2), ovarian (PA-1) and breast (MCF-7) cancer cell lines (81). Brucine (at doses of 12.5, 25, and 50 mg/kg, respectively, for 14 days) enhanced the survival time of mice with established ascites tumors (82). Brucine can reduce the expression of heat shock protein 70 and inhibit the mitochondrial apoptosis signaling pathway in human prostate cancer PC-3 cells, thereby playing an anti-apoptotic role. Therefore, brucine provides a new perspective and may serve potential therapeutic agent for the prevention and treatment of prostate cancer (83).

It has also been shown that brucine activates endoplasmic reticulum stress in glioma cells, leading to the upregulation of activating transcription factor 3 (ATF3) and nuclear translocation. ATF3 upregulates NOX4, and downregulates solute carrier family 7 member 11 and catalase expression to promote intracellular H_2O_2 accumulation. Furthermore, the knockdown of ATF3 was shown to prevent brucine-induced iron and H_2O_2 accumulation and glioma cell death. Ultimately, H_2O_2 causes glioma cell death by inducing iron overload regulated by follicular regulatory T-cells (84).

Immunoregulation. A previous study examined the effect of brucine on the non-specific immune function of normal mice and immunocompromised mice at an effective analgesic dose. It was found that brucine exerted a dose-dependent and function-dependent regulatory effect on the non-specific immune function of mice within the effective analgesic dose range (85). In another study, processed *Semen Strychni* reduced acetylcholine receptor antibody levels and regulated TGF-β1 content, thereby maintaining a dynamic balance between immune activation and immunosuppression (86).

Cardiovascular system protection. The impact of brucine on the action potential induced by high K^+ was previously assessed. It was found that this compound exerts a significant influence on the action potential, and the underlying mechanism was hypothesized to be associated with the blocking effect of brucine on myocardial tissue, which indicates that the substance possesses a certain antagonistic activity against arrhythmias (47). In another study, the cardioprotective potential of brucine was assessed by detecting myocardial infarct size, serum cardiac marker enzymes, endogenous antioxidants, inflammatory mediators and conducting histopathological analysis (87). The results revealed that brucine effectively reduced the infarct size and alleviated histopathological damage by enhancing endogenous antioxidants and lowering the levels of lipid peroxidation marker enzymes. These findings suggested that the myocardial protective effect of brucine may be linked to the activation of TNF-α and IL-6 signaling molecules (87).

Other applications. A previous study found that the extract of *Strychnos ligustrina* consisting of brucine and strychnine was effective in inhibiting the reproduction of *Plasmodium berghei*. These results open the possibility of further discovery of antimalarial drugs that may have more successful chemotherapeutic effects (88). The dimer analogues of strychnine, sungucine, isosungucine, hydroxyisosungucine, have considerable *in vitro* activity against *Plasmodium*, as these compounds have exhibited IC_{50} values in the high nanomolar/low micromolar range in various *Plasmodium falciparum* strains (89). The dimeric bisindole alkaloid strychnoflavine has also exhibited potent anti-plasmodial activity against these *Plasmodium falciparum* strains *in vitro*, with moderate to high molar IC_{50} values, and *in vivo* anti-malarial activity in *Plasmodium vinckei petteri* and *Plasmodium berghei* mouse models (90). As resistance to current antimalarial drugs has increased, the novel scaffold, which is a dimeric strychnine analogue, may serve as a potential avenue for the development of new antimalarial drugs.

Previous research has explored the effects of various concentrations of brucine on the proliferation of human chronic myeloid leukemia KCL-22 cells, using Annexin V-FITC/PI double staining flow cytometry and western blotting to detect apoptosis and protein expression levels. The findings indicated that brucine may inhibit the proliferation of KCL-22 cells by regulating the balance of Bax/Bcl-2, promoting the release of cytochrome *c*, and activating caspase-9 and caspase-3 (Fig. 5) (91). In a separate study, the CCK-8 assay and Annexin V-FITC/PI double labeling method were used to investigate the ability of brucine to induce apoptosis in human monocytic leukemia THP-1 cells and its potential underlying mechanism. Results showed that brucine suppressed the proliferation of THP-1 cells, which was hypothesized to be associated with the inhibition of Bcl-2 expression and the activation of Bax (92).

The effects *of Semen Strychni* at various concentrations on *Bacillus subtilis, Aeromonas hydrophila*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas marginata* have also been examined, and it was found that *Semen Strychni* was active against all tested microorganisms (93,94).

In another study, the antidiarrheal effect of a homeopathic preparation of *Semen Strychni* on young horses of different breeds was studied. The mean water content was reduced by 74% in the treatment group compared with the placebo group. In addition, a significant reduction in the total number of anaerobic bacteria in homeopathic preparations containing *Semen Strychni* extracts was found, which demonstrates the preventive application of homeopathic preparations for diarrhea in foals (95).

The uptake and intestinal permeability of strychnine, brucine, strychnine N-oxide and brucine N-oxide (the main alkaloids of strychnine) were studied in the human intestinal Caco-2 model and exhibited a clear concentration-dependent transport (96,97). Brucine has a high degree of brain permeability; thus, it is necessary to strictly control the clinical dosage and production quality of biological tracheal capsules to avoid side effects and obtain suitable therapeutic effects (98).

Clinical tests. The role of *Semen Strychni* in arthritis has been an ongoing field of research. In clinical practice, ankylosing

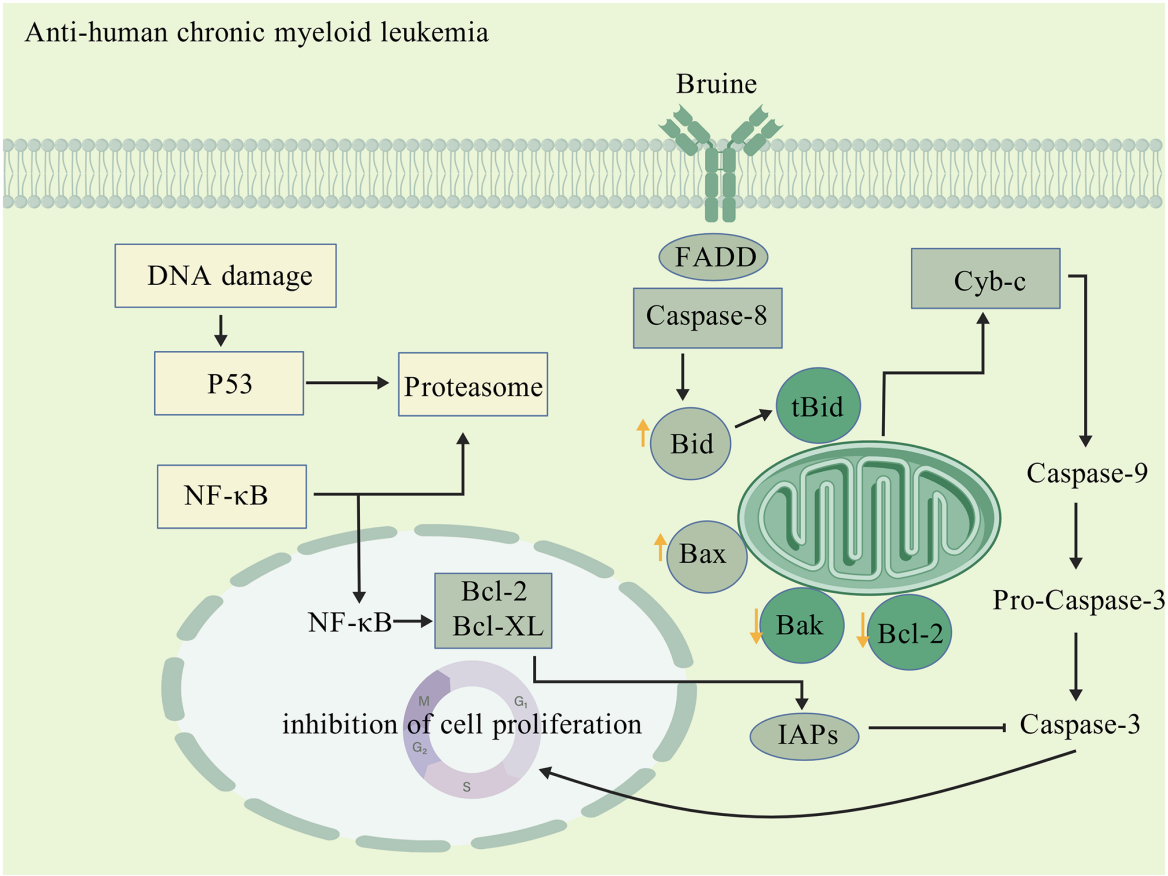

Figure 5. Schematic diagram illustrating the mechanistic pathway of brucine in the treatment of human chronic myeloid leukemia. The figure was created using BioGDP (https://biogdp.com/).

spondylitis (AS) is refractory to single-drug treatment. At present, there is still a lack of specific drugs for the treatment of AS. In a previous study, patients with AS were randomly divided into control group and observation group, and both groups were administered conventional Chinese and Western medicine treatment and routine nursing; in addition, the observation group was administered *Semen Strychni*, and the efficacy of the two groups was compared after treatment, significant difference, and no adverse consequences occurred. That study suggested that *Semen Strychni* is effective in the treatment of AS, safe and reliable and helpful for rehabilitation (99).

Bortezomib (BTZ) has shown significant efficacy in the treatment of MM; however, BTZ can cause related adverse reactions, such as bortezomib-induced peripheral neuropathy (BIPN). Therefore, exploring the clinical efficacy of roasted *Semen Strychni* capsule in the treatment of BIPN is helpful to provide research evidence for the clinical application of roasted *Semen Strychni* capsule. A total of 20 patients with MM diagnosed using TCM and Western practices and treated with BTZ and BIPN were enrolled in a prospective non-randomized controlled study. The TCM symptom score, neurotoxicity score, peripheral neuropathy grade and partial peripheral nerve conduction velocity were compared between the patients who did not receive *Semen Strychni* treatment and those who did not. Peripheral blood samples of control and treatment group patients were obtained and inflammation related factor expression levels were assessed using an ELISA. Roasted *Semen Strychni* capsules were shown to alleviate BIPN to a certain extent and was safe. Its mechanism may be related to regulating IncRNAXIST, promoting miR-96-5P/FN1 expression and inhibiting p-FAK-mediated neuroinflammation (100).

In vitro and in vivo experimental transformation differences. All of the aforementioned studies and experiments are single *in vitro* or *in vivo* experiments, and the translational differences form a great obstacle to the clinical trials carried out in the later stage. The exploration of molecular mechanisms relies on the collaborative verification of *in vitro* and *in vivo* experiments; however, key details of the translational differences between the two are commonly overlooked. This neglected difference not only affects the objective interpretation of the true effect of molecular mechanism, but also may reduce the reliability of the research results to clinical translation, which constitutes the core limitation of molecular research.

Firstly, the difference in the tumor microenvironment between *in vitro* and *in vivo* experiments and its regulatory effect on molecular mechanisms are the core source of the differences in transformation, which is also a key association that has not been focused on in previous studies (101,102). *In vitro* molecular experiments predominantly use a single tumor cell line culture system, and the experimental environment is highly controllable, which can accurately reveal the regulatory logic and molecular interactions of targets and pathways. However, this system is completely separated from the complex regulation of the tumor microenvironment

in vivo. *In vivo* experiments, the effect is weakened, offset or even reversed due to the regulation of microenvironment. This transformation difference has not been fully analyzed, affecting the integrity of molecular mechanism research.

Secondly, the heterogeneity differences of tumor cells between *in vitro* and *in vivo* experiments may be overlooked, further amplifying the translational gap between these models (103). The majority of the tumor cell lines used in the *in vitro* experiments have a relatively single genetic background and a stable phenotype after long-term passage, and their molecular characteristics are significantly different from those of primary tumor cells *in vivo*. However, tumor tissues *in vivo* are highly heterogeneous, and tumor cells in different regions have significant differences in gene expression, mutation characteristics, and molecular target expression levels. Furthermore, tumor cells *in vivo* can undergo adaptive evolution under physiological stress, resulting in inconsistent molecular regulation patterns with those of a single cell line *in vitro (*104).

Finally, molecular studies *in vitro* mostly focus on a single target or a single signaling pathway, while the occurrence and development of tumors *in vivo* are the result of the collaborative regulation of multiple molecules and multiple pathways (105). Furthermore, *in vitro* experiments can be controlled by a single variable to clarify the role of a molecule or signaling pathway, but it cannot simulate the cross-regulatory network of multi-molecules and multi-pathways *in vivo*. However, in the *in vivo* environment, there are close synergistic or antagonistic effects between the target or pathway and other related molecules or signaling pathways, and this cross talk directly alters the molecular mechanisms (106).

4. Toxicity

Toxic reactions. Animal experiments have demonstrated that *Semen Strychni* exhibits high levels of toxicity, as its components and extracts can induce toxic effects on multiple animal tissues and organs, including muscles, heart, kidneys, embryos and the brain. As previously demonstrated, a German shepherd dog displayed toxic symptoms, including pain, muscle tension, mild abdominal tension, reflux, salivation, septicemia, dyspnea and cyanosis, 10 h after ingesting *Semen Strychni* and died 15 min after the onset of these symptoms (107). Additionally, strychnine exerts embryotoxic effects in zebrafish and rats. Specifically, it was previously demonstrated that strychnine at a concentration of 200 μmol/l induced embryonic malformations and apoptosis in zebrafish (108), while doses of 5 or 8 mg/kg caused various abnormalities in rats, such as anencephaly, generalized hypoplasia and encephalocele. The potential mechanism underlying these malformations may be associated with the effects of strychnine on neurotransmitter receptors (109).

Autopsy after brucine or strychnine poisoning may reveal evidence of severe muscle spasms and cardiac arrest, congestion of the lungs, heart and stomach mucosa, and the long-term use of brucine at low doses (abuse) may trigger delirium, tremors and irritable, spasmic pain in the abdomen and legs (110). In addition, *Semen Strychni* may easily be confused with other plants; as demonstrated in a previous study, a 29-year-old male ingested an herbal preparation made from the bark of the *Semen Strychni*, which was mistaken for an herb obtained from the *Alstonia scholaris* (111). Another study demonstrated that a 24-year-old man who ingested an agent made from the bark and seeds of *Semen Strychni* developed muscle spasms and convulsions shortly after (112). A 34-year-old female patient also died after swallowing herbal powder containing *Semen Strychni* seeds (113).

Previous studies have determined the chronic toxicity of *Semen Strychni* through the circadian variation of cytochrome P450 3A11 metabolism, which helps to improve the efficacy of *Semen Strychni* by optimizing the administration time (114). Strychnine and brucine have similar tissue distribution characteristics, with the highest levels occurring in the kidney and the lowest levels found in the brain (115). Previous studies have found that soaking seeds in cow urine for 7 days followed by boiling them in milk for 3 h minimizes the amount of brucine and strychnine (116,117). In other research using these treatments, the greatest reductions in brucine and brucine levels were also observed in samples treated with milk and saline (118). It has also been shown that the content of toxic metabolites of *Strychnos* after boiling in milk is significantly lower compared with frying (119).

Antidote. Strychnine poisoning induces convulsions that lead to loss of airway muscle control, which can trigger respiratory arrest and eventual death. Current therapeutic options are scarce, requiring on-site medical intervention and placement of patients in a low-stimulation environment. Anticonvulsant and muscle relaxant medications demonstrate limited efficacy in cases of severe poisoning. Due to its high potency, accessibility and the absence of an effective antidote, strychnine presents a unique hazard in mass casualty incidents. To develop an anti-strychnine immunotherapeutic approach that can reduce or prevent strychnine-induced seizures, researchers synthesized a strychnine vaccine using the keyhole hemocyanin subunit (120).

Another study aimed to assess the protective effect of licorice against experimental renal injury induced by brucine (121). Rats were given brucine via intraperitoneal injection for 7 consecutive days, and the experimental groups received licorice extract during this period. The results indicated that licorice extract treatment significantly mitigated brucine-induced nephrotoxicity, along with reducing blood urea nitrogen and serum creatinine levels. Licorice alleviated brucine-induced nephrotoxicity by repairing the pathological imbalance of oxidative stress-achieved through inhibiting oxidative stress and mitochondria-mediated apoptosis. Notably, this renoprotective effect was accomplished, at least in part, by preventing the activation of STAT3 protein (121).

A separate investigation focused on exploring the neurotoxicity of brucine and its underlying mechanism (122). The results revealed that brucine significantly induced the death of neural-2A cells and primary astrocytes, as demonstrated by MTT assay and lactic dehydrogenase release detection. Transcriptome analysis revealed that the PPAR, NF-κB and apoptosis signaling pathways are involved in brucine-induced neurotoxicity. Specifically, brucine markedly inhibited PPARγ and promoted the phosphorylation of NF-κB. Furthermore, a PPARγ inhibitor exacerbated neurotoxicity, while an NF-κB inhibitor significantly reversed brucine-induced neurotoxicity. Additionally, TUNEL staining and western blot

confirmed that brucine notably induced neuronal apoptosis and triggered an increase in the Bax/Bcl2 ratio and cleaved caspase-3 levels. Molecular docking analysis indicated that brucine directly binds to caspase-3; notably, a caspase-3 inhibitor largely eliminated the neurotoxicity of brucine. Collectively, brucine induces neurotoxicity by activating the PPARγ/NF-κB/caspase-3-dependent apoptotic pathway, and these findings will provide a novel strategy for combating brucine-induced neurotoxicity (122).

Pretreatment methods for brucine and strychnine against toxicity. In recent years, a variety of pretreatment strategies have been investigated to improve the extraction efficiency or shorten the extraction time of brucine and strychnine in different samples. Appropriate pretreatment methods are not only beneficial to the separation, purification and concentration of target compounds, but also can remove impurities and interferences in the sample, reduce background noise or convert target substances into derivatives to achieve sensitive detection in subsequent analysis. These methods are as follows:

Liquid-liquid extraction (LLE) methods. In order to reduce the consumption of organic reagents and reduce the emulsification, the LLE process in microfluidic chips has been developed in recent years. In a previous study, a microfluidic LLE-UV method was developed for the analysis of strychnine and other alkaloids from Strychnine seeds (123). In that study, n-butyl acetate was optimized as the extraction solvent and the extraction efficiency >90%. In addition, microfluidic LLE has shown advantages in terms of time saving; in the aforementioned study, the results revealed that the extraction time was very short (only 25 sec) (123).

LPME methods. In order to improve extraction efficiency and mitigate the environmental impact due to the heavy use of toxic organic solvents, researchers have focused on simplifying and miniaturizing LLE methods (124). A notable advance in this field is the development of LPME, a novel sample pretreatment technique derived from the miniaturization of traditional LLE. This method effectively reduces the interference of impurities in complex matrices and facilitates the enrichment of target analytes, thereby improving detection sensitivity. A dispersive liquid-liquid microextraction (DLLME)-TLC method was developed for the detection of brucine and strychnine in blood samples. The extraction process was made using 100 µl chloroform and 1 ml methanol mixed with a 5 ml water sample. Following optimization, the recovery was between 82 and 94%. In addition, DLLME exhibited advantages in time-saving, low cost and environmental protection (125). Commonly used LPME methods for brucine and strychnine include DLLME and electric membrane extraction (EME). It was previously reported that brucine and strychnine were detected in human urine using the EME-HPLCDAD method (126). The ionic liquid was immobilized in the hollow fiber pore and used as a supporting liquid film. Following optimization, the whole extraction process takes only 5 min (126).

Analytical method. An HPLC-LTQ-Orbitrap mass spectrometry method was previously developed for the rapid analysis of brucine and strychnine in *Semen Strychni* seeds; in that study, the instrument was run in positive ionization mode for identification and quantification. Following optimization, a rapid comparison of compounds in crude and processed *Semen Strychni* was performed, resulting in efficient screening and characterization. In addition, HPLC-LTO-0rbitrap mass spectrometry exhibited high sensitivity; the detection limit of brucine and strychnine was shown to reach 60 mg/ml after optimization (127).

Capillary electrophoresis (CE) is an innovative liquid phase separation technique that uses capillaries as separation channels and high-voltage direct current electric fields as driving forces (128). The separation process is driven based on the differences in mobility and distribution properties of the components in the sample, enabling efficient and accurate analysis. Due to its high separation efficiency, CE is considered effective tool for the analysis of brucine and strychnine. A previously developed CE method for the separation of brucine and strychnine in Shufeng Dingtong pills was developed by using GO as the stationary phase of the separation. Following optimization, satisfactory separation results were obtained for all targets (129). In a previous study, brucine and strychnine in the strychnine samples were determined using the CE method. The analytical conditions were optimized in a NaH_2PO_4-Na_2HPO_4 buffer solution (pH 6.0) at 20 kV. Following optimization, the whole separation process took only 5 min in total (130).

5. Synopsis

Although *Semen Strychni* is a toxic herbal medicine, it has made notable contributions to the treatment of clinical diseases. Numerous studies have made continuous efforts to ensure the safety and efficacy of *Semen Strychni*. Among the components isolated and identified from *Semen Strychni*, apart from some bisindole alkaloids isolated from the stem bark or root, other compounds isolated from leaves and fruits, such as flavonoid glycosides, triterpenoids, sterols and organic acids (131) are also widely present in other plants. The main active ingredients of *Semen Strychni* are the total alkaloids, the content of which varies with species differences, geographical origin and processing methods, ranging from 1.5 to 5%, a notable consideration for clinical applications (132). Therefore, further research into the alkaloid components in the seeds of *Semen Strychni* is warranted. Analgesia is the primary clinical application of *Semen Strychni* in TCM; it has markedly greater analgesic effects than morphine (133). It also has notable antitumor potential, and its further study may be beneficial, due to the urgent need for highly effective anticancer drugs in clinical practice (134). Thus, the pharmacological effects of *Semen Strychni*, particularly the antitumor effects, remain the focus of research in this field. *Semen Strychni* can inhibit the proliferation of a variety of tumor cells, induce the apoptosis of tumor cells, can exert an anti-angiogenic effect, which can block the nutritional supply of tumor tissue and delay tumor progression, thus showing therapeutic potential.

Semen Strychni exerts therapeutic effects on a variety of malignant tumors, whereby brucine is the most potent component in *Semen Strychni*. The main mechanisms of brucine include the inhibition of tumor proliferation, the arrest of tumor cell growth cycle and antitumor angiogenesis. Through the synergistic action of multiple targets and multiple pathways, *Semen Strychni* can comprehensively correct the core molecular pathological abnormalities, such as the imbalance of tumor cell proliferation and apoptosis, inflammatory

microenvironment disorder, invasion and metastasis pathway activation and abnormal gene expression. *Semen Strychni* can exert multi-target and multi-pathway antitumor effects via the targeted regulation of the tumor molecular pathological network, which provides a solid molecular pathological basis for its clinical application in tumor treatment. In the future, the structure modification of the effective components of *Semen Strychni*, the construction of targeted delivery systems and the optimization of combination drug regimens can be further carried out, so as to improve its specificity for tumor molecular pathological targets while reducing drug toxicity. This may also provide more in-depth theoretical and experimental information for the development of novel antitumor TCM preparations based on *Semen Strychni* and promote its clinical standardized application. It also provides novel objectives and references for the study of the molecular mechanisms of the antitumor effects of TCM.

Herbal medicine poisoning caused by the misuse and wrong dosage of *Semen Strychni* is very common; thus, it should be used with caution in clinical practice (135). The toxic effects of *Semen Strychni* on the nervous, immune and other systems have been previously demonstrated. Research has indicated that brucine and strychnine are typical glycine postsynaptic membrane inhibitory antagonists, and their toxicological effects are mainly to stimulate the central nervous system (122). In order to mitigate its harmful effects and enhance its medicinal value, three main strategies have been adopted: i) Processing to achieve attenuation and synergistic effects; ii) combining with other herbs in prescription; iii) and developing new dosage forms. In the field of attenuation and synergism research, processing and other methods can improve the efficacy of toxicity reduction of *Semen Strychni*, which has good research potential and application prospects (136). It is used in combination with other herbs in the prescription, such as the combination of *Semen Strychni* and *Atractylodes rhizome* as aforementioned to achieve toxicity reduction. The first two strategies are relatively established, and the novel formulations also have the advantages of controlling drug concentration in blood, reducing adverse reactions and improving drug bioavailability. However, the novel formulations have not yet entered the clinical application or commercialization stage, and further experimental and clinical studies are warranted to support the methods.

The challenge of translating *in vitro* findings to *in vivo* and clinical settings is essentially a question of adaptation between standardized *in vitro* research and the complex *in vivo* and clinical environment. Future studies could optimize the experimental design, construct *in vivo* models closer to the characteristics of human diseases (such as human tumor xenograft models), use human primary tumor cells to carry out *in vitro* experiments, combine multi-omics technology to explore the cross-regulation of multi-molecules and multi-pathways, and strengthen the linkage analysis of *in vitro*, *in vivo* experiments and clinical sample data. These efforts will serve to gradually bridge the translational gap, thus improving the efficiency and reliability of the translation of basic research to clinical practice, so that the results of *in vitro* research can better serve the diagnosis and treatment of clinical diseases. AI algorithms (137,138) and big data mining (139) strategies can be combined to systematically screen key therapeutic targets (140), precisely predict the binding activity of active ingredients, and further elucidate the multi-target pharmacological characteristics and molecular mechanism of *Semen Strychni* against tumors.

6. Conclusion

The present study systematically reviewed the chemical constituents, pharmacological effects and toxicity characteristics of *Semen Strychni*. The aforementioned studies have shown that *Semen Strychni* is rich in active ingredients such as alkaloids and iridoid glycosides, and has shown significant pharmacological activities in the fields of antitumor, anti-inflammatory, anti-arrhythmia and analgesia. However, there are still numerous areas that warrant further research: The mechanisms of action and targets of its drug effect remain unclear, the risk of toxicity remains a bottleneck which restricts the wide clinical application of *Semen Strychni*, and there remains a substantial gap between the generation of experimental data *in vitro* and *in vivo* and its translation into practical applications. In the future, research may focus on attenuation and synergism, mechanistic analysis and research and the development of novel drug targets, relying on multi-omics and modern preparation technology to promote the safe and accurate application of *Semen Strychni* in the fields of analgesia, nerve repair and antitumor therapeutics.

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Availability of data and materials

Not applicable.

Authors' contributions

JM and YL conceived and designed the study. XD wrote the manuscript, DZ and XL created the figures and were

involved in the literature search. YL and JM critically revised the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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