Pharmacological Activities of Coumarin Compounds in Licorice: A Review

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Abstract
Licorice is a traditional medicine commonly used in China and many other countries. Over the last 50 years, the structure and pharmacological effects of coumarin compounds in licorice have been investigated. However, a comprehensive review of the literature summarizing current trends is currently lacking. Thus, the aim of the present review is to provide an up-to-date summary of the scientific literature regarding the pharmacological effects of coumarin compounds in licorice, thereby laying the foundation for further research and optimal utilization of licorice. We retrieved 111 articles on the coumarin components of licorice and their potential pharmacological effects, based on titles, keywords, and abstracts from databases (including PubMed and Web of Science). Glycyccoumarin, isoglycycoumarin, licoarylcoumarin, licopyranocoumarin, glycyrin, isotrifoliol, glycyrol, and glycyrurol have been investigated for their anticancer, hepatoprotective, antispasmodic, immunosuppressive, anti-inflammatory, and antibacterial properties, and use as therapeutic agents in metabolic syndrome, thereby demonstrating their potential for clinical applications. Future research should further explore the pharmacological mechanisms of action of coumarin compounds, including their antibacterial activities. Investigations into the pharmacological activities of different glycycoumarin isomers might open new research frontiers.

Keywords
licorice, coumarins, pharmacological activities, glycycoumarin, glycyrol

Received: February 11th, 2020; Accepted: August 4th, 2020.

Introduction
Licorice, or Gan-Cao in Chinese, is derived from the roots and rhizomes of Glycyrrhiza uralensis Fisch., G. inflata Bat., and G. glabra L. Licorice is an ancient Chinese ethnomedicine and its traditional benefits include tonifying the spleen and stomach, relieving pain, reducing phlegm, alleviating cough, and detoxification. Currently, licorice is used in many countries for the treatment of various digestive ailments (eg, stomach ulcers, hyperdipsia, flatulence, and colic), respiratory tract disorders (eg, coughs, sore throat, pneumonia, bronchitis, and bronchial asthma), fluid retention, low blood pressure, sexual debility, paralysis, rheumatism, psoriasis, malaria, jaundice, and certain viral infections. 

Coumarins are benzopyrone analogs that are secondary metabolites of many plant species, including those from the Clusiaceae, Umbelliferae, Rutaceae, and Leguminosae families. Coumarins such as novobiocin, coumermycin, and aflatoxin, have also been identified in bacteria and fungi. In addition, coumarins and their hybrids can be rationally designed and produced and their derivatives synthesized via Perkin condensation, Knoevenagel condensation, the Pechmann reaction, and metal-catalyzed cyclization. Based on their structural diversity, compounds in this family have been divided into various categories, including simple coumarins and polycyclic coumarins such as furanocoumarins, pyranocoumarins, and phenylcoumarins.

Coumarins and coumarin-based hybrids have demonstrated numerous biological properties, including anticancer, anti-inflammatory, antioxidant, antiviral, antimicrobial, antifungal, antitubercular, anticoagulant, antispasmodic, antihyperglycemic, antitubulin, immunosuppressive, hepatoprotective, and neuroprotective activities. Some mechanistic studies have also been performed. For example, coumarins have been evaluated as mitogen-activated and extracellular signal-regulated kinase inhibitors, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) inhibitors, and nucleotide excision repair inhibitors that interfere with cell growth, proliferation, differentiation, and other important cellular processes. Thus,
their activity has been associated with tumorigenesis.\textsuperscript{15} Coumarins and their derivatives also exhibit antimicrobial activities by blocking quorum-sensing signaling systems and inhibiting the formation of biofilms.\textsuperscript{25} The antioxidant action of some coumarins is dependent on the benzylic hydrogen atoms; the resonance involving these atoms promotes the release of hydrogen as a free radical, whereas the inductive effect of the benzene ring, oxygen, and nitrogen draws electrons to form a carbon-free radical, enhancing the stability of the molecule.\textsuperscript{37} The antiviral mechanisms of some coumarins involve either the inhibition of proteins essential for viral entry, replication, and infection, or the regulation of cellular pathways such as the Akt-mammalian target of rapamycin, NF-κB, and antioxidant pathways, which include nuclear factor erythroid 2-related factor 2 (Nrf2).

Licorice contains various naturally active compounds including flavonoids, triterpenoid saponins, coumarins, and phenolics. Of these, coumarins are one of the most important natural organic compounds.\textsuperscript{38-40} Since glycyrol and isoglycyrol were first separated from \textit{Glycyrrhiza} spp. in 1969,\textsuperscript{41} their structures and pharmacological effects, as well as those of other licorice-coumarin compounds, have been gradually elucidated. Coumarin compounds in licorice possess different structures and include simple coumarins (liqcoumarin\textsuperscript{38}), 3-arylcoumarins (glycycoumarin,\textsuperscript{42} isoglycycoumarin,\textsuperscript{43} licoarylcoumarin,\textsuperscript{44} 7,2′,4′-trihydroxy-5-methoxy-3-arylcoumarin,\textsuperscript{45} licycoplanocoumarin [also known as GU-7],\textsuperscript{46,47} glycyrin,\textsuperscript{48} and licofuranocoumarin\textsuperscript{49}], coumestans (isotrifoliol,\textsuperscript{49} glycyrol,\textsuperscript{41} isoglycyrol,\textsuperscript{41} neoglycyrol,\textsuperscript{50} glycyrol,\textsuperscript{51} gancaonin-F,\textsuperscript{52} hedysarimcoumestan B,\textsuperscript{45} hedysarimcoumestan E,\textsuperscript{45} and sophoracoumestan C\textsuperscript{53}], and 4-arylcoumarins (inflacoumarin A\textsuperscript{54}). Among these, glycycoumarin, isoglycycoumarin, licycoplanocoumarin, isotrifoliol, glycyrol, glycyrol, licoarylcoumarin, and glycyrin are the major coumarin components; their structures are presented in Figure 1.

Previously reported studies on the pharmacological effects of 8 coumarin compounds (glycycoumarin, isoglycycoumarin, licoarylcoumarin, licoparanocoumarin, glycyrin, isotrifoliol, glycyrol, and glycyrol) were identified using available online scientific databases such as PubMed and Web of Science, without a time restriction. The names of coumarin compounds in licorice, including “glycycoumarin” and “glycyrol,” were combined with words related to their pharmacological actions, including “anti-inflammatory” and “anticancer,” and searched in titles, keywords, and abstracts. The methods of biosynthesis, metabolic reactions, and coumarin products were not within the scope of this analysis. In addition, papers on compound medicines with an unclear chemical composition were excluded. Using this search strategy, 156 articles between 1964 and 2020 were identified, of which 45 were excluded. The pharmacological effects mentioned in the 111 articles included in this review are shown in Table 1. This review summarizes the findings of both in vivo and in vitro studies on coumarins from licorice.

\textbf{Anticancer Activities}

The anticancer activity of glycyrol was first reported in 2007,\textsuperscript{55} when it was demonstrated to dose-dependently decrease the viability of human gastric cancer cells (AGS and SNU638 cells). However, its mechanism of action was not determined at that time. Glycyrol was later found to induce apoptosis in human kidney epithelial 293 T cells (HEK 293 T cells) through

![Figure 1. Structures of 8 major coumarin compounds in licorice.](image-url)
endonuclease G. Glycyrol can significantly suppress the NF-κB-dependent transcriptional activity induced by phorbol ester (phorbol 12-myristate 13-acetate), as determined using luciferase reporter activity in HEK 293 T cells. Glycyrol also induces apoptosis by activating p53 through endonuclease G. Another study revealed that glycyrol induces apoptosis in human Jurkat cells through a membrane death receptor pathway that is independent of p53. This indicates that glycyrol induces the apoptosis of human Jurkat cells by NF-κB inhibition, S-phase arrest, caspase activation, and Fas enhancement, but not via either Bcl-2 or Bax proteins.

The anticancer activity of glycyrol, both in vitro and in vivo, was first evaluated in 2014, when it was found to induce cell death associated with apoptosis and autophagy, as evidenced by morphological changes in AGS and HCT-116 cells. In addition, glycyrol has been shown to suppress tumor growth in a nude mouse tumor xenograft model bearing HCT-116 cells.

Butyrate has been shown to exert anticancer activity by inducing apoptosis and inhibiting the growth of colon cancer cells. Lu et al. attempted to combine butyrate with glycyrol to reduce the proliferation of cancer cells. These results demonstrated that the combination greatly enhanced the apoptotic effect of butyrate owing to the benzofuranyl, isopentenyl, and furan groups of glycyrol.

Wang et al. showed that glycyrol exerts higher cytotoxicity than liquiritcoumarin, crotoliquiritin, ammopiptanoside A, glycyrin, hedysanincomestan B, glycyrrhisoflavone, licoisoflavone A, isolicoflavonol, licoflavonol, isoliquiritigenin, licochalcone, licoricone, and gabroly. Glycyrol exhibits a moderate antiproliferative effect with a half-maximal inhibitory concentration (IC50) of 11.46 µM for A549 cells and 7.38 µM for NCI-H292 cells, following treatment for 48 hours; however, the underlying mechanism for this effect was not determined.

Glycycomarin, another coumarin component extracted from licorice, was found to exert potent antitumor effects, as demonstrated through the induction of apoptosis in vitro (HepG2, Huh7, DU-145, and HCT-116 cells) and reduction of tumor size in vivo (male BALB/c athymic nude mice). The treatment of HepG2 cells with glycycomarin for 36 hours leads to a concentration-dependent increase in cell death.

ABT-737 is an inhibitor of the Bcl-2 family of proteins that leads to the disruption of mitochondrial membrane potential. The protective effects of glycycomarin inhibited ABT-737-mediated toxicity of platelets against liver cancer significantly in both cell culture and xenograft animal models.

The specific mechanisms underlying the anticancer activities of these coumarin compounds are shown in Table 2.

### Hepatoprotective Activities

Recent studies have used glycycomarin to ameliorate alcoholic liver disease, combat acetaminophen-induced acute liver injury (AILI), and prevent the development of nonalcoholic fatty liver disease (NAFLD).

Glycycomarin exerts a strong preventive effect against alcohol-induced hepatotoxicity in mouse models of chronic and acute alcohol-induced liver injury. However, a study found a clear decrease in steatosis induced by chronic alcohol exposure following co-treatment with glycycomarin and alcohol.

Another study demonstrated that glycycomarin (50 mg/kg) is effective in acetaminophen-induced hepatotoxicity in C57BL/6N mice. AILI is dose-dependently ameliorated following treatment with glycycomarin, as demonstrated by a progressive reduction in the serum levels of alanine aminotransferase. Moreover, glycycomarin is superior to N-acetylcysteine, a modified amino acid clinically used as the only standard treatment for AILI, in terms of effective dosage and therapeutic time window.

Two later studies revealed that glycycomarin could effectively prevent NAFLD by suppressing endoplasmic reticulum (ER) stress and inducing lipoapoptosis. In in vitro models, treatment with 10-40 μM glycycomarin was found to suppress apoptosis significantly in HepG2 cells; at 20 μM, and it was highly effective in preventing oleic acid/palmitic acid-induced lipid accumulation. In in vivo models, glycycomarin reverses the biochemical and pathological changes in methionine/choline-deficient diet-fed mice, such as a marked
Table 2. Summary of Studies Investigating the Anticancer Activities of Coumarin Compounds From Licorice.

| Property                  | Coumarin compound | Experimental subject                                      | Experimental mode | Mechanism of action                                                                 | Reference |
|---------------------------|-------------------|-----------------------------------------------------------|-------------------|-------------------------------------------------------------------------------------|-----------|
| Anticancer (gastric)      | Glycyrol          | AGS and SNU638 cells                                      | In vivo           | Not determined                                                                      | 55        |
| Apoptosis of tumor cells  | Glycyrol          | HEK 293 T cells                                           | In vitro          | Downregulation of NF-κB-dependent genes; inducing apoptosis through activation of p53 via endonuclease G | 56        |
| (kidney)                  |                   |                                                           |                   | Enhanced cleavage of procaspases-8, 9, and 3, and decreased caspase-3 activity       | 57        |
| Apoptosis of human Jurkat | Glycyrol          | Human Jurkat T lymphocytes                                | In vitro          | Induction of G0/G1 phase arrest by increasing p21; activation of JNK/p38 MAPKs and induction of caspase-dependent apoptosis, accompanied by AMPK activation | 58        |
| T cells                   |                   |                                                           |                   | Suppression of IAPs, combined with butyrate-induced mitochondrial pathway leading to the synergistic induction of cell death | 61        |
| Apoptosis of tumor cells  | Glycyrol          | Human gastric cancer AGS, human colon cancer HCT-116 cells | In vitro; in vivo | Induction of G0/G1 phase arrest by increasing p21; activation of JNK/p38 MAPKs and induction of caspase-dependent apoptosis, accompanied by AMPK activation | 58        |
| (gastric, kidney)         |                   |                                                           |                   | Induction of G0/G1 phase arrest by increasing p21; activation of JNK/p38 MAPKs and induction of caspase-dependent apoptosis, accompanied by AMPK activation | 58        |
| Anticancer (colon)        | Glycyrol          | Human colon cancer cells HCT-116, HT-29                  | In vitro          | Suppression of IAPs, combined with butyrate-induced mitochondrial pathway leading to the synergistic induction of cell death | 61        |
| Cytotoxicity              | Glycyrol          | A549 cells and NCI-H292 cells                             | In vitro          | Not determined                                                                      | 62        |
| Anticancer (liver)        | Glycycoumarin     | Human hepatocellular carcinoma cells                      | In vitro; in vivo | Binds to and inactivates oncogenic TOPK, leading to activation of the p53 pathway    | 63        |
|                           |                   | HepG2, Huh7, and human prostate cancer DU-145 cells; human colon cancer HCT-116 cells; male BALB/c athymic nude mice |                   | Inactivates the TOPK-survivin axis and inhibits de novo lipogenesis                  | 64        |

Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; IAPs, inhibitors of apoptosis proteins; JNK, c-Jun N-terminal kinase; MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TOPK, T-LAK cell-originated protein kinase.
increase in alanine transaminase, a key marker of liver injury, and the accompanying profound hepatic steatosis. In addition, glycyounarin leads to a reduction in body weight. In a recent study by the same group, glycyrol was also found to suppress fumonisin B1 (FB1)-induced ER stress and protect against apoptosis via the inactivation of inositol-requiring transmembrane kinase/endoribonuclease 1α (IRE1α). Glycyrol (10 µM) significantly reduces the apoptosis of AML12 cells following exposure to 300 µM FB1 for 48 hours. Furthermore, FB1-induced IRE1α phosphorylation and Bip upregulation are suppressed following treatment with glycyrol at this concentration for 24 hours ($P < 0.01$).

The specific mechanisms underlying the hepatoprotective activities of these coumarin compounds are presented in Table 3.

### Antispasmodic Activities

The antispasmodic activity of the coumarin compounds found in licorice can help control abdominal cramping, fecal urgency, and postprandial lower-abdominal discomfort associated with diarrhea. Glycycoumarin was found to inhibit contractions induced by various types of stimulants, including carbachol, potassium chloride, barium chloride, and A23187 (calcium ionophore III), with similar efficacy as papaverine, a representative antispasmodic drug targeting the smooth muscle. In addition, the antispasmodic potency of cultivated and wild licorice was found to be directly dependent on glycycoumarin content, according to a study published by Nagai et al. They also reported that boiled-water extracts from 4-year-old cultivated and wild licorice exerted relaxant activity on carbachol-induced contractions in mouse jejunum (median effective dose: 134 ± 21 and 134 ± 16 µg/mL, respectively). However, the mechanism of action underlying this activity was not determined.

Shaoyaogancao-tang, a formulation that contains powdered extracts of the roots and rhizomes of licorice, is prescribed to provide rapid relief of muscle cramps arising from different causes. Researchers found that a low intravenous dose (2.7 mmol/kg) of glycycoumarin (1 of 8 active constituents) attenuated tetanus-induced contractions in mouse jejunum (median effective dose: 134 ± 21 and 134 ± 16 µg/mL, respectively). However, the mechanism of action underlying this activity was not determined.

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### Therapeutic Activities Toward Metabolic Syndrome

Metabolic syndrome refers to a cluster of conditions that occur simultaneously and increase the risk of heart disease, stroke, and type 2 diabetes. Ligands, such as those targeting peroxisome proliferator-activated receptor (PPAR)-γ, are effective in metabolic syndrome, including type 2 diabetes. Glycycoumarin,
glycyrrin, dehydroglyasperin C (flavonoid), and dehydroglyasperin D (flavonoid) have been reported as PPAR-γ ligands. When these were mixed at the same concentrations found in licorice ethanolic extract, the effective PPAR-γ ligand-binding activity was found to be 90% of the extract-binding activity. Glycyrrin markedly reduces blood glucose levels in genetically diabetic KK-Ay mice. In addition, glycyrin exhibits significant PPAR-γ ligand-binding activity in vitro. However, after 4 days of feeding, the blood glucose levels of glycyrin-treated and pioglitazone-treated animals were markedly reduced relative to those in the control group. In an oral sucrose tolerance test, glycyrin and pioglitazone significantly inhibited the increase in blood glucose levels in mice after sucrose loading. The specific mechanisms underlying the therapeutic activities of these coumarin compounds on metabolic syndrome are shown in Table 5.

**Immunosuppressive and Anti-Inflammatory Activities**

Calcineurin (CN) is a protein phosphatase that plays an important role in immune regulation. Glycyrol (IC$_{50}$ = 84.6 µM) was found to dose-dependently inhibit CN activity in an enzymatic assay. At a nontoxic concentration, glycyrin markedly reduced the proliferation of murine splenic T lymphocytes induced by concanavalin A and the mixed lymphocyte reaction. The delayed hypersensitivity (DTH) of glycyrin-treated mice decreased in a dose-dependent manner, whereas graft survival (BALB/c mice with skin grafts from male donor C57BL/6 mice) increased by 59% compared with that of the control group ($P < 0.05$). Another study examined the interaction between glycyrin with calcineurin A (CNA) and demonstrated that glycyrin binding changes the secondary structure of CNA, which may inhibit CN activity.

Glycycoumarin has also been shown to induce autoimmune regulation and inflammatory responses. A study demonstrated that the anti-inflammatory effect of glycycoumarin is caused by the inhibition of nuclear factor-kappa B alpha (IkBα) phosphorylation. However, another study reported that the peroral administration of glycycoumarin is effective in slowing down collagen-induced arthritis in male DBA/1J mice, a model of rheumatoid arthritis, as it decreases serum inflammatory cytokine levels. Glycycoumarin reduces DTH, improves carbon clearance and decreases acetic acid-induced capillary permeability.

Glycycoumarin is another coumarin constituent that exerts anti-inflammatory activity. This compound inhibited prostaglandin E2 (PGE2) secretion by more than 80% at a concentration of 10 µM in RAW 264.7 murine macrophages.

Glycycoumarin and glycyrin are 2 of the main compounds in San’ao decoction (SAD), an extract formulation prescribed for the treatment of asthma. The ethyl acetate fraction of SAD has a dramatic effect on PPAR-γ activation and may have anti-inflammatory properties during various chronic
inflammatory processes. Glycycoumarin (5 µM) also exerted significant activity on PPAR-γ; however, this was found to be less than that of formononetin in the SAD ethyl acetate fraction. The specific mechanisms underlying the immunosuppressive and anti-inflammatory activities of these coumarin compounds are shown in Table 6.

Neuroprotective Activities

Licorice has been shown to possess protective effects against amyloid β (Aβ) oligomer-induced apoptosis.91 This study also demonstrated that glycycoumarin markedly reduced Aβ oligomer-induced neuronal death at concentrations of 10, 30, and 50 mM ($F_{[6, 35]} = 64.584, P < 0.001$).

Parkinson’s disease is a neurodegenerative disease characterized by the progressive death of dopaminergic neurons in the substantia nigra. Licopyranocoumarin and glycyrurol have demonstrated cytoprotective effects in neuronal cells. These compounds block 1-methyl-4-phenylpyridinium (MPP+) induced neuronal PC12D cell death and the loss of mitochondrial membrane potential, which are mediated by c-Jun N-terminal kinase.92

The specific mechanisms underlying the neuroprotective activities of these coumarin compounds are shown in Table 7.

Antimicrobial Activities

Glycycoumarin was first reported to exert activity against microorganisms including bacteria, yeast, and fungi, in 1988.93 Approximately, 13 years later, a study revealed that glycyrol, glycyrin, and glycycoumarin display antibacterial activity against *Streptococcus pyogenes*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and other bacteria of the upper respiratory tract.94 The minimum inhibitory concentrations (MICs) of these 3 compounds against microorganisms are listed in Table 8. A previous study showed that glycyrin has weak activity against *Helicobacter pylori*, similar to that reported for glycyrhetinic acid and liquiritigenin.95 Eerdunbayaer et al found that licoarylcoumarin and glycycoumarin have moderate antibacterial activity toward vancomycin-resistant *Enterococci*.96 Low MIC values (16 µg/mL) were found for *Enterococcus faecium* and *Enterococcus faecalis* via the liquid dilution method. However, the mechanisms underlying the antimicrobial activity were not elucidated in the above-mentioned studies.

In 2015, a study revealed that activity-guided compounds from *G. glabra* significantly decreased the virulence factor of *Acinetobacter baumannii*, including motility ($P < 0.05$), which regulates quorum sensing and the production of antioxidant enzymes.97 Glycyrin was also identified as a coumarin compound responsible for quorum quenching against *A. baumannii*. Another study found that glycyrin extracted from *G. glabra* possessed activity against *Bacillus subtilis* FtsZ (BsFtsZ) guanosine triphosphate (GTPase), with efficacy levels similar to those reported for the synthetic FtsZ inhibitor, Zantrin.
Only 1 in vivo experiment involving coumarin in licorice indicates that glycyrol may contribute to the development of a novel agent with antifungal activity against cutaneous candidiasis. The comparison of infected sites on the dorsal sides of treated and untreated mice showed that glycyrol treatment of the infected sites reduced colony-forming units by up to 60% and 85.5% at 20 and 40 µg/mouse of glycyrol, respectively (P < 0.01). To the best of my knowledge, coumarins from licorice are safe for human consumption, but a thorough analysis of their potential cytotoxicity has not been performed to date. Two of the studies mentioned above have reported underlying mechanisms of action of coumarin compounds, whereas the other studies have not demonstrated any mechanism, as summarized in Table 9.

### Antioxidant Activities

A previous study has indicated that some coumarin compounds possess the significant antioxidant ability for scavenging peroxyl radicals in experiments involving reactive oxygen species.

The first study to report the antioxidant activity of glycycomarin derived from licorice also elucidated its antimicrobial activity. However, the data indicate that the peroxidase activity of glycycomarin is close to that of the blank control, suggesting that...
glycycoumarin does not exert significant peroxidase activity. Nevertheless, another study revealed that glycycoumarin has strong scavenging activity against 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)+ radicals and could inhibit lipid peroxidation in rat liver microsomes relative to ascorbic acid. The specific mechanisms underlying the antioxidant activities of these coumarin compounds are shown in Table 10.

**Antiviral Activities**

Many coumarin compounds such as dicamphanoyl-khellactone and calanolide A have been reported to exhibit anti-human immunodeficiency virus (HIV) effects via unique mechanisms dependent on the different stages of HIV replication.

A study published in 1988 revealed the anti-HIV activity of licopyranocoumarin and glycycoumarin. These compounds showed inhibitory activity against giant cell formation at 20 µg/mL without any observable cytotoxicity. To date, however, there has been no other report of licorice-based coumarins exerting this effect. Nonetheless, glycycoumarin was reported to possess activity against the hepatitis C virus (HCV), with a half-maximal effective concentration of 15.5 ± 0.8 mg/mL. In addition, a subsequent report revealed that glycycoumarin, glycyrin, and glycyrol are anti-HCV constituents, with IC_{50} values of 8.8, 7.2, and 4.6 mg/mL, respectively.

Neuraminidase (NA) is an enzyme involved in the release of progeny virus from infected cells and is known to cleave sugars that bind to mature viral particles. Glycyrol, which has a 5-membered closed B ring, demonstrated high (IC_{50} = 3.1 µM) activity against NA.

The specific mechanisms underlying the antiviral activities of these coumarin compounds are shown in Table 11.

**Antiplatelet and Antithrombotic Activities**

Through in vitro experiments, licopyranocoumarin was found to inhibit progressively the aggregation of platelets induced by 0.01 U/mL of thrombin. Furthermore, licopyranocoumarin can suppress the phosphorylation of 40 and 20 kDa proteins, production of inositol 1,4,5-trisphosphate, increase intracellular calcium ions, and activity of phosphodiesterase in platelets. Isotrifoliol is reported to significantly prolong thrombin time (TT) with a good dose-effect relationship (dosage = 2.5 µg/mL, TT prolongation = 10.35 ± 2.38%).

The specific mechanisms underlying these activities of coumarin compounds are shown in Table 12.

**Other Activities**

The specific mechanisms underlying other activities of coumarin compounds are described below and summarized in Table 13.

**Estrogenic activity.** Glycycoumarin is an estrogen agonist that can stimulate the expression of estrogen-regulated genes; however, the potency and efficacy of glycycoumarin in stimulating the expression of such genes are lower than those of methoxychalcone and vestitol.

**Protecting against acute lung injury.** Paraquat (PQ) is one of the most widely used herbicides in developing countries and a highly toxic compound capable of causing acute lung injury.

### Table 7. Summary of Studies Investigating the Neuroprotective Activities of Coumarin Compounds From Licorice.

| Property                                      | Coumarin compound | Experimental subject | Experimental mode | Mechanism of action                                                                 | Reference |
|-----------------------------------------------|-------------------|----------------------|-------------------|-------------------------------------------------------------------------------------|-----------|
| Amyloid β oligomer-induced apoptosis          | Glycycoumarin     | Sprague-Dawley rat embryos | In vivo          | Attenuation of amyloid β oligomer-induced activation of caspase-3, but not caspases-8 and 9 | 91        |
| Cytoprotective treatment for Parkinson's disease | Licopyranocoumarin and glycryrrol | PC12 pheochromocytoma cells | In vitro          | Inhibition of reactive oxygen species generation and the subsequent suppression of MPP+-induced JNK activation; attenuation of MPP+-induced neuronal PC12D cell death | 92        |

### Table 8. Minimum Inhibitory Concentrations (MICs; µg/mL) of 3 Licorice Constituents Tested Against Microorganisms.

| Substance | Glycycoumarin | Glycyrol | Glycryrrol |
|-----------|---------------|----------|------------|
| Bacteria  |
| Streptococcus mutans | 12.5 | - | - |
| Staphylococcus aureus | 3.13 | - | - |
| Bacillus subtilis | 6.25 | - | - |
| Streptococcus pyogenes | 25 | 50 | 50 |
| Haemophilus influenzae | 25 | 100 | 20 |
| Moraxella catarrhalis | 100 | >100 | 50 |
| Yeasts |
| Saccharomyces cerevisiae | 25 | - | - |
| Candida utilis | 50 | - | - |
| Pichia nankawae | 25 | - | - |

Note: “-“ Denotes not determined.
Glycyrol has been shown to decrease the accumulation of PQ in vivo in Kunming mice (oral bioavailability = 90.8%, drug-likeness >0.1). In addition, glycyrol has been used to inhibit PQ-induced cell death associated with the cytochrome P450 (CYP450) and Nrf2 pathways in vitro.

Use as a selective probe.

CYP2A6 is an important hepatic phase I detoxifying enzyme with a polymorphism that may be related to smoking and hepatomas. Coumarin compounds have been used as probe substrates for CYP2A6 and are subsequently metabolized to 7-hydroxycoumarin. CYP2A6 was identified as the major enzyme involved in the metabolism of isoglycycoumarin, and the catalytic activity of CYP2A6 can be determined by its hydroxylation of isoglycycoumarin to generate licopyranocoumarin.

Discussion

Licorice has been used since ancient times as a common medicinal ingredient and is favored owing to its beneficial activities in the treatment of numerous diseases. To date, the chemistry and pharmacology of licorice have been investigated in many studies in different countries. Although the content of coumarin compounds in licorice is relatively low, an increasing number of researchers are now focusing on their pharmacological activities.

The studies discussed herein have investigated the activities of some coumarin compounds present in licorice such as glycycoumarin, isoglycycoumarin, glycyrol, glycyrin, licopyranocoumarin, licoarylcoumarin, glycyrurol, and Gu-7. These compounds have been reported to exhibit anticancer, hepatoprotective, antispasmodic, immunosuppressive, anti-inflammatory, and antimicrobial activities, as well as therapeutic effects on metabolic syndrome components. In recent years, coumarins in licorice have drawn significant attention owing to their potential therapeutic applications in cancer, hepatic disease, and viral infections.

While performing this literature review, in some cases, the structure presented as glycyrol was found to be |

| Property                      | Coumarin compound      | Experimental subject                                                                 | Experimental mode | Mechanism of action                                                                 | References |
|-------------------------------|------------------------|--------------------------------------------------------------------------------------|-------------------|--------------------------------------------------------------------------------------|------------|
| Antimicrobial activity        | Glycycoumarin          | Microorganisms listed in Table 8                                                    | In vitro          | -                                                                                    | 93         |
| Antibacterial activity against upper respiratory tract pathogens | Glycyrol, glycyrin, and glycycoumarin | *Streptococcus pyogenes, Haemophilus influenza,* and *Moraxella catarrhalis*                  | In vitro          | -                                                                                    | 94         |
| Inhibition of *Helicobacter pylori* activity | Glycyrin               | *H. pylori* ATCC 43504, ATCC 43526, *H. pylori* ZLM 1007, ZLM 1200, clarithromycin-resistant *H. pylori* GP98 | In vitro          | -                                                                                    | 95         |
| Inhibition of *Enterococcus* species activity | Licoarylcoumarin and glycycoumarin | *E. faecalis* FN-1 and *E. faecalis* NCTC 12201                                      | In vitro          | -                                                                                    | 96         |
| Attenuation of quorum sensing-mediated virulence of *Acinetobacter baumannii* | Glycyrin               | *Acinetobacter baumannii* strains and *Acinetobacter tumefaciens* A136                 | In vitro          | Reduced expression of the autoinducer synthase gene, *abaI,* and decreased production (92%) of 3-OH-C12-HSL | 97         |
| Inhibition of *Bacillus subtilis* FtsZ (FtsZ) GTPase activity | Glycyrin               | Plasmid DNA encoding BsFtsZ, EcFtsZ, and the BsFtsZ V307R mutant protein              | In vitro          | Binds to the cleft on BsFtsZ as an uncompetitive FtsZ inhibitor, PC190723; anti-BsFtsZ inhibitory activity | 98         |
| Inhibition of *Candida albicans* activity | Glycyrol               | BALB/c female mice; *C. albicans* yeast cells                                       | In vivo and in vitro | Damages the cell wall to enhance the response of *C. albicans* to fluconazole        | 99         |

Abbreviation: 3-OH-C12-HSL, N-(3-hydroxydodecanoyl)-L-homoserine lactone.

"-" Denotes not determined.
neoglycyrol,\textsuperscript{58,59,86-89} as shown in Figure 2. Glycyrrol and neoglycyrol are isomers; glycyrrol contains 5′-hydroxy and 7′-methoxy in its structure, whereas neoglycyrol is the converse. Therefore, the structure of glycyrrol in those studies should be verified. Additionally, further studies are needed to confirm whether neoglycyrol exerts similar pharmacological effects as glycyrrol. Since isoglycycomarin and glycycoumarin are isomers, they may display similar pharmacological effects, which warrant further study.

Similarly, isotrifoliol was misidentified as glycycoumarin in 2 studies.\textsuperscript{119,120} Its structure is shown in Figure 1. The structures of these 2 compounds are different; glycycoumarin is chemically 3-arylcoumarin, while isotrifoliol is a coumestan. Isotrifoliol has shown significant anti-thrombotic activity, but the mechanisms underlying this activity were not elucidated. Another study showed that isotrifoliol isolated from soy leaves exhibits anti-inflammatory effects, specifically inhibiting lipopolysaccharide (LPS)-induced NF-κB and mitogen-activated protein kinase activation by attenuating Toll-like receptor (TLR) signaling in macrophages.\textsuperscript{121} Since both licorice and soy belong to the Leguminosae family, it could be inferred that isotrifoliol is present in many plants belonging to this family. Therefore, isotrifoliol could be extracted from more plants from the

| Table 10. Summary of Studies Investigating the Antioxidant Activities of Coumarin Compounds From Licorice. |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Property                                      | Coumarin compound                           | Experimental subject                          | Experimental mode   | Mechanism of action                           | References |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Antioxidant activity in lard                  | Glycycoumarin                                | 1 g of mixed lard                             | In vitro          | No significant activity                        | 93          |
| Scavenging activity                           | Glycycoumarin                                | RAW 264.7 murine macrophages                  | In vitro          | Exerts protective effects by chelating metals or altering iron redox chemistry | 101         |

| Table 11. Summary of Studies Investigating the Antiviral Activity of Coumarin Compounds From Licorice. |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Property                                      | Coumarin compound                           | Experimental subject                          | Experimental mode   | Mechanism of action                           | References |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Anti-HIV activity                             | Lacopyranocoumarin; glycycoumarin             | Human lymphoblastic leukemia cell line; OKM-3T | In vitro          | Not determined                                | 46          |
| Inhibition of hepatitis C viral replication   | Glycycoumarin                                | Human hepatoma cell line; Huh7                | In vitro          | Decreased translation of the HCV nonstructural protein, NS5A, from the HCV replicon | 104         |
| Anti-HCV                                      | Glycycoumarin; glycuryl; glycyrin             | Huh 7.5 cells                                 | In vitro          | Inhibition of HCV ribonucleic acid replication and HCV protein synthesis to produce HCV infectious particles | 105         |
| Inhibition of neuraminidase activity         | Glycuryl                                     | rvH1N1 (A/Bervig-Mission/1/18) neuraminidase  | In vitro          | A 5-membered ring between C-4 and C-20 in coumestan is critical for neuraminidase inhibition | 107         |

| Table 12. Summary of Studies Investigating the Anticoagulative Activities of Coumarin Compounds in Licorice. |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Property                                      | Coumarin compound                           | Experimental subject                          | Experimental mode   | Mechanism of action                           | References |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| Antiplatelet activity                        | Licopyranocoumarin                           | Blood obtained from healthy volunteers       | In vitro          | Inhibition of platelet aggregation by increased intracellular cyclic adenosine monophosphate concentration | 47          |
| Antithrombotic activity                      | Isotrifoliol                                 | Blood obtained from rabbits’ common carotid artery | In vitro          | -                                             | 51          |

Abbreviations: HCV, hepatitis C virus; HIV, human immunodeficiency virus.

\textsuperscript{“-”} Denotes not determined.
Glycycoumarin, glycyrol, and glycyrin have been utilized for their anticancer, hepatoprotective, antispasmodic, and antibacterial properties; however, further studies are required to understand their antibacterial mechanism(s) of action. I anticipate that these future studies will contribute to the development and utilization of licorice resources.

**Acknowledgments**

I would like to express my gratitude to all those who assisted in the preparation of this review. My deepest gratitude goes first to Professor Chunsheng Liu, my supervisor during my Master's degree, who helped me to identify this review topic. Second, I would like to express my heartfelt gratitude to the Biomedicine College of Beijing City University for providing the resources to complete this review. Finally, I would like to thank “Editage” for their excellent assistance in language editing.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.

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**Table 13. Summary of Studies Investigating Other Activities of Coumarin Compounds in Licorice.**

| Property                          | Coumarin compound | Experimental subject | Experimental mode             | Mechanism of action                                                                 | Reference |
|----------------------------------|-------------------|----------------------|-------------------------------|-----------------------------------------------------------------------------------|-----------|
| Estrogenic activity              | Glycycoumarin     | MCF-7 cells          | In vitro                      | Binds with approximately equal affinity to ERα and ERβ (beta/alpha ratio, approximately 1); increased expression of PgR and GREB1 | 109       |
| Acute lung injury                | Glycyrol          | Human type II alveolar adenocarcinoma basal epithelial, A549 cells; HepG2 cells; KM mice | In vitro; in vivo | Increased levels of CYP3A4, Nrf2, and its downstream detoxification genes GR, GPX, and NQO1 | 110       |
| Selective probe                  | Isoglycycoumarin  | Human CYP2A6 cells; recombinant CYP2A6; human liver microsomes | In vitro | Cyclize isoprenyl group of isoglycycoumarin entering the active cavity of CYP2A6 | 111       |

Abbreviations: ER, estrogen receptor; GPX, glutathione peroxidase; GR, glutathione reductase; GREB1, growth regulation by estrogen in breast cancer 1; KM, Kun Ming; NQO1, NAD(P)H quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; PgR, progesterone receptor.
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