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Diosgenin: an important natural pharmaceutical active ingredient

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Abstract

Diosgenin is a steroidal saponin isolated from dioscorea opposita, which has been proven to have anti-cancer, anti-inflammatory, anti-thrombotic and immune system modulating effects in a large number of pharmacological tests. Diosgenin is also one of the important raw materials for the synthesis of steroidal drugs, and structural modification of diosgenin to obtain diosgenin derivatives can not only improve the solubility and dissolution, but also enhance their pharmacological effects. This paper reviews the synthetic reactions, pharmacological effects of diosgenin and its derivatives, aiming to provide detailed information for the further development and application of diosgenin, which is important to improve the utilization value of diosgenin.

Keywords: diosgenin; anti-cancer; anti-inflammatory; anti-thrombotic.

Practical Application: An important natural pharmaceutical active ingredient.

1 Introduction

Diosgenin (Dio) is a steroidal saponin isolated from *Dioscorea nipponica Makino* (Chinese Pharmacopoeia Commission, 2020), an endemic dioscorea plant in China, commonly known as dioscorea, which is known as (3β,25R)-spirost-5-en-3-ol in IUPAC, CAS No.:512-04-9, is a spirostanol glycosides, obtained by hydrolysis and extraction from raw material of *Sichuan Dioscorea* or *Huang Jiang*. Several studies have shown that diosgenin have a variety of biological activities, such as anti-bacterial (Cong et al., 2020), anti-inflammatory, anti-cardiovascular disease (Chen et al., 2015), hypoglycemic, and can be used as an effective antioxidant (Jesus et al., 2016); at the same time, diosgenin can act on cells to inhibit the proliferation and induce apoptosis of many cancer cells (Digbeu et al., 2013; Leonel et al.,2006), such as hepatocellular carcinoma, breast cancer, gastric cell carcinoma, and prostate cancer; in addition, it also have the ability to delay aging, anti-pulmonary fibrosis (Sun et al., 2019), immunomodulation, anti-hyperthyroidism (Wang et al., 2016), anti-platelet aggregation and hypolipidemic effects. Diosgenin also have a wide range of applications in pharmaceutical, functional food and skin care industries, especially the pharmaceutical industry uses diosgenin as the main precursor compounds to make steroid drugs (Chen et al., 2015). In this paper, we review the progress of domestic and international research in recent years regarding the synthesis of steroidal drugs, pharmacological effects and related mechanisms in anti-tumor, immunomodulatory and anti-inflammatory and anti-thrombotic aspects.

2 Synthesis of diosgenin derivatives

Diosgenin is a white needle-like crystal or light powder, as the active ingredient of Chinese medicine steroidal saponin, widely existed in legumes and dioscorea. It is a precursor of many steroidal drugs synthesis, an important raw material for artificial synthesis of steroid hormones and steroidal contraceptives. It has good anti-tumor, anti-inflammatory, hypolipidemic and other effects, also has a certain neuroimmune and behavioral improvement effects on neurological diseases such as Alzheimer's disease (AD) (Qi et al., 2019; Zhang et al., 2019).

2.1 Glucocorticoids

The glucocorticoid class of drugs can be synthesized using diosgenin as raw material (Herráiz, 2017). Commonly, prednisone, prednisolone, dexamethasone, betamethasone, methylprednisolone, and hydrocortisone are synthesized from diosgenin, and all of them are synthesized by a combination of chemical and biological (fermentation) methods.

2.2 Diosgenin derivatives

Diosgenin meta-carbamates

(Yang et al., 2020) synthesized a series of diosgeninogen carbamate derivatives from diosgenin and tested them for anti-inflammatory and anti-oxidant activities, and the results showed that among all the synthesized carbamate derivatives, compound (22*R*,25*R*)-3β-(*N*-benzylcarboxylate)-5-en-20α-spirostan is the most valuable derivative for anti-inflammatory and anti-AD activity. The synthetic pathway is as follows: diosgenin is the first reacted with p-nitrophenyl chloroformate to form intermediate S1 with a carbonyl group, and then compound S1 is converted to this compound using amine, pyridine and dichloromethane (DCM) at room temperature. The synthetic pathway is shown in Figure 1.

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Amino acid derivatives of diosgenin

(Huang et al., 2017) synthesized a series of diosgenin derivatives from diosgenin in order to develop new anti-cancer and anti-inflammatory drugs. Among them, the compound 6-aminohexanoic acid diosgenyl ester, synthesized by the reaction of 6-aminohexanoic acid with $(Boc)_2O$ in a mixed acetone-water solvent in the presence of triethylamine (Figure 2 for the synthetic route), showed the highest anti-cancer activity and was considered as a promising new anti-cancer drug. In addition, the compound diosgenyl salicylate conjugates, obtained by the pyridine-catalyzed esterification of diosgenin with 2-acetoxybenzoyl chloride, showed the highest anti-inflammatory activity.

(Cai et al., 2019) improved the solubility and physicochemical properties of diosgenin by introducing different amino acids at the C-3 position of diosgenin, due to the neuroprotective effect of amino acids, and a series of amino acid derivatives of diosgenin were synthesized, which were tested to show that most of the derivatives were better than the raw material, with 3β-(L-isoleucine)-diosgenin having neuro dual role of neuroprotection and angiogenesis. The synthetic pathway is shown in Figure 3.

Michalak et al. (2020) also synthesized a series of amino acid derivatives such as the introduction of amino acids at the C-3 position, among which (25*R*)-5α-spirostan-3β-yl L-serinate hydrochloride, (25*R*)-5α-spirostan-3β-yl L-glutamate hydrochloride, (25*R*)-spirost-5-en-3β-yl (*E*)-3-(3,4-dihydroxyphenyl)acrylate, three derivatives with the best *in vitro* activities such as anti-tumor and immunomodulation, can be used as potential lead compounds for further development and research of novel anti-cancer and immunomodulatory agents. The synthetic pathways are shown in Figure 4.

2.3 Dihydrodiosgenin

(Shen et al., 2018) used the anti-inflammatory activity of diosgenin to treat acute pancreatitis (AP), and due to its toxicity and side effects, dihydrodiosgenin, another parent glycoside of diosgenin, was synthesized by opening the spiroacetal ring of diosgenin (Figure 5 for the reaction pathway). It was shown that dihydrodiosgenin, after the opening of diosgenin and its spiroacetal ring, significantly reduced the activation of oxymethane induced necrotic cell death pathway in isolated pancreatic follicles, and

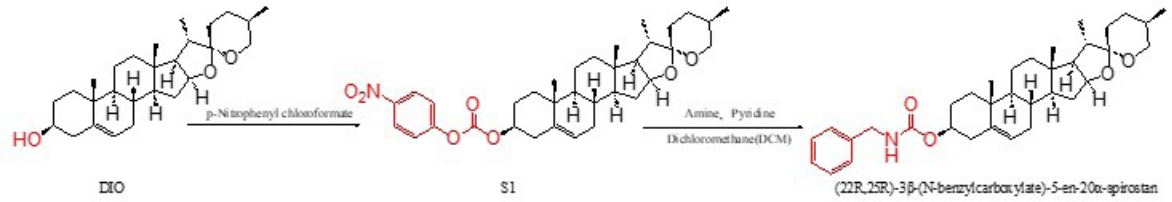

Figure 1. The synthetic pathway of (22*R*,25*R*)-3β-(*N*-benzylcarboxylate)-5-en-20α-spirostan.

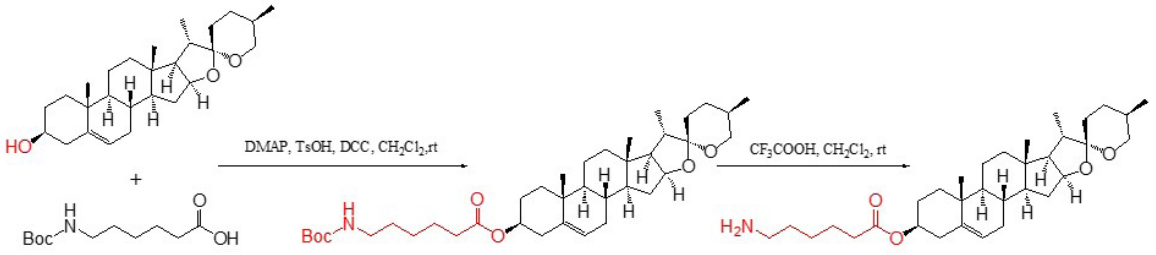

Figure 2. The synthetic pathway of 6-aminohexanoic acid diosgenyl ester.

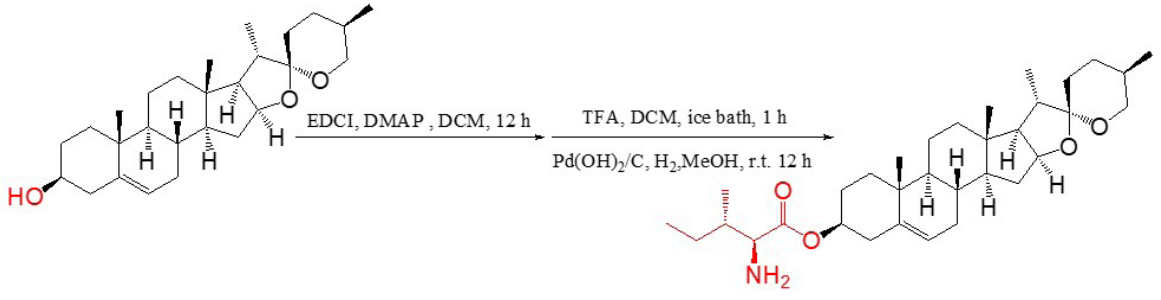

Figure 3. The synthetic pathway of 3β-(L-isoleucine)-diosgenin.

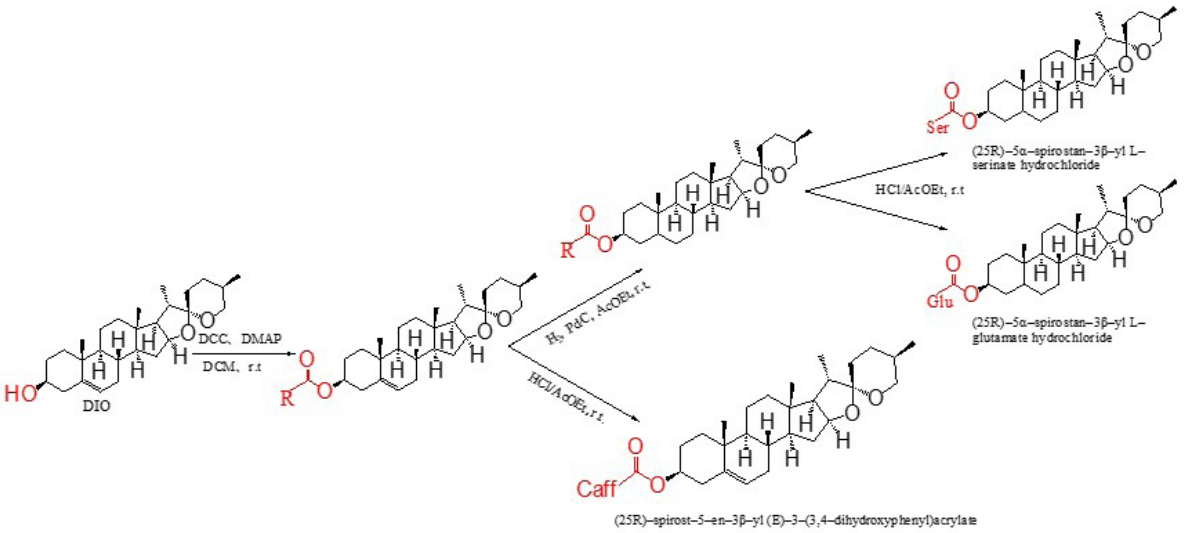

Figure 4. The synthetic pathway of (25*R*)–5α–spirostan–3β–yl L–serinate hydrochloride, (25*R*)–5α–spirostan–3β–yl L–glutamate hydrochloride, (25*R*)–spirost–5–en–3β–yl (*E*)–3–(3,4–dihydroxyphenyl)acrylate.

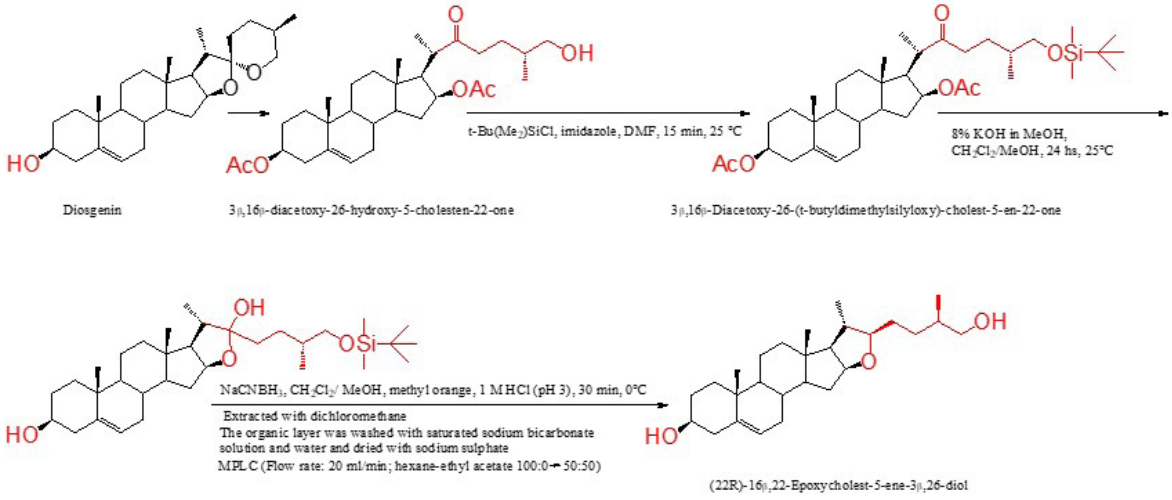

Figure 5. The synthetic pathway of dihydrodiosgenin.

dihydrodiosgenin showed a more pronounced protective effect compared with diosgenin at all tested concentrations, and no toxic effects were observed.

3 Pharmacological effects of diosgenin

Diosgenin, commonly known as "saponin", is a natural steroidal saponin, and numerous studies have shown that diosgenin has a good anti-tumor activity to block tumor progression, inhibit tumor metastasis, and improve the survival rate of tumor patients. Currently, cancer remains a serious threat to human health and death (Torre et al., 2016). Cancer is usually detected at an advanced stage and in most cases, the primary cancer has metastasized to other sites (Zeeshan & Mutahir, 2017). Clinically, many patients with malignant tumors already have metastases at the time of diagnosis. Therefore, there is a need to focus on the prevention and inhibition of tumor metastasis. Despite the availability of semi-synthetic and synthetic anti-cancer drugs in clinical use, the therapeutic advantages are limited due to the chemotherapy resistance and highly toxic side effects they produce. In contrast, natural products are a good source of compounds with novel chemical structures, effective and less toxic side effects (Newman & Cragg, 2016; Shanmugam et al., 2017). Diosgenin act through multiple mechanisms and have significant inhibitory effects on tumor cells both *in vivo* and *in vitro*, which have the prospect of in depth research and development value.

3.1 Anti-tumor

Diosgenin have proapoptotic and anti-cancer properties against various cancers *in vitro* and *in vivo*. *In vitro*, diosgenin can effectively inhibit the proliferation of various cancer cells, suppress inflammation and induce apoptosis. (Wang et al., 2018a) used cellular metabolomics to confirm that diosgenin

inhibit proliferation, induce apoptosis, and suppress invasion and metastasis, while providing its anti-tumor mechanism at the cellular level and metabolic pathway *in vitro*. In chronic myeloid leukemia cells, diosgenin metabolites produce reactive oxygen species (ROS), induce autophagy to inhibit mammalian target of rapamycin (mTOR) signaling pathway and cause cell death (Jiang et al., 2016). In addition to inhibiting cancer cell proliferation *in vitro*, several studies have demonstrated that diosgenin can selectively inhibit tumor cell growth, cause cell morphological changes and DNA fragmentation, and exert anti-tumor effects by inducing apoptosis in animal cancer models *in vivo*. Diosgenin and thymoquinone (TQ) alone or synergistically acted significantly to inhibit tumor growth and induce apoptosis in a mouse xenograft (Das et al., 2012) model. In the *N*-nitroso-*N*-methylurea (NMU) induced rat breast cancer (Jagadeesan et al., 2013) model, diosgenin has also been shown to inhibit the proliferation of Michigan Cancer Foundation-7 (MCF-7) cells in dose dependent manner. Thus, *in vitro* and *in vivo*, diosgenin can modulate multiple targets and inhibit tumor growth. On the downside, the poor solubility and low bioavailability of diosgenin in organic solvents greatly hinder its conversion into a therapeutic compound. Therefore, further trials are needed to evaluate its potential as a preventive or therapeutic anti-cancer agent.

Effects on breast cancer

Breast cancer is a common malignant tumor in women and its incidence is increasing year by year. Age factors can affect the occurrence of breast cancer and the disease is becoming younger with the influence of poor lifestyle in younger age groups. The results of numerous studies have shown that diosgenin has a significant inhibitory activity against breast cancer and induces apoptosis. Diosgenin could effectively inhibit the proliferation, invasion and migration of cancer cells (Figure 6). In human breast cancer cell line MCF-7 experiment (Li et al., 2019), diosgenin could significantly inhibit the proliferation of breast cancer cell line MCF-7 by regulating the methylation status and activating microRNA-145 (miR-145), and the treatment was safe and effective. Also, the migration ability of MCF-7 cells was reduced with increasing concentration of diosgenin. Another study reported that diosgenin was shown to be sensitive to the

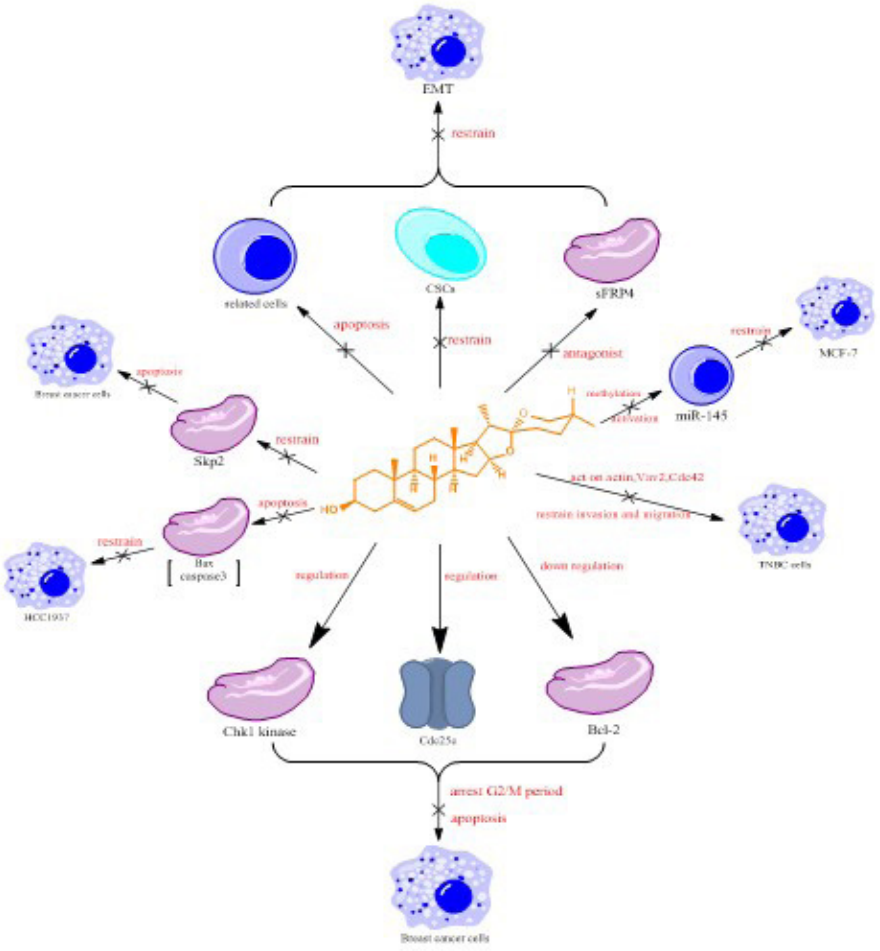

Figure 6. Pharmacological effects of diosgenin on the production of breast cancer cells, "↠" indicates that the former has a negative effect on the latter, producing a negative impact.

triple-negative breast cancer cell line HCC1937 (Deng et al., 2017), inhibiting proliferation in dose and time dependent manner, reducing invasiveness, and inducing apoptosis of Bcl-2 associated X protein (Bax), caspase3 protein. S-phase kinase-associated protein 2 (Skp2) is a key oncoprotein in human breast cancer, and inactivation of Skp2 may be a new way to combat breast cancer. A study showed that diosgenin inhibited the expression of Skp2 in breast cancer cells (Liu et al., 2020b), leading to reducing cell viability and motility and induction of apoptosis. It was also found to have an inhibitory effect on cell viability and promote apoptosis, and also reduce the invasion of breast cancer cells. In addition, diosgenin inhibited the invasion and migration of triple-negative breast cancer cells with inhibition of actin polymerization, vav guanine nucleotide exchange factor 2 (Vav2) phosphorylation, and cell division cycle protein 42 (Cdc42) activation. These proteins have been shown to be involved in the initiation of metastatic potential of cancer cells (He et al., 2014a). Also, diosgenin has been reported to lead to attenuation of epithelial-mesenchymal transition (EMT) and invasion in breast cancer through induction of apoptosis and inhibition of CSCs-related phenotypes by Wnt antagonists targeting the connexin signaling pathway of secreted frizzled related protein 4 (sFRP4) (Bhuvanalakshmi et al., 2017). From this, we can observe that diosgenin may work against breast cancer cells through invasive and migratory potential. In addition, diosgenin may inhibit breast cancer cell proliferation by inducing cell cycle arrest. A study found that diosgenin has caused G2/M phase arrest and mediated apoptosis in human breast cancer cell lines by regulating checkpoint kinase 1 (Chk1), the Cdc25c pathway, downregulating B-cell lymphoma-2 (Bcl-2) protein (Liao et al., 2019).

Effects on liver cancer

Hepatocellular carcinoma (HCC) is one of the most serious cancers with rapid disease progression and high malignancy, and the incidence of hepatocellular carcinoma has been slowly increasing in recent years. Although surgical resection is still the best option, the early diagnosis rate is not high, and some of the patients are already in the middle and late stages when they are diagnosed, with metastatic tumors and lost surgical opportunities. It was found that diosgenin have a preventive and delaying effect on the formation of liver cancer, inhibiting cell growth (Figure 7). It is well known that cell cycle dysregulation is a hallmark of tumor cells. Diosgenin can induce apoptosis through cell cycle arrest at different stages of hepatocellular carcinoma. In Bel-7402, SMMC-7721 and HepG2 human hepatocellular carcinoma cells (Li et al., 2015) studies, it was shown that diosgenin have a strong growth inhibitory effect and induce G2/M cell cycle arrest and apoptosis through upregulation of p21 and p27 proteins and activation of cystathionin. Similarly, in a study with HepG2 and SMMC-7721 cells (Yu et al., 2018), diosgenin promoted DEAD box polypeptide 3 (DDX3) expression, decreased cell cycle protein D1 levels and induced

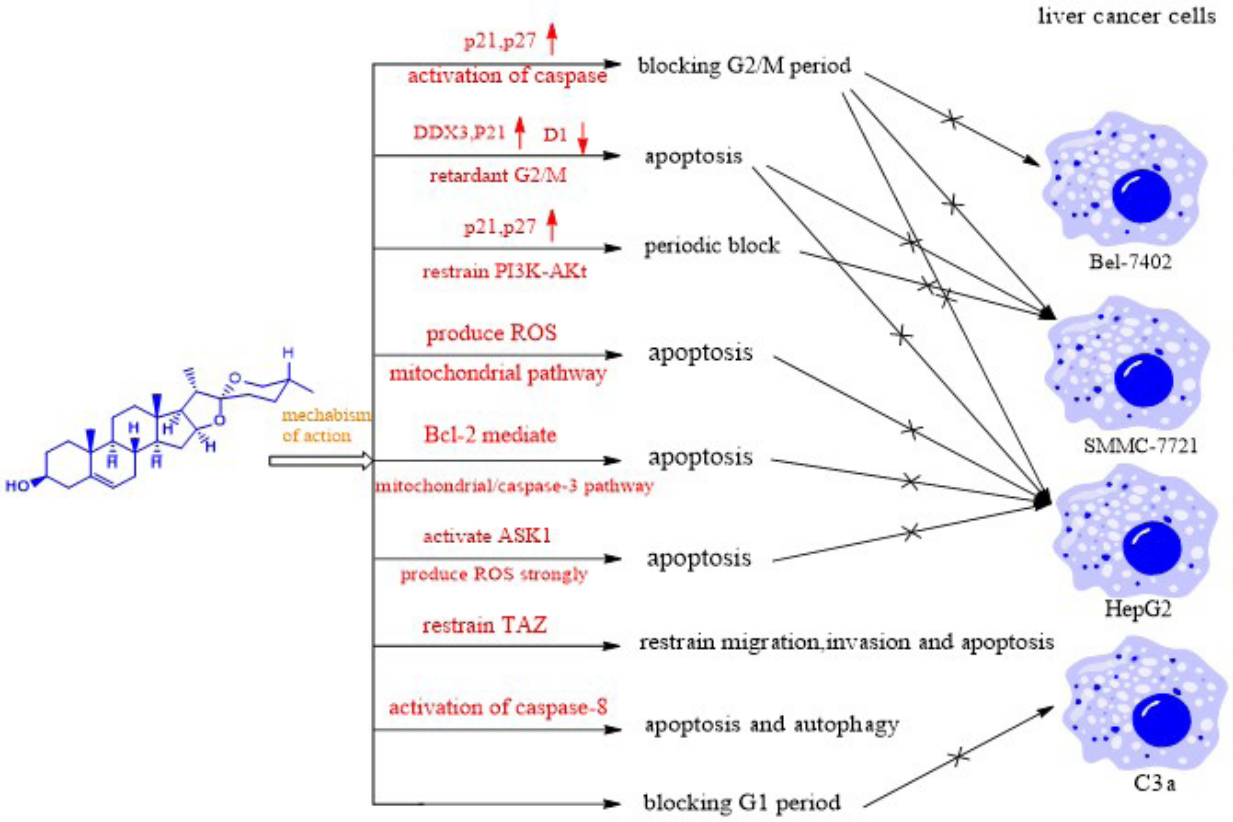

Figure 7. Pharmacological effects of diosgenin on the production of liver cancer cells, "↠" indicates that the former has a negative effect on the latter, producing a negative impact.

p21 expression, which significantly inhibited cell proliferation and triggered apoptosis, induced G2/M phase cell cycle arrest, and lead to inhibition of cell migration and invasion capacity. In contrast, diosgenin was described to block the cell cycle in the G1 phase in complement component 3a (C3a) hepatocellular carcinoma cells (Li et al., 2010). This suggests that the effect of diosgenin on the cell cycle of hepatocellular carcinoma and the associated mechanisms may be determined by the cell type, which ultimately leads to apoptosis. In addition, cell proliferation and apoptosis play an important role in maintaining homeostasis *in vivo* (Chen et al., 2015), and diosgenin inhibit cancer cell proliferation and induce apoptosis in various hepatocellular carcinoma cell lines. A study of (Wang, 2014) reported that diosgenin induced cycle arrest and inhibited cell proliferation in human hepatocellular carcinoma cells SMMC-7721 by inhibiting phosphoinositide 3-kinase/serine threonine kinase (PI3K-Akt) and upregulating p21 and p27 expression. At the same time, it activates caspase-8 to induce apoptosis and autophagy in concentration dependent manner. In another study by (Chen et al., 2018) diosgenin was found to inhibit the expression of tafazzin (TAZ) in hepatocellular carcinoma cells, thereby inhibiting cell growth, inducing apoptosis, and suppressing cell migration and invasion. In addition, diosgenin can induce apoptosis in HepG2 cells through the production of ROS and mitochondrial pathways. (Kim et al., 2012) have demonstrated that diosgenin (40 μmol/L) strongly produces ROS to induce hepatocellular carcinoma HepG2 by activating apoptosis signal-regulating kinase (ASK)-1 (a key upstream signal for JNK/p38 MAPK activation in HepG2 cancer cells) cell apoptosis. Also, it can induce HepG2 cell apoptosis through the Bcl-2 protein family mediated mitochondrial/caspase-3 dependent pathway.

Effects on gastric cancer

Gastric cancer is one of the common gastrointestinal malignant tumors, and the incidence of gastric cancer has shown a trend of youthfulness and expansion in recent years, but there is no fundamental change in its treatment methods. Therefore, the search for natural plant components with anti-tumor effects has become a hot spot for gastric cancer treatment. Chinese herbal medicine can alleviate the clinical symptoms of various types of gastric cancer, reduce the adverse effects and improve the treatment rate. Diosgenin can exert anti-tumor effects by regulating various intracellular signaling pathways and molecules related to cell growth, invasion, migration and apoptosis (Figure 8). It has been reported that the action of diosgenin on AGS cells significantly increased the expression level of miR-34a and decreased the expression levels of miR-34a target genes E2F transcription factor 1 (E2F1), E2F3 and cyclin D1 (CCND1), which exerted the ability to inhibit the proliferation

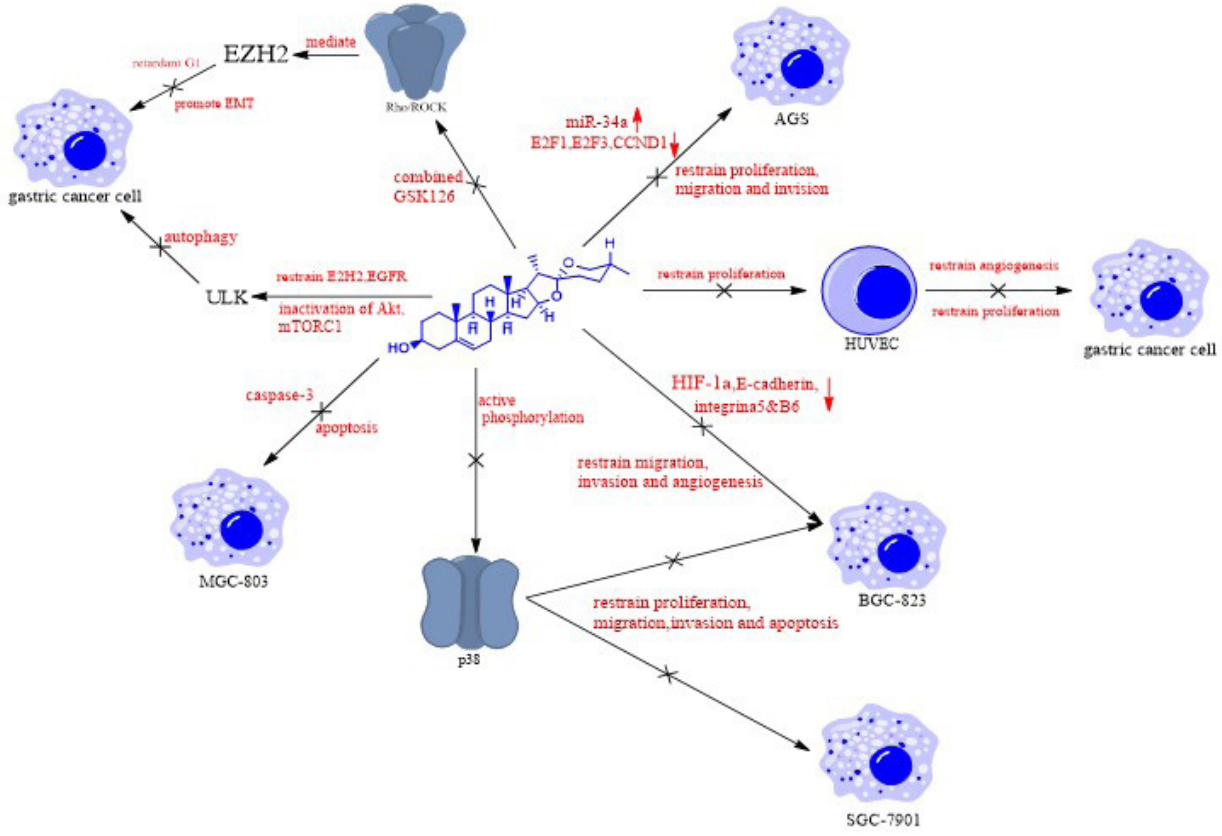

Figure 8. Pharmacological effects of diosgenin on the production of gastric cancer cells, "⇸"indicates that the former has a negative effect on the latter, producing a negative impact.

and clone formation of gastric cancer AGS cells and attenuated their migratory and invasive effects (Li et al., 2020). Similarly, another study showed that diosgenin inhibited the proliferation of gastric cancer cells by reducing the proliferative activity of human umbilical vein endothelial cells (HUVEC), inhibiting intratumor angiogenesis, and affecting the blood supply to the tumor (Huo et al., 2014). And it was also found that it could significantly inhibit the migration ability of BGC-823 cells. Meanwhile, it was confirmed that in gastric cancer BGC-823 and SGC-7901 cells (Wu et al., 2014), diosgenin may act through the activation of phosphorylation of p38 pathway, thus regulating cell proliferation, apoptosis, migration and invasion. In addition, it was shown that diosgenin can induce apoptosis in MGC-803 cells through the caspase-3 pathway, thus affecting the suppression of gene expression in human gastric hypofractionated mucinous adenocarcinoma cells and killing the cells (He et al., 2014b). And it can inhibit the growth, migration and spread of tumor cells by affecting the G protein, Wnt signaling pathway, producing anti-tumor effects.

Many molecules contribute to the invasion and metastasis of cancer cells, and the EMT process can enhance this ability of cancer cells. Diosgenin may act against gastric cancer cells by regulating EMT. (Liu et al., 2020a) demonstrated that the combination of diosgenin and GSK126 mediated enhancer of zeste homology 2 (EZH2) through the Ras homolog gene/Rho-associated coiled coil-forming protein kinase (Rho/ROCK) signaling pathway, which exerted a synergistic effect on EMT in gastric cancer cells and altered the expression of EMT markers (E-cadherin, N-cadherin, waveform protein and fibronectin). It was also found that EZH2 overexpression reversed the anti-tumor effects of diosgenin by inducing cell survival, blocking G1 phase block, and promoting EMT. In line with this, (Yang et al., 2018) demonstrated that inhibition of both EZH2 and epidermal growth factor receptor (EGFR) inactivated protein kinase B and mammalian target of rapamycin complex 1 (mTORC1) to activate unc-51-like autophagy activating kinase (ULK), which led to increased autophagy and reduced survival of gastric cancer cells producing a synergistic effect. In addition, drug resistance is a major factor in the limited efficacy of gastric cancer chemotherapy. HIF-1α is a central transcription factor for hypoxia and is thought to be involved in drug resistance. (Mao et al., 2012) have shown that the combination of diosgenin and downregulated hypoxia-inducible factor-1α (HIF-1α) can have a synergistic effect. It was also found that 10 μmol/L diosgenin inhibited invasion, migration and angiogenesis of gastric cancer BGC-823 cells by decreasing the expression levels of E-cadherin, integrin α5, and integrin β6 in a hypoxic simulated microenvironment.

3.2 Immunomodulation and anti-inflammation

Rheumatoid arthritis (RA) is a common chronic autoimmune dysfunctional disease with a complex pathogenesis, mainly manifesting as synovial inflammation and leading to destruction of articular cartilage and bone, which can lead to loss of abnormal joint extent in severe cases. Recent studies have identified the role of diosgenin on immune regulation and inflammatory cells. Diosgenin can affect cells and transfer factors in collagen-induced arthritis (CIA) mice, exerting immunosuppressive effects and reducing inflammatory responses. (Zhao, 2016) found that diosgenin specifically transcribe $CD4^{+}$T cells, suppress helper T (Th) cell 1 and Th17 cells and promote Th2 cells in mice. Another study showed that diosgenin could modulate vascular endothelial growth factor (VEGF) expression by inhibiting the regulation of two subunits of activator protein-1 (AP-1) in rat synovial cells, thereby attenuating the angiogenic and inflammatory response and treating RA (Guo et al., 2015). In addition, diosgenin inhibited the expression of IL-1β induced inflammatory mediators. Diosgenin significantly inhibited IL-1β-induced nitric oxide (NO) and prostaglandin E2 (PGE2) production, and protein levels of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) in human chondrocytes, preventing IL-1β-induced IκBα degradation in human osteoarthritic chondrocytes (Wang et al., 2015a). In addition, in a recent study, it was also reported that diosgenin could inhibit the proliferation and migration of RA synoviocytes and reduce the inflammatory response of RA synoviocytes through downregulation of phosphodiesterase type 3B (PDE3B) (Wang et al., 2021).

The lysosomal enzyme myeloperoxidase (MPO) is involved in diseases such as inflammation, vasculitis and atherosclerosis. Diosgenin reduces MPO levels and attenuates the inflammatory response by inhibiting inflammatory mediator release and neutrophil infiltration. Diosgenin inhibits the inflammatory response and infiltration of neutrophil polymorphonuclear leukocyte (PMN) by reducing proinflammatory factors and MPO expression during ischemia/reperfusion (I/R), while inhibiting phosphorylated NF-κb induced activation of p38 mitogen activated protein kinase (p38-MAPK) and c-Jun N-terminal kinase (JNK) signaling pathways, thereby reducing inflammation and acting as a protective agent against injured myocardium (Wang, 2018b; Wang et al., 2018c). Similarly, in a study of diosgenin ameliorated testicular injury in diabetic rats, it was confirmed that diosgenin could significantly improve serum insulin and testosterone levels, attenuate testicular inflammatory markers tumor necrosis factor α (TNFα) and interleukin 6 (IL-6), and reduce MPO activity, a biomarker of neutrophil infiltration in diabetic rats (Khosravi et al., 2019).

3.3 Anti-thrombotic

Among peripheral vascular diseases, thrombophilia is a common and frequent disease that endangers people's health and affects their daily quality of life. NO is an important cellular messenger molecule in the body, which is closely related to platelet adhesion and inflammatory response. Diosgenin can inhibit platelet aggregation by increasing serum NO, reduce myocardial infarction area, lower whole blood viscosity, and thus anti-thrombosis. Diosgenin has been found to inhibit platelet aggregation and thrombosis by improving anticoagulation, dependent prolongation of activated partial thromboplastin time (APTT), prothrombin time (PT) and thrombin time (TT), and anti-thrombotic activity in a mouse thrombosis model (Gong et al., 2011). Another study also reported that diosgenin caused elevated serum NO and MPO, decreased NO in the vessel wall, and inhibited thrombosis in mice with ligated inferior vena cava (Zhang, 2014). In addition, (Ning et al., 2008) found that

diosgenin could reduce serum creatine kinase (CK) and lactate dehydrogenase (LDH) levels, and had a significant inhibitory effect on the elevation of plasma thromboxane (TXB2) caused by myocardial ischemia, prolonging thrombus formation time. In addition, tissue factor (TF) has also been reported to contribute to thrombosis, which causes cardiovascular disease. (Yang et al., 2013) demonstrated that diosgenin (0.01-1 μmol/L) downregulated the phosphorylation of NF-κb/p65, inhibitor κB kinase-β (IKK-β), Akt, ERK, and JNK, significantly inhibited TNF-α induced TF procoagulant activity and decreased TF expression.

4 Pharmacological effects of diosgenin derivatives

Diosgenin is a class of steroidal saponins naturally synthesized by plants, with anti-tumor, hypoglycemic, hypolipidemic, anti-inflammatory and other pharmacological activities. Although it has good physiological activity, but clinically due to its high hydrophobicity, low solubility in the digestive fluid after oral administration, so its bioavailability is very low, almost difficult to absorb into the blood, which makes diosgenin can not play its pharmacological role, largely limiting the clinical application of diosgenin preparations. In contrast, the type of adulterants synthesized by diosgenin can produce synergistic effects and enhance the pharmacological activity of their components (Özdemir et al., 2020). Therefore, it is necessary to synthesize different structural diosgenin derivatives by chemically modification and transformation of the structure to improve the solubility and dissolution of diosgenin and enhance the pharmacological effects.

4.1 Anti-tumor

Studies have shown that diosgenin itself has anti-cancer activity and can induce apoptosis in a variety of tumor cell lines. This suggests that diosgenin and its derivatives can be investigated as potential anti-tumor active substances. The Bcl-2 protein is mainly involved in the mitochondrial pathway, and mitochondria is the main target for diosgenin to exert its anti-tumor effects. (Dong et al., 2012) used diosgenin as a raw material to target Bcl-2 protein, which decreased the activity of Bcl-2 protein by inhibiting the binding of proapoptotic protein ligands to Bcl-2, thus leading to apoptosis.

Diosgenin derivatives

Most of the diosgenin esters have better biological activity than diosgenin itself, including diosgenin 2-tetrahydrofuroic acid esters, diosgenin furoate esters, and furan-3-carboxylic acid diosgenin esters, among others. (Guo, 2016) selected DMAP and EDCI as dehydrating agent and catalyst, respectively, and esterified diosgeninogen with carboxylic acidic components by the synthetic principle of esterification reaction to finally obtain compounds, whose structures were determined using IR as well as HNMR and CNMR. In addition, carbamate derivatives were synthesized at the C26 of the furanone ring using the helical ketone bond (F-ring) of diosgenin as the starting point, which inhibited the G1 phase population of the cell division cycle, activated caspase-3, induced apoptosis, and showed significant antiproliferative activity against human breast cancer cells (Pathak et al., 2019).

Diosgenin amino acid derivatives

Due to its strong hydrophilicity and lipophilic effect, amino acid molecule, as the basic component unit of biofunctional macromolecule protein, can be introduced into the molecular structure of insoluble drugs, which can essentially improve the solubility of drugs in water and enhance the active transport process in the intestine, thus promoting drug absorption, solving the phenomenon of low oral bioavailability and improving its utilization value (Figure 9). Among the series of DG amino acid derivatives that have been synthesized, they have been shown to be used in the treatment of cancer, inflammation, diabetes, thrombosis and neurodegenerative diseases (Huang et al., 2017). (Li, 2016) found that L-valine diosgenin ester inhibited the growth of human tumor cells *in vitro* and *in vivo*, and inhibited the proliferation of human salivary gland adenoid cystic carcinoma ACCM and S-180 mouse transplanted tumor cells *in vitro*, while it inhibited the proliferation of mouse S-180 tumor cells *in vivo*. In addition, the derivatives synthesized from diosgenin linked with levulinic acid, 3,4-dihydroxycinnamic acid, dipeptides and various amino acids through ester bonds on the C3-oxygen atom of steroid backbone. Its most representative L-serine derivative has antiproliferative activity and induces apoptosis in MCF-7 cells by activating caspase-3/7 (Michalak et al., 2020). Another study showed that the presence of succinic or valeric acid junction, piperazinyl amide terminus and lipophilic cations all favor the promotion of cytotoxic activity, and compound (25*R*)-Spirost-5-ene-3β-yl 4-oxo-4-(Piperazin-1-yl)butanoate, synthesized on the basis of this study, exhibited excellent cytotoxic activity against HepG2 cells by inducing G0 /G1 cell cycle arrest and apoptosis (Yin et al., 2020).

Diosgenin F ring-opening derivatives

Diosgenin F-ring is less affected by the spatial site resistance because it is at the edge of the molecule, so the F-ring semi-ring opening reaction is easy. The F-ring of diosgenin was opened to induce hydrophilic groups for the design and selective synthesis of new compounds.(Li, 2016) used imidazole as a linking group at the 26-position of the lost F-ring diosgenin, and then introduced hydrophobic groups to open the F-ring, brominated the hydroxyl group at the 26-position of the lost F-ring of diosgenin, and then attached imidazole, and finally the 3-position of 1-steroidal imidazole reacted with the corresponding brominated substituents thus forming diosgenin open F-ring imidazole derivatives, and the obtained derivatives showed good *in vitro* anti-tumor activity. Similarly, new compound obtained after another F-ring opening showed significant antiproliferative activity against human breast cancer cells and its mechanism of action was to block the cell division cycle in G1 phase and induce apoptosis (Pathak et al., 2019).

Diosgenin glycosylated derivatives

The glycosyl structure is influential on the biological activity and modification of the glycosyl group can alter the activity.

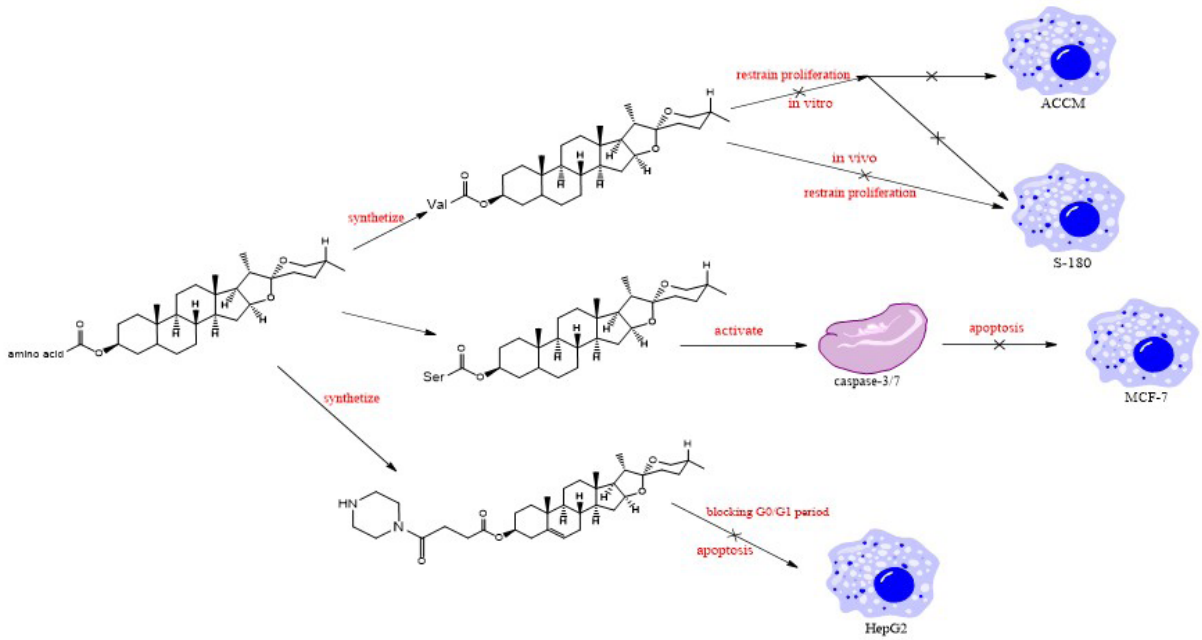

Figure 9. Anti-tumor cellular effects of amino acid derivatives of diosgenin, "↠" indicates that the former has a negative effect on the latter, producing a negative impact.

The carbohydrate fraction plays a key role in the biological function of steroidal saponins (Wang et al., 2012). Therefore, the modification of the glycosylated structure of diosgenin also leads to the formation of derivatives with anti-tumor activity. These compounds produce certain apoptosis-inducing activity against tumor cells.(Chang, 2019) prepared derivatives of diosgenin with different sugars by various methods, and the activity of the synthesized partial nitrogen glycosides was significantly increased, and the anti-tumor activity of the compounds was stronger than the α-configuration products when the glycosidic configuration was β. Also, the activity could be improved if rare sugars were introduced into the glycosyl group. In addition, the antiproliferative, necrotic and apoptotic activities of diosgenin (DSG) and its glycoside derivatives (25*R*)-spirost-5-en-3β-yl *O*-β-D-glucopyranoside (3GD), (25*R*)-spirost-5-en- 3β-yl *O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside (3GRD) and dioscin (DSC) have been reported. In both cancer cell lines, 3GD and 3GRD were less potent than DSG, whereas DSC was more potent than DSG. DSG glycosides induced apoptosis more than DSG, suggesting that glucose and rhamnose residues play a central role in enhancing the apoptotic activity of DSG cells (Hernández-Vázquez et al., 2020). In addition, a unique disaccharide saponin was synthesized from the natural product β-hederin along with 12 glycosylated derivatives, which were found to be cytotoxic to A549 cells, and this cytotoxicity was associated with apoptotic cell death characterized by morphological alterations, chromatin condensation, DNA fragmentation, and phosphatidylserine externalization. One of these compounds: diosgenyl α-L-rhamnopyranosyl- (1→2)-[α-L-arabinopyranosyl-(1→4)]-α-L-arabinopyranoside, induces apoptosis in A549 cells via caspase-8 mediated exogenous pathway and caspase-9 mediated endogenous pathway (Wang et al., 2012). Another study reported that diosgenin altered the biofilm affinity of cytarabine (Ara-C) during liposome preparation to exert anti-tumor activity, and DG-Ara-C liposomes showed superior anti-tumor activity than free DG and Ara-C liposomes against leukemia cells and solid tumor cells (Liao et al., 2021). Furthermore, (Meng et al., 2019) synthesized an additional fluorophore derivative of diosgenin (GND) from diosgenin and glucosamine hydrochloride in 7-10 steps. GND induces endoplasmic reticulum swelling, mitochondrial damage and autophagic vesicles, inhibits cleaved caspase-8, and upregulates inositol requiring-protein 1 (IRE-1) to inhibit induction of autophagy and apoptosis.

4.2 Anti-inflammatory and analgesic

Inflammation is a defense response of living tissues with a vascular system to tissue damage or invasion by pathogenic factors, and many chronic diseases are mediated by chronic inflammation, such as gout and joint pain. Studies have shown that diosgenin derivatives have anti-inflammatory and analgesic activity. Aspirin was chloroformylated and then reacted with diosgenin to produce 3β-2-acetoxy-benzoyl-diosgenin ester (ABDE), a derivative with twin drug properties, and it was found that the degree of auricular swelling in xylene induced mice and the number of writhing reactions in acetic acid induced mice decreased, confirming that ABDE has some anti-inflammatory and analgesic effects (Ma et al., 2011). In addition, the synthesized new derivative of saponin containing primary amine, (3β,25*R*) spirost5en3oxyl (2((2((2aminoethyl)amino)ethyl)amino)ethyl) carbamate (DGP), blocked p65 nuclear translocation and NF-κB p65/DNA binding activity through NF-κB signaling pathway and also blocked the phosphorylation of JNK, inhibited NO synthesis in lps stimulated microglial BV2 cells, downregulated

iNOS and COX-2, produced anti-inflammatory effects (Cai et al., 2018). In addition, another study reported that one compound could rescue neuroinflammation caused by endotoxemia, which triggers an inflammatory cascade involving the neurotransmitters 5-hydroxytryptamin (5-HT) and high mobility group protein B1/Toll-like receptor 4 (HMGB-1/TLR4), and has a neuroprotective effect on hippocampal neurogenesis damaged by neuroinflammation (Yang et al., 2017).

4.3 Anti-thrombotic

The steroidal saponins chemical components in natural products generally have cardiovascular pharmacological effects such as anti-platelet aggregation and anti-thrombosis, and making them into diosgenin derivatives improves the aqueous solubility of diosgenin to some extent and enhances its anti-thrombogenic activity. Fu (2009) modified the hydroxyl group of diosgenin at the C-3 position with groups of different polarity, volume and acidity. The synthesized derivatives were found to improve the anti-thrombotic activity of diosgenin in animal anti-thrombotic models with the modification of appropriate hydrophilic groups on the C-3 position hydroxyl group. On this basis (Fu et al., 2011) also synthesized the target compounds by esterification reaction and found that the compounds with salicylic acyl groups attached to the synthesized compounds, showed better activity in anti-thrombotic activity screening assays. These results suggest that the introduction of appropriate active groups can enhance the anti-thrombotic activity of diosgenin, and the number of active groups is closely related to the activity intensity. In addition, a new diosgenin derivative compound was reported to confirm significant anti-inflammatory activity superior to that of aspirin, prolonged bleeding time, reduced regulatory factor VIII activity and inhibited adenosine diphosphate (ADP) induced platelet aggregation (Zheng et al., 2016). And it also significantly reduced the mean length and weight of arterial and venous thrombi. In addition, a helical ketone structure of 6-aminohexanoic acid residue was prepared from diosgenin, which had better anti-thrombotic activity and prevented thromboembolism (Huang et al., 2017).

4.4 Effects on tracheal smooth muscle

Diosgenin has various pharmacological activities, but its effect on tracheal smooth muscle was not significant, and its design and synthesis of diosgenin derivatives increased the action activity on tracheal smooth muscle. (Liu et al., 2009) found that diosgenin and its derivatives had different degrees of diastolic effects on guinea pig isolated tracheal smooth muscle through experiments with guinea pig isolated tracheal smooth strips, and their structural modification at the 3-position could increase their activities. And alkane straight chain esters have a tendency to increase the diastolic activity of tracheal smooth muscle with increasing chain length, but the longer the carbon chain is not better, 5-6 carbon atoms is better, and for branched esters containing benzene ring, the activity varies more, presumably the larger site resistance of naphthalene ring affects the activity.

In addition to the application of diosgenin in the treatment of various diseases, the corresponding derivatives of diosgenin also have some medicinal value and use significance. (Zeng et al., 2017) reported that diosgenin and its derivatives can inhibit the growth of cancer cells by inhibiting cell proliferation and inducing apoptosis. Diosgenin as a precursor can combine with amides and then react with sodium azide and triphenylphosphine to form intermediates of anti-tumor derivatives, and 15 novel amide derivatives were synthesized, and different derivatives showed different degrees of anti-tumor activity. Another study by (Wang et al., 2015b) summarized the current conformational relationships between diosgenin and its derivatives, obtaining the corresponding activities of different derivatives of this substance, different derivatives as intermediates of active ingredients of different drugs or final forms of activity presentation. In addition, it was shown that the amino acid derivatives of diosgenin have neurosynthetic and angiogenic effects, and their corresponding series of derivatives target enhanced neuroprotection compared to diosgenin, especially the compound DG-15, which has stronger activity and lower cytotoxicity than diosgenin, and is an important direction for the future development of drugs such as vascular activators (Cai et al., 2019). It has been studied that diosgenin–betulinic acid conjugates have the ability to enhance and/or alter the pharmacological properties of the substance's own components (Özdemir et al., 2020).

5 Discussion

Natural products are the main source and raw material of drugs, and diosgenin is a natural pharmaceutical active ingredient found in dioscorea plant, which has great potential for development. In addition, diosgenin has been shown to have a variety of pharmacological activities such as anti-tumor, immunomodulatory, anti-inflammatory and anti-thrombotic. In particular, its anti-tumor effects and mechanism of action have been studied in depth, providing a strong basis for its clinical treatment of tumors. However, the oral absorption of diosgenin is slow and the bioavailability is low, which greatly affects the therapeutic effect. The current method to improve the solubility of diosgenin is mainly to modify the structure of diosgenin during the drug design stage. The corresponding derivatives of diosgenin can improve the pharmacological activity of diosgenin and improve its water solubility to increase the bioavailability, which has a good development prospect, and the research on the relationship between its derivatives and conformation should be strengthened. Meanwhile, diosgenin is an important precursor for the synthesis of steroidal drugs, and structural modification of diosgenin to derive more compounds synthesized to find efficient and low toxic derivatives with operability is to be studied in depth.

In summary, diosgenin is an important natural pharmaceutical active ingredient. Meanwhile, extensive studies have revealed the pharmacological effects of diosgenin and its derivatives, which should be given more attention for further pharmacological and related mechanism studies, which will help diosgenin to be used for the treatment of human diseases with clinical value. In conclusion, the value of diosgenin is not to be ignored, and will certainly attract more and more attention from the scientific community.

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References

Bhuvanalakshmi, G., Basappa, Rangappa, K. S., Dharmarajan, A., Sethi, G., Kumar, A. P., & Warrier, S. (2017). Breast cancer stem-like cells are inhibited by diosgenin, a steroidal saponin, by the attenuation of the Wnt β-Catenin signaling via the Wnt antagonist secreted frizzled related Protein-4. *Frontiers in Pharmacology*, 8, 124. http://dx.doi.org/10.3389/fphar.2017.00124. PMid:28373842.

Cai, B., Seong, K. J., Bae, S. W., Chun, C., Kim, W. J., & Jung, J. Y. (2018). A synthetic diosgenin primary amine derivative attenuates LPS-stimulated inflammation via inhibition of NF-κB and JNK MAPK signaling in microglial BV2 cells. *International Immunopharmacology*, 61, 204-214. http://dx.doi.org/10.1016/j.intimp.2018.05.021. PMid:29890414.

Cai, D., Qi, J., Yang, Y., Zhang, W., Zhou, F., Jia, X., Guo, W., Huang, X., Gao, F., Chen, H., Li, T., Li, G., Wang, P., Zhang, Y., & Lei, H. (2019). Design, synthesis and biological evaluation of diosgenin-amino acid derivatives with dual functions of neuroprotection and angiogenesis. *Molecules*, 24(22), 4025. http://dx.doi.org/10.3390/molecules24224025. PMid:31703284.

Chang, X. (2019). *Synthesis and antitumor activity of glycosylated derivatives of diosgenin* (Master Dissertation). Beijing Univ Chin Med, Bei jing.

Chen, Y., Tang, Y. M., Yu, S. L., Han, Y. W., Kou, J. P., Liu, B. L., & Yu, B. Y. (2015). Advances in the pharmacological activities and mechanisms of diosgenin. *Chinese Journal of Natural Medicines*, 13(8), 578-587. http://dx.doi.org/10.1016/S1875-5364(15)30053-4. PMid:26253490.

Chen, Z., Xu, J., Wu, Y., Lei, S., Liu, H., Meng, Q., & Xia, Z. (2018). Diosgenin inhibited the expression of TAZ in hepatocellular carcinoma. *Biochemical and Biophysical Research Communications*, 503(3), 1181-1185. http://dx.doi.org/10.1016/j.bbrc.2018.07.022. PMid:30005871.

Chinese Pharmacopoeia Commission. (2020). *Chinese Pharmacopoeia* (Vol. 1). Beijing: China Med Sci Press.

Cong, S., Qi, S., Peng, Q., Zhou, R., Tong, Q., & Xu, Y. (2020). A study on diosgenin antibacterial activity against Enterococcus faecalis in vitro. *Journal of Clinical Stomatology*, 36, 7-10.

Das, S., Dey, K. K., Dey, G., Pal, I., Majumder, A., MaitiChoudhury, S., Kundu, S. C., & Mandal, M. (2012). Antineoplastic and apoptotic potential of traditional medicines thymoquinone and diosgenin in squamous cell carcinoma. *PLoS One*, 7(10), e46641. http://dx.doi.org/10.1371/journal.pone.0046641. PMid:23077516.

Deng, Y. Z., Sun, X. D., Guo, H. B., Kang, F., Wang, H. L., & Wang, Y. Y. (2017). Influence of diosgenin on the proliferation and apoptosis of triple-negative breast cancer cell line HCC1937. *Journal of Chinese Practical Diagnosis and Therapy*, 31, 657-659.

Digbeu, D. Y., Due, A. E., & Dabonne, S. (2013). Biochemical characteristics of composite flours: influence of fermentation. *Food Science and Technology*, 33(4), 599-604. http://dx.doi.org/10.1590/S0101-20612013000400001.

Dong, J. H., He, G., Wu, Y. K., Wan, J. F., & Fan, J. Z. (2012). Synthesis and Anticancer Activity Study of Diosgenin Derivatives. *Zhongguo Yao Xue Za Zhi*, 47, 1407-1414.

Fu, X. L. (2016). *Synthesis of diosgenin derivatives and antithrombotic activity*. (Master Dissertation). Tianjin Univ., Tianjin.

Fu, X. L., Han, Y. M., & Zhang, S. J. (2011). Synthesis of diosgenin derivatives and their antithrombotic activity (II). *Chinese Traditional and Herbal Drugs*, 42, 1683-1688.

Gong, G., Qin, Y., & Huang, W. (2011). Anti-thrombosis effect of diosgenin extract from dioscorea zingiberensis C.H. Wright in vitro and in vivo. *Phytomedicine*, 18(6), 458-463. http://dx.doi.org/10.1016/j.phymed.2010.08.015. PMid:21036572.

Guo, S. (2016). *Synthesis of diosgenin in Dioscoreae nipponicae rhizoma and screening the activity in vitro*. (Master Dissertation). Jilin Agric Uni., Jilin.

Guo, Y., Liang, X., Gao, Y., & Song, H. (2015). Effects of Diosgenin on VEGF and AP-1 Expression in Synovial Tissues of CIA Rats. *Modernization of Traditional Chinese Medicine and Materia Medica-World Science and Technology*, 17, 1801-1805.

He, Z., Chen, H., Cai, E., Li, C., Wang, Y., & Gao, Y. (2014b). Effects of Diosgenin on Gene exression in human gastric cancer cell MGC-803. *Journal of Ginseng Research*, 26, 44-46.

He, Z., Chen, H., Li, G., Zhu, H., Gao, Y., Zhang, L., & Sun, J. (2014a). Diosgenin inhibits the migration of human breast cancer MDA-MB-231 cells by suppressing VAV2 activity. *Phytomedicine*, 21(6), 871-876. http://dx.doi.org/10.1016/j.phymed.2014.02.002. PMid:24656238.

Hernández-Vázquez, J. M. V., López-Muñoz, H., Escobar-Sánchez, M. L., Flores-Guzmán, F., Weiss-Steider, B., Hilario-Martínez, J. C., Sandoval-Ramírez, J., Fernández-Herrera, M. A., & Sánchez Sánchez, L. (2020). Apoptotic, necrotic, and antiproliferative activity of diosgenin and diosgenin glycosides on cervical cancer cells. *European Journal of Pharmacology*, 871, 172942. http://dx.doi.org/10.1016/j.ejphar.2020.172942. PMid:31972180.

Herráiz, I. (2017). Chemical pathways of corticosteroids, industrial synthesis from sapogenins. *Methods in Molecular Biology,* 1645, 15-27. http://dx.doi.org/10.1007/978-1-4939-7183-1_2. PMid:28710618.

Huang, B. Z., Xin, G., Ma, L. M., Wei, Z. L., Shen, Y., Zhang, R., Zheng, H. J., Zhang, X. H., Niu, H., & Huang, W. (2017). Synthesis, characterization, and biological studies of diosgenyl analogs. *Journal of Asian Natural Products Research*, 19(3), 272-298. http://dx.doi.org/10.1080/10286020.2016.1202240. PMid:27380052.

Huo, Z. H., Hu, J., Chu, Z. L., Lv, S., Yin, P., & Zhang, L. (2014). Mechanism underlying inhibition of migration of gastric carcinoma by diosgenin in vitro and vivo. *Journal of Jiangsu University*, 24(5), 394-398.

Jagadeesan, J., Langeswaran, K., Gowthamkumar, S., & Balasubramanian, M. P. (2013). Diosgenin exhibits beneficial efficiency on human mammary carcinoma cell line MCF-7 and against N-nitroso-N-methylurea (NMU) induced experimental mammary carcinoma. *Biomedicine & Preventive Nutrition*, 3(4), 381-388. http://dx.doi.org/10.1016/j.bionut.2013.06.009.

Jesus, M., Martins, A. P., Gallardo, E., & Silvestre, S. (2016). Diosgenin: recent highlights on pharmacology and analytical methodology. *Journal of Analytical Methods in Chemistry*, 2016(4156293), 1-16. http://dx.doi.org/10.1155/2016/4156293. PMid:28116217.

Jiang, S., Fan, J., Wang, Q., Ju, D., Feng, M., Li, J., Guan, Z. B., An, D., Wang, X., & Ye, L. (2016). Diosgenin induces ros-dependent autophagy and cytotoxicity via mtor signaling pathway in chronic myeloid leukemia cells. *Phytomedicine*, 23(3), 243-252. http://dx.doi.org/10.1016/j.phymed.2016.01.010. PMid:26969378.

Khosravi, Z., Sedaghat, R., Baluchnejadmojarad, T., & Roghani, M. (2019). Diosgenin ameliorates testicular damage in streptozotocin-diabetic rats through attenuation of apoptosis, oxidative stress, and inflammation. *International Immunopharmacology*, 70, 37-46. http://dx.doi.org/10.1016/j.intimp.2019.01.047. PMid:30785089.

Kim, D. S., Jeon, B. K., Lee, Y. E., Woo, W. H., & Mun, Y. J. (2012). Diosgenin induces apoptosis in HepG2 cells through generation of reactive oxygen species and mitochondrial pathway. *Evidence-Based Complementary and Alternative Medicine*, 2012, 981675. http://dx.doi.org/10.1155/2012/981675. PMid:22719792.

Leonel, M., Mischan, M. M., Pinho, S. Z., Iaturo, R. A., & Duarte, J. Fo. (2006). Efeitos de parâmetros de extrusão nas propriedades físicas de produtos expandidos de inhame. *Food Science and Technology,* 26(2), 459-464. http://dx.doi.org/10.1590/S0101-20612006000200033.

Li, F., Fernandez, P. P., Rajendran, P., Hui, K. M., & Sethi, G. (2010). Diosgenin, a steroidal saponin, inhibits STAT3 signaling pathway leading to suppression of proliferation and chemosensitization of human hepatocellular carcinoma cells. *Cancer Letters*, 292(2), 197-207. http://dx.doi.org/10.1016/j.canlet.2009.12.003. PMid:20053498.

Li, K. (2016). *Studies on the antitumor activities and pharmacokinetics of amino acid derivatives of diosgenin*. (Master Dissertation). Jilin Agric Univ., Jilin.

Li, Y., Li, R., Shi, F., Chen, X., Lyu, L., Zhao, H., Li, X., & Hou, L. (2020). Anti-cancer effect of diosgenin through regulating miR-34a and its target genes in gastric cancer. *Journal of Beijing University of Traditional Chinese Medicine*, 43, 108-114.

Li, Y., Lyu, P., Hou, L., Zhang, Y., Wang, C., Fan, Q., Chen, X., & Shi, F. (2019). Diosgenin's inhibitory effects on proliferation and migration of MCF-7 breast cancer cells through demethylation of microRNA-145. *Journal of Beijing University of Traditional Chinese Medicine*, 42(8), 662-666.

Li, Y., Wang, X., Cheng, S., Du, J., Deng, Z., Zhang, Y., Liu, Q., Gao, J., Cheng, B., & Ling, C. (2015). Diosgenin induces G2/M cell cycle arrest and apoptosis in human hepatocellular carcinoma cells. *Oncology Reports*, 33(2), 693-698. http://dx.doi.org/10.3892/or.2014.3629. PMid:25434486.

Liao, A. M., Cai, B., Huang, J. H., Hui, M., Lee, K. K., Lee, K. Y., & Chun, C. (2021). Synthesis, anticancer activity and potential application of diosgenin modified cancer chemotherapeutic agent cytarabine. *Food and Chemical Toxicology*, 148, 111920. http://dx.doi.org/10.1016/j.fct.2020.111920. PMid:33346046.

Liao, W. L., Lin, J. Y., Shieh, J. C., Yeh, H. F., Hsieh, Y. H., Cheng, Y. C., Lee, H. J., Shen, C. Y., & Cheng, C. W. (2019). Induction of G2/M Phase Arrest by Diosgenin via Activation of Chk1 Kinase and Cdc25C Regulatory Pathways to Promote Apoptosis in Human Breast Cancer Cells. *International Journal of Molecular Sciences*, 21(1), 172. http://dx.doi.org/10.3390/ijms21010172. PMid:31881805.

Liu, C., Wang, Z. Z., Zheng, W., Bao, X., & Fan, J. Z. (2009). Effects of diosgenin and its derivatives on isolated guinea pig trachea smooth muscle. *Huaxi Yaoxue Zazhi*, 24, 483-486.

Liu, S., Rong, G., Li, X., Geng, L., Zeng, Z., Jiang, D., Yang, J., & Wei, Y. (2020a). Diosgenin and GSK126 produce synergistic effects on epithelial–mesenchymal transition in gastric cancer cells by mediating EZH2 via the Rho/ROCK signaling pathway. *OncoTargets and Therapy*, 13, 5057-5067. http://dx.doi.org/10.2147/OTT.S237474. PMid:32606728.

Liu, Y., Zhou, Z., Yan, J., Wu, X., & Xu, G. (2020b). Diosgenin Exerts Antitumor Activity via Downregulation of Skp2 in Breast Cancer Cells. *BioMed Research International*, 2020, 8072639. http://dx.doi.org/10.1155/2020/8072639. PMid:32626765.

Ma, M. H., Wu, X. H., He, Y., & Huang, W. (2011). Anti-inflammatory and analgesic effects of saponins from D. zingiberensis C.H. wright and diosgenin derivative on mice. *Journal of Sichuan University*, 42(4), 494-497. PMid:21866633.

Mao, Z. J., Tang, Q. J., Zhang, C. A., Qin, Z. F., Pang, B., Wei, P. K., Liu, B., & Chou, Y. N. (2012). Anti-proliferation and anti-invasion effects of diosgenin on gastric cancer BGC-823 cells with HIF-1α shRNAs. *International Journal of Molecular Sciences*, 13(5), 6521-6533. http://dx.doi.org/10.3390/ijms13056521. PMid:22754381.

Meng, X., Dong, H., Pan, Y., Ma, L., Liu, C., Man, S., & Gao, W. (2019). Diosgenyl saponin inducing endoplasmic reticulum stress and mitochondria-mediated apoptotic pathways in liver cancer cells. *Journal of Agricultural and Food Chemistry*, 67(41), 11428-11435. http://dx.doi.org/10.1021/acs.jafc.9b05131. PMid:31589037.

Michalak, O., Krzeczyński, P., Cieślak, M., Cmoch, P., Cybulski, M., Królewska-Golińska, K., Kaźmierczak-Barańska, J., Trzaskowski, B., & Ostrowska, K. (2020). Synthesis and anti–tumour, immunomodulating activity of diosgenin and tigogenin conjugates. *The Journal of Steroid Biochemistry and Molecular Biology*, 198, 105573. http://dx.doi.org/10.1016/j.jsbmb.2019.105573. PMid:32017993.

Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products*, 79(3), 629-661. http://dx.doi.org/10.1021/acs.jnatprod.5b01055. PMid:26852623.

Ning, K., Li, Y., Gao, H., & Li, L. (2008). Effects of methyl protodioscin on in-vivo and in-vitro thrombosis and blood viscosity in rats. *Traditional Chinese Drug Research & Clinical Pharmacology*, 19, 3-5.

Özdemir, Z., Rybková, M., Vlk, M., Šaman, D., Rárová, L., & Wimmer, Z. (2020). Synthesis and pharmacological effects of diosgenin-betulinic acid conjugates. *Molecules,* 25(15), 3546. http://dx.doi.org/10.3390/molecules25153546. PMid:32756514.

Pathak, N., Fatima, K., Singh, S., Mishra, D., Gupta, A. C., Kumar, Y., Chanda, D., Bawankule, D. U., Shanker, K., Khan, F., Gupta, A., Luqman, S., & Negi, A. S. (2019). Bivalent furostene carbamates as antiproliferative and antiinflammatory agents. *The Journal of Steroid Biochemistry and Molecular Biology*, 194, 105457. http://dx.doi.org/10.1016/j.jsbmb.2019.105457. PMid:31454535.

Qi, Y., Li, R., Xu, L., Yin, L., Xu, Y., Han, X., & Peng, J. (2019). Neuroprotective Effect of Dioscin on the Aging Brain. *Molecule*, 24(7), 1247. http://dx.doi.org/10.3390/molecules24071247. PMid:30935017.

Shanmugam, M. K., Warrier, S., Kumar, A. P., Sethi, G., & Arfuso, F. (2017). Potential role of natural compounds as anti-angiogenic agents in cancer. *Current Vascular Pharmacology*, 15(6), 503-519. http://dx.doi.org/10.2174/1570161115666170713094319. PMid:28707601.

Shen, Y., Wen, L., Zhang, R., Wei, Z., Shi, N., Xiong, Q., Xia, Q., Xing, Z., Zeng, Z., Niu, H., & Huang, W. (2018). Dihydrodiosgenin protects against experimental acute pancreatitis and associated lung injury through mitochondrial protection and PI3Kγ/Akt inhibition. *British Journal of Pharmacology*, 175(10), 1621-1636. http://dx.doi.org/10.1111/bph.14169. PMid:29457828.

Sun, R., Niu, J., Cao, F., Xia, Y., & Jiao, Y. (2019). Research on intervention mechanism of diosgenin on MRC-5 fibrosis induced by LPS. *Global Traditional Chinese Medicine*, 12, 988-993.

Torre, L. A., Siegel, R. L., Ward, E. M., & Jemal, A. (2016). Global cancer incidence and mortality rates and trends-an update. *Cancer Epidemiology, Biomarkers & Prevention*, 25(1), 16-27. http://dx.doi.org/10.1158/1055-9965.EPI-15-0578. PMid:26667886.

Wang, B., Chun, J., Liu, Y., Han, L., Wang, Y. S., Joo, E. J., Kim, Y. S., & Cheng, M. S. (2012). Synthesis of novel diosgenyl saponin analogues and apoptosis-inducing activity on A549 human lung adenocarcinoma. *Organic & Biomolecular Chemistry*, 10(44), 8822-8834. http://dx.doi.org/10.1039/c2ob26579f. PMid:23042047.

Wang, F., Zhao, Y., & Yang, Y. (2016). Effects of diosgenin on hepatic function and states of oxidative stress in hyperthyroidism rats. *Pharmacology and Clinics of Chinese Materia Medica*, 32, 39-42.

Wang, H. (2018b). *Diosgenin protects rats from myocardial inflammatory injury induced by ischemia-reperfusion*. (Master Dissertation). Nanjing Med Univ., Nanjing.

Wang, H. W., Liu, H. J., Cao, H., Qiao, Z. Y., & Xu, Y. W. (2018c). Diosgenin protects rats from myocardial inflammatory injury induced by ischemia-reperfusion. *Medical Science Monitor*, 24, 246-253. http://dx.doi.org/10.12659/MSM.907745. PMid:29329279.

Wang, H., Hu, J. H., Liu, C. C., Liu, M., Liu, Z., & Sun, L. X. (2018a). Analysis of antitumor mechanism of diosgenin by cell metabonomics strategy. *Chinese Journal of Experimental Traditional Medical Formulae*, 24, 95-101.

Wang, L., Ma, T., Zheng, Y., Lv, S., Li, Y., & Liu, S. (2015a). Diosgenin inhibits IL-1β-induced expression of inflammatory mediators in human osteoarthritis chondrocytes. *International Journal of Clinical and Experimental Pathology*, 8(5), 4830-4836. PMid:26191174.

Wang, M. Z., Li, Q., & Zhao, Y. Q. (2015b). Advances in the structure-activity relationship study of diosgenin and its derivatives. *Journal of Shenyang Pharmaceutical University*, 32(2), 154-160.

Wang, R., Sun, Y., Jin, X., Wen, W., & Cao, Y. (2021). Diosgenin inhibits excessive proliferation and inflammatory response of synovial fibroblasts in rheumatoid arthritis by targeting PDE3B. *Inflammation*, 44(3), 946-955. http://dx.doi.org/10.1007/s10753-020-01389-5. PMid:33237390.

Wang, X. (2014). *Mechanisms of inhibitory effect of diosgenin on human hepatic carcinoma cell SMMC-7721*. (Master Dissertation). Nanjing Univ Chin Med., Nanjing.

Wu, Y. Y., Cui, G. X., Ma, T. L., Ding, W. L., Ge, Z. J., & Tang, Z. A. Z. A. (2014). Diosgenin affect human gastric cancer BGC-823 and SGC-7901 cells through MAPK pathways. *Journal of Jiangsu University*, 24, 207-210.

Yang, G. X., Huang, Y., Zheng, L. L., Zhang, L., Su, L., Wu, Y. H., Li, J., Zhou, L. C., Huang, J., Tang, Y., Wang, R., & Ma, L. (2020). Design, synthesis and evaluation of diosgenin carbamate derivatives as multitarget anti-Alzheimer's disease agents. *European Journal of Medicinal Chemistry*, 187, 111913. http://dx.doi.org/10.1016/j.ejmech.2019.111913. PMid:31837501.

Yang, H. P., Yue, L., Jiang, W. W., Liu, Q., Kou, J. P., & Yu, B. Y. (2013). Diosgenin inhibits tumor necrosis factor-induced tissue factor activity and expression in THP-1 cells via down-regulation of the NF-κB, Akt, and MAPK signaling pathways. *Chinese Journal of Natural Medicines*, 11(6), 608-615. http://dx.doi.org/10.3724/SP.J.1009.2013.00608. PMid:24345501.

Yang, R., Chen, W., Lu, Y., Li, Y., Du, H., Gao, S., Dong, X., & Yuan, H. (2017). Dioscin relieves endotoxemia induced acute neuro-inflammation and protect neurogenesis via improving 5-HT metabolism. *Scientific Reports*, 7, 40035. http://dx.doi.org/10.1038/srep40035. PMid:28059131.

Yang, Y., Zhu, F., Wang, Q., Ding, Y., Ying, R., & Zeng, L. (2018). Inhibition of EZH2 and EGFR produces a synergistic effect on cell apoptosis by increasing autophagy in gastric cancer cells. *OncoTargets and Therapy*, 2018(11), 8455-8463. http://dx.doi.org/10.2147/OTT.S186498. PMid:30555238.

Yin, H., Zhang, M. J., An, R. F., Zhou, J., Liu, W., Morris-Natschke, S. L., Cheng, Y. Y., Lee, K. H., & Huang, X. F. (2020). Diosgenin derivatives as potential antitumor agents: synthesis, cytotoxicity, and mechanism of action. *Journal of Natural Products*, 84(3), 616-629. PMid:33381964.

Yu, H., Liu, Y., Niu, C., & Cheng, Y. (2018). Diosgenin increased DDX3 expression in hepatocellular carcinoma. *American Journal of Translational Research*, 10(11), 3590-3599. PMid:30662610.

Zeeshan, R., & Mutahir, Z. (2017). Cancer metastasis-tricks of the trade. *Bosnian Journal of Basic Medical Sciences*, 17(3), 172-182. PMid:28278128.

Zeng, Q. Q., Sun, B. B., Luo, Z. M., Chen, F., Yang, X. J., Yang, H. J., & Feng, Y. C. (2017). Synthesis, antitumor activity and cytotoxicity of diosgenin amide derivatives. *Natural Product Research and Development*, 29, 1455-1463.

Zhang, Y. (2014). Study and analysis of antithrombotic mechanism and bioactivity of Chinese patent medicine diosgenin. *Journal of North Pharmacy*, 11(52), 4.

Zhang, Y. Q., Zhu, X. Y., Wang, J., & Guan, F. (2019). Review of effects and mechanisms of diosgenin on Alzheimer's disease. *Chemical Engineering*, 33, 53-56.

Zhao, X. (2016). *Effect of diosgenin on expression of Th1, Th2, Th17 cell and its specific transcription factors in collagen-induced arthritis mice in vitro*. (Master Dissertation). Chengde Med Univ., Hebei.

Zheng, H., Wei, Z., Xin, G., Ji, C., Wen, L., Xia, Q., Niu, H., & Huang, W. (2016). Preventive effect of a novel diosgenin derivative on arterial and venous thrombosis in vivo. *Bioorganic & Medicinal Chemistry Letters*, 26(14), 3364-3369. http://dx.doi.org/10.1016/j.bmcl.2016.05.032. PMid:27217000.