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Review

Bioactive components, pharmacological properties and underlying mechanism of *Ganoderma lucidum* spore oil: A review

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ABSTRACT

Ganoderma lucidum is a Chinese medicinal fungus with a long history of use in healthcare and disease treatment. *G. lucidum* spores (GLS) are tiny germ cells released from the mushroom cap during the mature stage of growth. They contain all the genetic active substances of *G. lucidum*. *G. lucidum* spore oil (GLSO) is a lipid component extracted from broken-walled *Ganoderma* spores using supercritical CO_2 extraction technology. GLSO contains fatty acids, *Ganoderma* triterpenes, sterols and other bioactive compounds. Previous studies have demonstrated that GLSO has a wide range of pharmacological properties, including anti-tumor, anti-aging, neuroprotection, immunomodulation, hepatoprotection and modulation of metabolic diseases. This review summarizes the research progress of GLSO over the past two decades in terms of its bioactive components, extraction and processing techniques, pharmacological effects and safety evaluation. This provides a solid foundation for further research and application of GLSO.

Contents

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1. Introduction

Ganoderma spp. is a long-established and precious polyporaceae plant with an umbrella-like shape. Due to its wide range of pharmacological effects, its value status is widely recognized. *Ganoderma* spp. has a usage history of over two thousand years in China. Many ancient Chinese literatures mentioned *Ganoderma lucidum* (Leyss. Ex Fr.) Karst. It was first recorded in the *Shennong's Classic of Materia Medica* (*Shen Nong Ben Cao Jing*), the first Chinese pharmacological monograph. This book divided medicines into three classes, upper, middle and lower, according to their efficacy and toxicity. The upper grades are all effective and non-toxic, while *G. lucidum* is listed as the upper grade. Ancient Chinese scholars had some preliminary understanding of the biological properties of *G. lucidum*. "*Liezi-Tang wen*", one of the chapters of "*Liezi*", a famous Taoist philosophical text in ancient China, recorded that: "Above the rotten soil, there is a fungus *Ganoderma*." In the Eastern Han Dynasty, Chong Wang pointed out in "*Lunheng*" that "Mushrooms are born in the soil, and the rustic atmosphere is harmonious, so the grass of mushrooms thrives"; Hongjing Tao also pointed out that "Purple *Ganoderma* spp. is born on a rotten wood plant, shaped like a wooden barrel." These discourses all indicate that that *Ganoderma* spp. grows on "rotten soil" or "rotten wood" and requires suitable growing conditions. When describing *G. lucidum* in "Baopuzi - Xian Yao Pian", it states: "The red ones are like coral, the white ones are like fat cutting, the black ones are like lacquer, the green ones are like jade feathers, and the yellow ones are like purple and gold, all of which are as bright as ice, with the largest being more than ten kilograms and the smallest being three or four kilograms", which accurately describes the color, appearance characteristics and weight of *Ganoderma* spp. fruiting body. Regarding its efficacy, in the "*Liezi -Tangwen*" it is described that "When boiled for hundreds of times, its taste is clear and fragrant, and drinking it can improve vision, clear the mind, calm the heart, and strengthen the kidneys, making it a treasure." The *Compendium of Materia Medica* (*Ben Cao Gang Mu*) also states that "It grows in Huoshan. Its smell is mild and bitter, non-toxic, and it is mainly used for treating chest congestion, enhancing the heart and *qi*, invigorating the spleen, increasing wisdom, preventing forgetfulness, and long-term consumption can lighten the body and prevent aging." (He et al., 2023). It can be seen that in ancient times, *Ganoderma* spp. was regarded as a great product to enhance physical fitness and prolong lifespan.

Ganoderma (Lingzhi in Chinese) is the dried substrate of *G. lucidum*. or *Ganoderma sinense* Zhao, Xu et Zhang, according to *Chinese Pharmacopoeia* (2015 Edition). It is harvested throughout the year, with impurities removed, cut off the lower stipe with rotten wood, sediment or culture medium, and dried in shade or at 40–50 °C. The spores, mycelium and fruiting bodies of *G. lucidum* are the different stages of growth of *G. lucidum*. *G. lucidum* spores (GLS) are mature germ cells excreted from the mycelia of *G. lucidum*. They contain all the genetic active substances of *G. lucidum* and are concentrated into a powder form known as *G. lucidum* spore powder. The development and use of spore powder began in the 1990s. It is difficult to draw general conclusions as to whether GLS are more optimized than the fruiting bodies in terms of chemical composition or viability. This is because it depends specifically on the purpose of the different functions and applications. For example, in terms of immune enhancement, the two have similar effects; but in terms of anti-tumor, the results may be different (Gao, Bao, & Bau, 2013; Yue, Fung, Leung, & Lau, 2008). For liver protection, there are no controlled studies of the two. However, with a higher absorption rate and overall good health benefits, *G. lucidum* spore powder is gradually gaining popularity among consumers and becoming a common product in the modern health food market.

With the development of modern technology in recent years, research on *G. lucidum* spore oil (GLSO) has gradually gained attention (Liu et al., 2005; Wang et al., 2023). GLSO is a fat-soluble substance extracted from GLS, which is rich in terpenoids, sterols, fatty acids, polysaccharides and other active ingredients (Yuan et al., 2007). Modern pharmacological experiments have proven that GLSO has anti-tumor, neuroprotective, anti-aging, immunomodulatory, hepatoprotective and hypolipidemic effects (Deng, Yuan, & Lü, 2017; Huang, Wang, & Huang, 2007; Li, Wu, Xi, & Zhao, 2006; Li, Xiao, Mao, & Huang, 2006; Liu, Yuan, Chung, & Chen, 2002; Yuan, Wang, & Liu, 2007; Zhang, Cai, Tao, Yuan, & Jiang, 2021; Li, Xie, Zhou, & Luo, 2006). Due to its richness in *Ganoderma* triterpenoids, GLSO can be regarded as the "mainstay" of *Ganoderma* spores against diseases and has a higher biological activity and medicinal value than the *Ganoderma* spores themselves (Luo et al., 2021; Wang et al., 2005). GLSO is a yellow to golden translucent liquid at room temperature and is mainly available in capsule form (Zhang, 2013) (Fig. 1). GLSO has good health functions, and its market demand potential is huge. GLSO has become a hot spot in health care drug research due to its excellent stability, safety and broad application prospects. As a listed health product, the main functions of GLSO are now focused on two areas, one is to improve immunity and the other is to protect against chemical liver damage. The vast majority of companies are declaring their products as "immunity boosting". However, in our research on the effects of GLSO on acute liver injury and immunity caused by CCl_4, ethanol and drugs, we found that GLSO has a significant protective effect on the liver. In particular, in studies on chemical liver injury, including acute liver injury caused by CCl_4 and ethanol, GLSO has shown better effects. Therefore, this paper provides some suggestions and references for comprehensive development or expansion of new applications through a comprehensive review of the current uses and mechanisms of GLSO.

2. Extraction

GLS have a tenuous shell that need to be broken to extract their lipid content. The wall-breaking process enhances both the oil yield and the absorption of the active ingredients in GLS in the human body (Li et al., 2015; Liu et al., 2002). The wall-breaking methods include biological methods (e.g., enzymatic digestion and sprouting) (Tian et al., 2016; Xia et al., 2005), chemical methods (e.g., solvent extraction and acid-base degradation) (Zhang et al., 2012), and physical–mechanical methods (e.g., low-temperature freezing, ultra-micro crushing and impact) (Xiao et al., 2015; Yang et al., 2010; Zhou et al., 2002). Physical and mechanical methods are commonly used among single methods. However, they cannot completely destroy the walls of GLS due to their small size and hardness. Therefore, a combination of methods

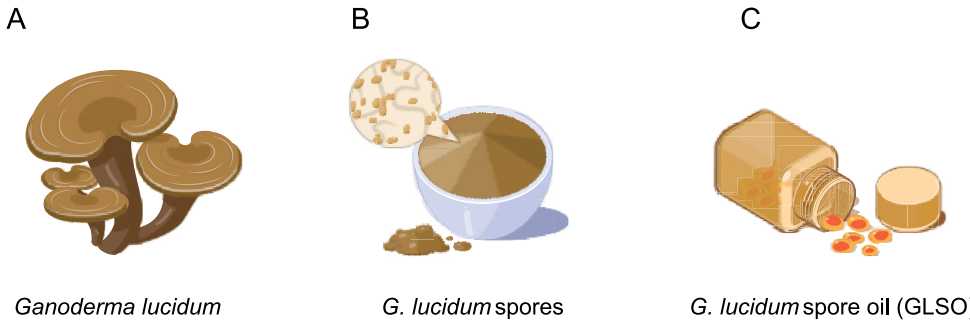

Fig. 1. Characteristics of *G. lucidum* (A), *G. lucidum* spores (B), and *G. lucidum* spore oil (C).

is usually applied. For example, GLS were broken by germination, ultra-micro pulverization and enzymatic digestion, achieving a wall-breaking rate of 85.38% (Li, Liang, He, & Qian, 2008; Li, Zhang, & Li, 2008). Moreover, cellulase pretreatment with high pressure air impact crushing method and variable temperature differential pressure embrittlement with ultrafine crushing method increased the wall-breaking rate of GLS to over 99% (Xiao et al., 2015; Zhou et al., 2012). These results indicate that the combined method is more effective in breaking the wall and maximizing the use of the active ingredients in GLS.

GLSO is mainly extracted by aqueous enzymatic method, Soxhlet extraction method, ultrasonic extraction method and supercritical CO_2 extraction method (Li et al., 2016; Wang et al., 2006; Yang et al., 2018). Supercritical CO_2 extraction technology is preferred for producing GLSO because it has high oil yield, low energy consumption, no pollution and prevents spore oil oxidation. This technology uses supercritical fluid (CO_2) as extractant to separate the extract from the mixture (Han et al., 2016). Several studies have reported different optimal extraction conditions of GLSO by supercritical technology. We found that the best conditions for achieving high extraction quality and oil yield were: extraction pressure 14 MPa, extraction temperature 34 °C, separation pressure (5.5 ± 0.2) MPa, separation temperature 32 °C, CO_2 flow rate 25 L/h and extraction time 270 min (Liu et al., 2009; Mao et al., 2012; Zhu et al., 2009). Adding entrainer ethanol as a related variable to optimize the extraction process can improve the ability of supercritical CO_2 to extract polar compounds and increase the oil extraction rate and clarity (Li, Zhang, & Liu, 2016; Li, Liang, He, & Qian, 2008; Li, Zhang, & Li, 2008). Moreover, using static expansion process before supercritical CO_2 dynamic circulation extraction can also obtain the desired extraction results with an extraction rate over 90% (Luo et al., 2008). Processing GLSO can protect unsaturated fatty acids from being degraded by air oxidation and ensure its quality. GLSO is usually marketed as processed soft capsules (Yang, 2014). Microencapsulation of GLSO with gelatin and arabic gum or soy protein isolate and maltodextrin as capsule materials is another new technology to preserve its active substances (Gao et al., 2014; Li et al., 2021). Processing GLSO can also reduce the loss of active ingredients and facilitate their storage, which broadens the application field of GLSO.

3. Bioactive components

GLSO has three main bioactive components: fatty acids, steroids and triterpenoids. Unsaturated fatty acids are the main and most important components of GLSO, as they enable GLSO to have various pharmacological effects.

3.1. Fatty acids

GLSO mainly contains triglycerides (74.25%), which are important for quality control of GLSO (Liu, 2017). Four triglycerides (**1**–**4**) (Table 1 and Fig. 2) were isolated and identified from GLSO using modern chromatographic and wave spectrometry techniques (Zhou, 2012). Lu et al. further studied the glycerol composition of GLSO and isolated five more triglycerides (**5**–**9**) (Lu et al., 2013). Monoarachidin (**10**) was also reported in GLSO (Li et al., 2011). Methylating GLSO can yield free fatty acids, which can make up to 94% of GLSO (Wang et al., 2017). Thirty-seven fatty acid components (**11**–**47**) have been isolated and identified from GLSO (Chen & Wu, 2006; Gao et al., 2012; Li et al., 2011; Liu et al., 2007; Meng et al., 2013; Tang et al., 2017; Tian & Li, 2003; Wang et al., 2006; Zhou, 2012). The main fatty acid components of GLSO are C18:1 (oleic acid), C16:0 (palmitic acid), C18:2 (linolenic acid) and C18:0 (stearic acid) (Chen et al., 2013; Liu et al., 2007; Tian & Li, 2003). Unsaturated fatty acids such as oleic acid and linoleic acid have various functions such as preventing thrombosis and arteriosclerosis, purifying blood, eliminating cell free radicals and preventing fatty liver. Fukuzawa et al. found that C_{19} fatty acids extracted from GLS inhibited the proliferation of human cancer cell lines. They also identified nonadecanoic acid and *cis*-9-nonadecenoic acid as anti-tumor compounds from 19-carbon fatty acids (Fukuzawa et al., 2008; Gao et al., 2012). Unsaturated fatty acids account for more than 60% of GLSO (Tian & Li, 2003). Therefore, the rich unsaturated fatty acids in GLSO are highly related to its extensive pharmacological activities.

3.2. Triterpenoids

Triterpenoids are important active ingredients in GLSO and have higher concentrations in spore oil than in GLS powder (Yang & Zhu, 2011). An early study reported that the maximum content of triterpenoids in GLSO extracted by supercritical CO_2 was 30.25% (Wang et al., 2006). More than 150 triterpenoids have been isolated from GLS and spore powder, but they are highly lipid soluble and hard to isolate from GLSO (Ma et al., 2011; Zhang, 2013). Thus, few studies have isolated and identified triterpenoids in GLSO. Triterpenoids have complex structures, mainly tetracyclic triterpenoids with lanostane as the basic structure and some pentacyclic triterpenoids (Fig. 3) (Zhang, 2013). The triterpenoid *G. lucidum* acid has various physiological activities, especially antitumor, due to its diverse functional groups and complex structures. Therefore, exploring the important medicinal value of GLSO requires in-depth research on the species and structures of *G. lucidum* triterpenoids.

3.3. Sterols

Ergosterol is the main form of sterols in GLSO and an important phytosterol. It affects the integrity and fluidity of fungal cell membrane, the activity of membrane-bound enzymes and the transport of cellular substances (Deng et al., 2001; Li et al., 2016). *G. lucidum* sterol has both free and esterified ergosterol forms, with free

Table 1
Summary of fatty acid composition in GLSO.

No.	Compounds	Chemical formulas	Molecular weights	References
1	1,3-Dipalmitoyl-2-oleoyl-glycerol	$C_{53}H_{100}O_6$	833.36	Zhou, 2012
2	1-Stearoyl-2-oleoyl-3-palmitoyl-glycerol	$C_{55}H_{104}O_6$	861.41	Zhou, 2012
3	1,2-Dioleoyl-3-palmitoyl-glycerol	$C_{55}H_{102}O_6$	859.39	Zhou, 2012
4	1,2,3-Trioleoyl-glycerol	$C_{57}H_{104}O_6$	885.43	Zhou, 2012
5	1-Stearoy-2,3-trioleoyl-glycerol	$C_{57}H_{106}O_6$	887.45	Lu et al., 2013
6	1-Dipalmitoyl-2-oleoyl-3-*O*-(9,12*Z*-octadecadienyl)-glycerol	$C_{55}H_{100}O_6$	857.38	Lu et al., 2013
7	1,2-Trioleoyl-3-*O*-(9,12*Z*-octadecadienoyl)-glycerol	$C_{57}H_{102}O_6$	883.42	Lu et al., 2013
8	1-*O*-(11*Z*-Octadecenyl)-2,3-trioleoyl- glycerol	$C_{57}H_{104}O_6$	885.43	Lu et al., 2013
9	1-*O*-(11*Z*-Octadecenyl)-2- trioleoyl-3-*O*-(9,12*Z*-octadecadienoyl)-glycerol	$C_{57}H_{102}O_6$	883.42	Lu et al., 2013
10	Monoarachidin	$C_{23}H_{46}O_4$	386.6	Li et al., 2011
11	Dodecanoic acid	$C_{12}H_{24}O_2$	200.32	Tian & Li, 2003
12	Tridecanoic acid	$C_{13}H_{26}O_2$	214.34	Tian & Li, 2003
13	Myristic acid	$C_{14}H_{28}O_2$	228.37	Tian & Li, 2003
14	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	Tian & Li, 2003
15	Palmitoleic acid	$C_{16}H_{30}O_2$	254.41	Tian & Li, 2003
16	Palmitic acid	$C_{16}H_{32}O_2$	256.42	Tian & Li, 2003
17	*cis*-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	268.43	Tian & Li, 2003
18	Heptadecanoic acid	$C_{17}H_{34}O_2$	270.45	Tian & Li, 2003
19	Linolic acid	$C_{18}H_{32}O_2$	280.45	Tian & Li, 2003
20	Oleic acid	$C_{18}H_{34}O_2$	282.46	Tian & Li, 2003
21	Stearic acid	$C_{18}H_{36}O_2$	284.48	Tian & Li, 2003
22	*cis*-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310.51	Tian & Li, 2003
23	Eicosanoic acid	$C_{20}H_{40}O_2$	312.53	Tian & Li, 2003
24	11(*Z*)-Docosenoic acid	$C_{22}H_{42}O_2$	338.57	Tian & Li, 2003
25	Docosanoic acid	$C_{22}H_{44}O_2$	340.58	Tian & Li, 2003
26	Tricosanoic acid	$C_{23}H_{46}O_2$	354.61	Tian & Li, 2003
27	Nervonic acid	$C_{24}H_{46}O2$	366.62	Tian & Li, 2003
28	Tetracosanoic acid	$C_{24}H_{48}O_2$	368.64	Tian & Li, 2003
29	1,3,5-Cycloheptatriene	C_7H_8	92.14	Chen & Wu, 2006
30	Hexyl octyl ether	$C_{14}H_{30}O$	214.39	Chen & Wu, 2006
31	Ethyl nonanoate	$C_{11}H_{22}O_2$	186.29	Chen & Wu, 2006
32	Tridecanol	$C_{13}H_{28}O$	200.36	Chen & Wu, 2006
33	*Z,Z*-10,12-Hexadecadienoic acid	$C_{16}H_{28}O$	236.39	Chen & Wu, 2006
34	Ethyl hexadecanoate	$C_{18}H_{36}O2$	284.48	Chen & Wu, 2006
35	Linolenic acid	$C_{18}H_{30}O_2$	278.43	Chen & Wu, 2006
36	9,12,15-Octadecatrien-1-ol	$C_{18}H_{32}O$	264.45	Chen & Wu, 2006
37	Arachidonic acid	$C_{20}H_{32}O_2$	304.47	Wang et al., 2006
38	Methyl linoleate	$C_{19}H_{34}O_2$	294.47	Liu et al., 2007
39	*cis*-10-Heptadecenoic acid	$C_{17}H_{32}O_2$	268.43	Liu et al., 2007
40	Dibutyl terephthalate	$C_{16}H_{22}O_4$	278.34	Li et al., 2011
41	Methyl hexacosanoate	$C_{27}H_{54}O_2$	410.72	Zhou, 2012
42	Nonadecanoic acid	$C_{19}H_{38}O_2$	298.5	Gao et al., 2012
43	10-*cis*-Nonadecenoic acid	$C_{19}H_{36}O_2$	296.49	Gao et al., 2012
44	Phthalic acid	$C_8H_6O_4$	166.13	Meng et al., 2013
45	*L*-Ascorbic acid	$C_6H_8O_6$	176.12	Meng et al., 2013
46	Methyl tetracosanoate	$C_{25}H_{50}O_2$	382.66	Tang et al., 2017
47	Timnodonic acid	$C_{20}H_{30}O_2$	302.45	Tang et al., 2017

ergosterol being the major part of GLSO (de Sio et al., 2000; Yuan et al., 2007). The content of ergosterol in GLSO ranges from 0.65 to 2.79 mg/g and can be used as a quality indicator for GLSO products (Wang et al., 2016; Yuan et al., 2006). Fourteen sterols (**48**–**60**) have been isolated from GLSO (Table 2 and Fig. 4) (Ge et al., 2017; Liao, 2016; Yu, 2014; Zhou, 2012). The sterols in GLSO also have potential health benefits such as anti-inflammatory, anti-aging and antitumor effects (Subbiah & Abplanalp, 2003; Xu et al., 2021; Zhang, Mills, & Nair, 2002). Therefore, studying the sterol component of GLSO is very important.

4. Pharmacological effects

4.1. Antitumor

The improvement of living standards in recent years has also increased the incidence of cancer. The standard clinical treatments are surgery, radiotherapy and chemotherapy. However, these treatments can have side effects that can lower the quality of life of patients. *G. lucidum* has been shown to have a wide range of anti-cancer effects which is used as an adjunctive therapy to reduce pain and improve quality of life for patients (Ahmad, 2020; Jiang, Slivova, Valachovicova, Harvey, & Sliva, 2004; L. Jin, Huang, Wu, Li, & Chen, 2016; Jin, Ruiz Beguerie, Sze, & Chan, 2016; Lu et al., 2004; Tang et al., 2020; Wu, Ye, Xu, Zhang, & Tang, 2018; Zhao & He, 2018; Zhong et al., 2023). GLSO also has various anticancer effects. The current evidence shows that GLSO acts against cancer through four main pathways: activating apoptosis-related molecules to induce tumor cell death, blocking tumor cell spread by inhibiting blood vessel formation, killing cancer cells by boosting immune function and regulating genes related to tumor growth.

4.1.1. Apoptosis

The active lipids of GLSO have been shown to be a promising adjuvant therapy for malignant hematological diseases. GLSO induces apoptosis in human monocytic leukemia cells (THP-1) by inhibiting the ERK1/2 and Akt pathways, activating the JNK1/2 pathway, and triggering Caspase-3, -8, and -9 activation (Wang et al., 2012). Studies have compared the anti-tumor effects of GLSO

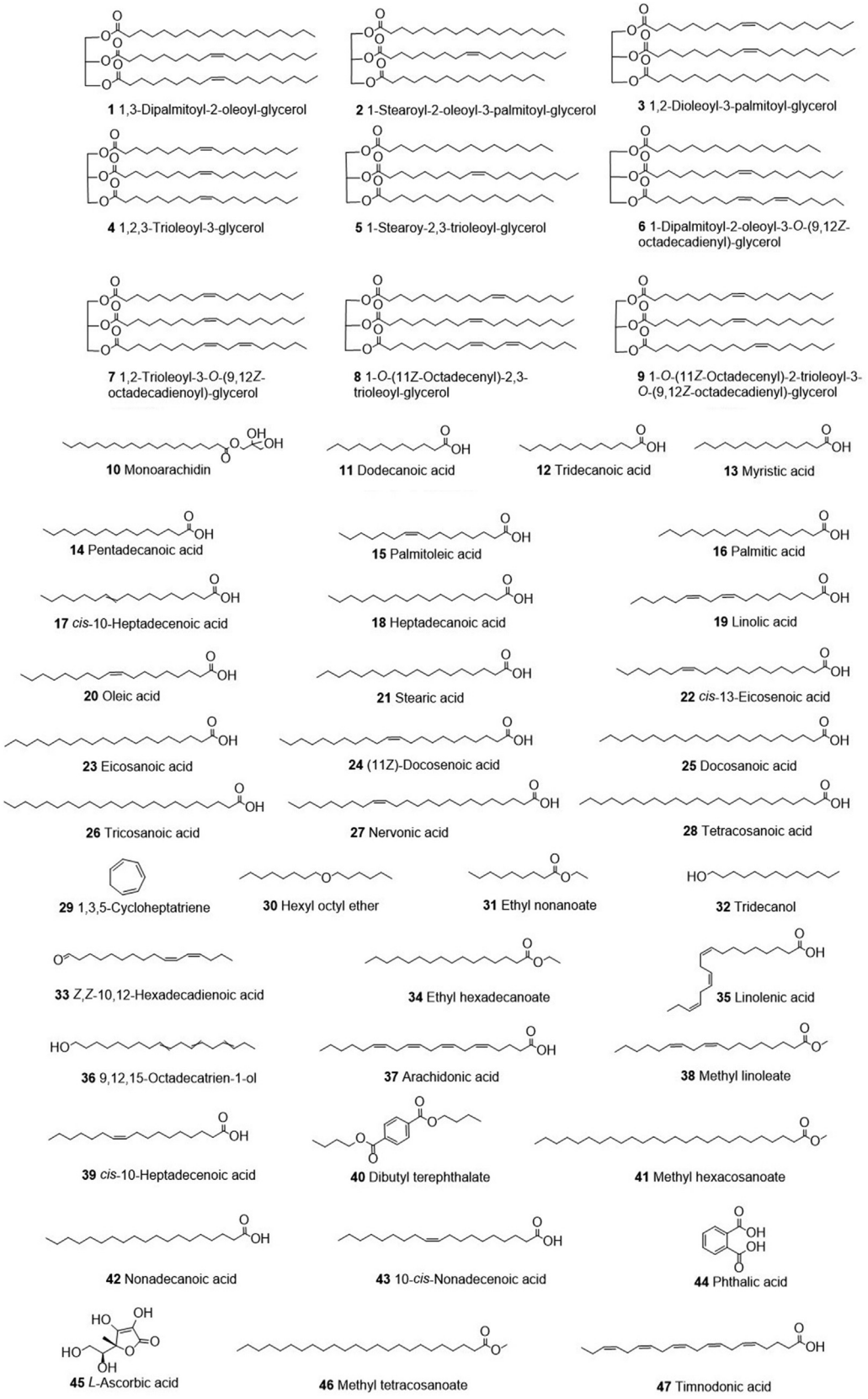

Fig. 2. Chemical structures of fatty acids from GLSO.

on various human cancer cell lines, including hepatocellular carcinoma (HepG2), non-small cell lung adenocarcinoma (A549), and colorectal adenocarcinoma (HCT116). Results showed that GLSO inhibited these tumor cells by activating the NF-κB pathway to increase inflammatory damage and by activating the Caspase-3 apoptotic pathway to accelerate tumor cell death (Peng et al.,

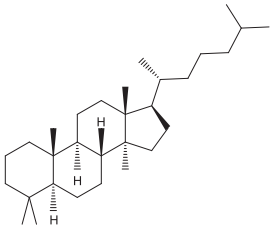

Fig. 3. Basic chemical structure of lanosterane triterpenes.

2019). Another possible mechanism is that GLSO-triggered apoptosis may be linked to an NF-κB-induced inflammatory response causing reactive oxygen species (ROS) accumulation and indirectly triggering apoptosis. Additionally, GLSO has been shown to promote tumor cell autophagy or inhibit tumor regeneration in human lung adenocarcinoma cells (SPC-A1) by down-regulating miR-21 and up-regulating PTEN and PDCD4 (Zhao, Guo, Zhao, Wang, & Hou, 2011). GLSO may also induce Bcl-2-mediated apoptosis by down-regulating miR-21 expression (Si et al., 2007). Furthermore, GLSO has been found to treat breast cancer by regulating the mitochondrial apoptosis pathway, specifically by up-regulating Bax, Caspase-3, and Caspase-9 while down-regulating XIAP in breast adenocarcinoma (MDA-MB-231) (Jiao et al., 2020). The modification of GLSO using modern nanotechnology can achieve powerful anti-tumor effects. A 40 nm GLSO nanosystem (40 nm-GLSO@NEs) designed by high-pressure homogenization can prevent cancer cell migration and invasion and induce cancer cell death through cell cycle arrest mediated by the p53 signal and apoptosis mediated by Caspase-3 and Caspase-9 (Dai et al., 2021). Primary tumor cells were isolated from the cancerous pleural fluid of 12 lung adenocarcinoma patients. After GLSO intervention, tumor cell inhibition was observed at 48.7%, mainly due to the upregulation of the *Bax* gene and the downregulation of the *Bcl-2* gene by GLSO (Lü et al., 2011). In conclusion, apoptosis is an important defense mechanism in cancer prevention and treatment, and GLSO acts against cancer by directly or indirectly regulating complex apoptosis signaling pathways (Fig. 5A).

4.1.2. Angiogenesis

Vascular endothelial growth factor (VEGF) is a highly specific glycoprotein that promotes the migration, proliferation, and angiogenesis of vascular endothelial cells. It is a key participant in tumor vascular growth and has become a key target for anti-tumor therapy. Tumor neovascularization provides nutrition and metastasis channels for solid tumors, making it crucial for tumor occurrence, infiltration, and metastasis. *In vitro* studies have reported that GLSO inhibited the proliferation of human lung adenocarcinoma (LTEP-a2) cells in a time- and dose-dependent manner by promoting the expression of the tumor suppressor miR-16 and inhibiting the expression of Bcl-2 and VEGF (Wang et al., 2011). In gastric adenocarcinoma (BGC823) cells, GLSO inhibited tumor cell proliferation and migration and induced apoptosis by down-regulating VEGF, Bcl-2, matrix metalloproteinase-2 (MMP2), and matrix metalloproteinase-9 (MMP9) gene expression while up-regulating tissue inhibitor of metalloproteinase-2 (TIMP-2) and *Bax* gene expression (He et al., 2011). Additionally, GLSO has been shown to inhibit the activation and transcription activity of the androgen receptor (AR) in prostate cancer by inhibiting protein kinase D phosphorylation, thereby blocking dihydrotestosterone (DHT)-induced proliferation of prostate carcinoma (LNCaP) cells (Zhang et al., 2015). However, the exact mechanism by which protein kinase D and AR inhibit LNCaP cell proliferation remains unclear. In a mouse model bearing H22 tumor cells, GLSO intervention inhibited tumor tissue growth in a dose–effect relationship by suppressing VEGF production in tumor tissues and inhibiting vascular endothelial cell proliferation and migration (Bian et al., 2007). GLSO has also been shown to impede angiogenesis around human-derived highly malignant breast cancer in nude mice by inhibiting epithelial growth factor receptor (EGFR) and VEGF expression while promoting thrombospondin-1 (TSP-1) expression (Zhang et al., 2014). VEGF binds to its corresponding receptors and induces vascular regeneration through the PI3K/AKT or Raf/MEK/ERK pathway (Karar & Maity, 2011; Yang et al., 2008). However, the specific pathway by which GLSO blocks vascular regeneration by inhibiting VEGF remains unclear. In conclusion, inhibiting VEGF expression has become a new approach to tumor treatment and is one of the important anticancer mechanisms of GLSO (Fig. 5B).

4.1.3. Immunity boosting

Improving the immune system's ability to fight cancer is a promising approach to anticancer therapy. Several experimental studies have investigated the specific anti-tumor mechanisms of GLSO from an immunological perspective. In a preliminary study on the treatment of hepatoma ($HepG_2$) cells cultured *in vitro* with GLSO, tumor cells showed dose- and time-dependent inhibition of proliferation, down-regulation of *VEGF*, *Bcl-2*, and *Bax* gene expression, and changes in Toll-like receptor expression on the cell surface (Sun et al., 2011). GLSO has been shown to activate monocytes/macrophages differently from lipopolysaccharide (LPS)-related mechanisms at low concentrations, promoting the production of pro-inflammatory factors such as tumor necrosis factor-α (TNF-α) and interleukin-6/10 (IL-6/10) and the expression of antigen presentation-related molecules such as antigen differentiation cluster 86 (CD86) and human leukocyte DR antigen (HLA-DR), effectively exerting anti-tumor immune effects (Zhang,

Table 2
Summary of sterol components in GLSO.

No.	Compounds	Chemical formulas	Molecular weights	References
48	Ergosterol	$C_{28}H_{44}O$	396.66	Zhou, 2012
49	Ergosterol peroxide	$C_{28}H_{44}O_3$	428.65	Zhou, 2012
50	Ergosta-7-en-3*β*-ol	$C_{28}H_{48}O$	400.68	Yu, 2014
51	Ergosta-4,6,8(14),22-tetraen-3-one	$C_{28}H_{40}O$	392.62	Yu, 2014
52	*β*-Sitosterol	$C_{29}H_{50}O$	414.71	Yu, 2014
53	Isofucosterol	$C_{29}H_{48}O$	412.69	Yu, 2014
54	Ergosta-7,22-diene-3*β*,5*α*,6*β*-triol	$C_{28}H_{46}O_3$	430.66	Yu, 2014
55	Ergosta-7,22-diene-3*β*,5*α*,6*α*-triol	$C_{28}H_{46}O_3$	430.66	Yu, 2014
56	(22*E*,24*R*)-Ergosta-5*α*,6*α*-epoxide-8,22-diene-3*β*,7*α*-diol	$C_{28}H_{44}O_3$	428.65	Yu, 2014
57	Ganoderin A	$C_{28}H_{47}O_4$	447.67	Ge et al., 2017
58	Chaxine B	$C_{28}H_{42}O_5$	458.63	Ge et al., 2017
59	Stellasterol	$C_{28}H_{46}O$	398.67	Ge et al., 2017
60	Ergosta-4,6,8(14),22-tetraen-3-one	$C_{28}H_{40}O$	392.62	Liao, 2016

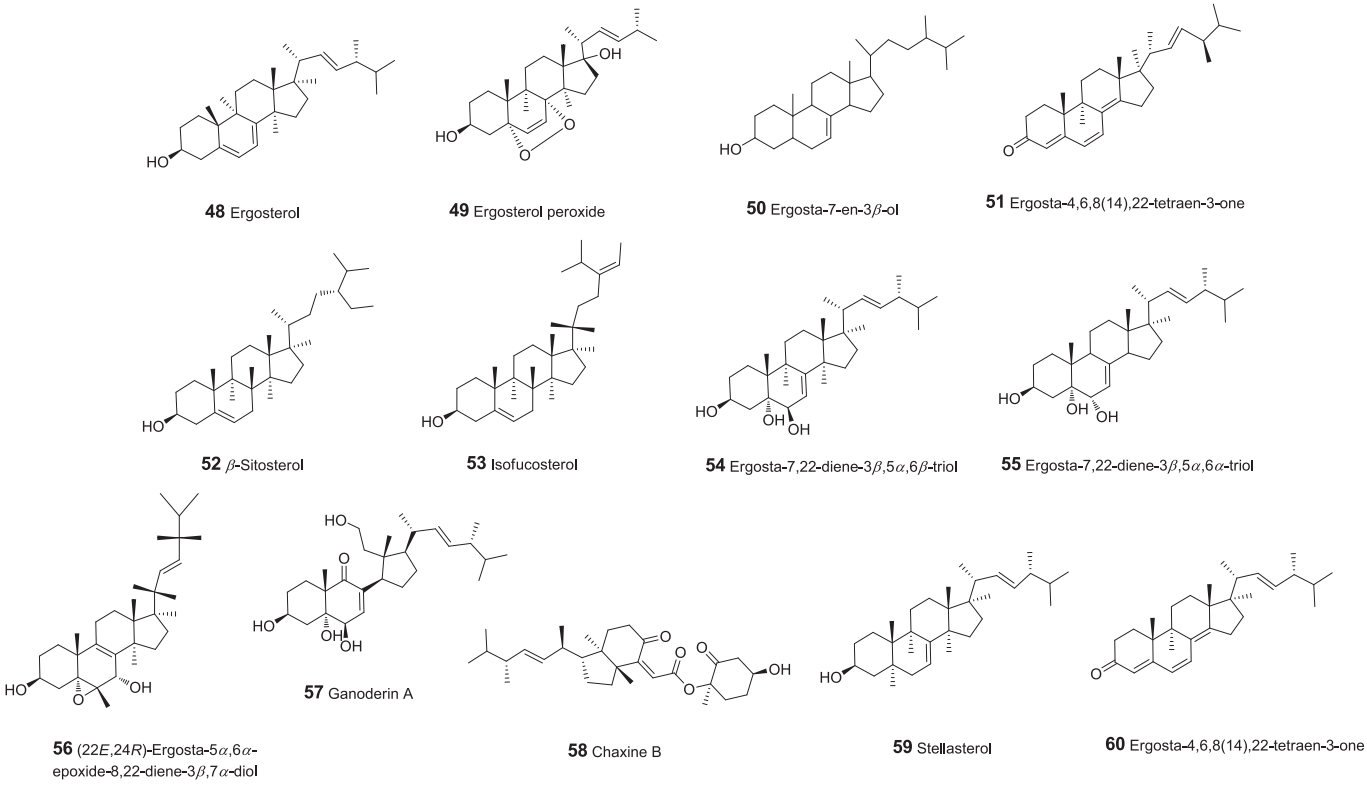

Fig. 4. Chemical structures of sterols from GLSO.

Zheng, Wang, Magnusson, & Liu, 2009). *In vivo* studies have shown that gavage administration of low, medium, and high doses of GLSO to H22 tumor-bearing mice resulted in inhibition of tumor growth and a significant increase in survival by improving the immune function of tumor-bearing mice. This was achieved by improving spleen swelling, increasing lymphocyte and $CD4^+$ T cell ratios in peripheral blood, enhancing CD80 and CD86 molecule expression on dendritic cell (DC) surfaces, and facilitating cancer cell antigen presentation by DCs to antigen-presenting cell (APC) cells (Nie et al., 2010). This initiates T cell-mediated cellular immunity to kill cancer cells and provides an adjuvant basis for the anti-tumor effects of GLSO. Further *in vivo* experiments have shown that GLSO effectively inhibited tumor growth, increased macrophage phagocytosis and T-cell immunity in liver cancer mice, and significantly increased cytokine levels such as gamma interferon (IFN-γ) and TNF-α in liver cancer mouse blood (Jin et al., 2011). GLSO also improved leukocyte and platelet levels and immune organ quality in S180 tumor-bearing mice treated with *trans*-fluorouracil, indirectly promoting chemotherapeutic agent tumor suppression by restoring immune function (Chen et al., 2008). In conclusion, enhancing liver cancer mouse immune function through various channels is an important mechanism for GLSO's anti-hepatocellular carcinoma efficacy (Fig. 5C).

4.1.4. Genomic DNA stability

DNA topoisomerases are enzymes involved in regulating DNA supercoiling and are responsible for DNA replication, transcription, translation, recombination, and chromosome separation. Inhibition of topoisomerase activity can lead to abnormal DNA structures, blocking replication forks, and inhibiting tumor cell proliferation. *In vitro* and *in vivo* studies have confirmed the anti-tumor effect of GLSO on mice inoculated with primary 180 sarcoma cells and H22 liver cancer cells and on human cancer cell lines such as acute and chronic leukemia (HL60 and K562) cells and gastric carcinoma (SGC-7901) cells. This effect is related to the inhibition of topoisomerase I and II activity and reduction of cell cycle protein D1 levels in K562 cells, inducing G1 arrest in the cell cycle (Chen et al., 2016). Telomerase, a nuclear protein reverse transcriptase responsible for telomere elongation in cells, adds telomeric DNA to eukaryotic chromosome ends, repairs telomeres lost during DNA replication, keeps cells dividing, and plays a decisive role in tumor cell growth and proliferation. The anti-tumor effect of GLSO on transplanted mouse liver cancer has been shown to be related to the inhibition of hepatoma cell telomerase activity in tumor-bearing mice (Liu, Zhong, Yuan, & Huang, 2000; Yuan, Liu, & Zhong, 2000). In summary, GLSO inhibits tumorigenesis at a fundamental level by affecting DNA replication through multiple pathways and blocking tumor cell division and proliferation (Fig. 5A). Regulation at the gene level is key to GLSO's remarkable anti-tumor effect. However, the exact mechanisms of DNA regulation by GLSO remain unclear. Further research is needed to determine which specific components of GLSO regulate genomic DNA stability to disrupt tumor cell proliferation.

According to the current research, most researchers have adopted the *in vitro* experiment of cancer cell lines and the *in vivo* experiment of tumor-bearing mice to study the anti-cancer mechanism of GLSO. We summarize the *in vitro* and *in vivo* experiments on the anticancer effects of GLSO, respectively (Tables 3 and 4, Fig. 5).

4.2. Protecting nervous system

GLSO has a certain effect on the treatment of nervous system diseases. Regarding the mechanism of GLSO on Parkinson's disease (PD), Zhu's team has done extensive research. In the early stage, the experimental group made a preliminary study on the effects

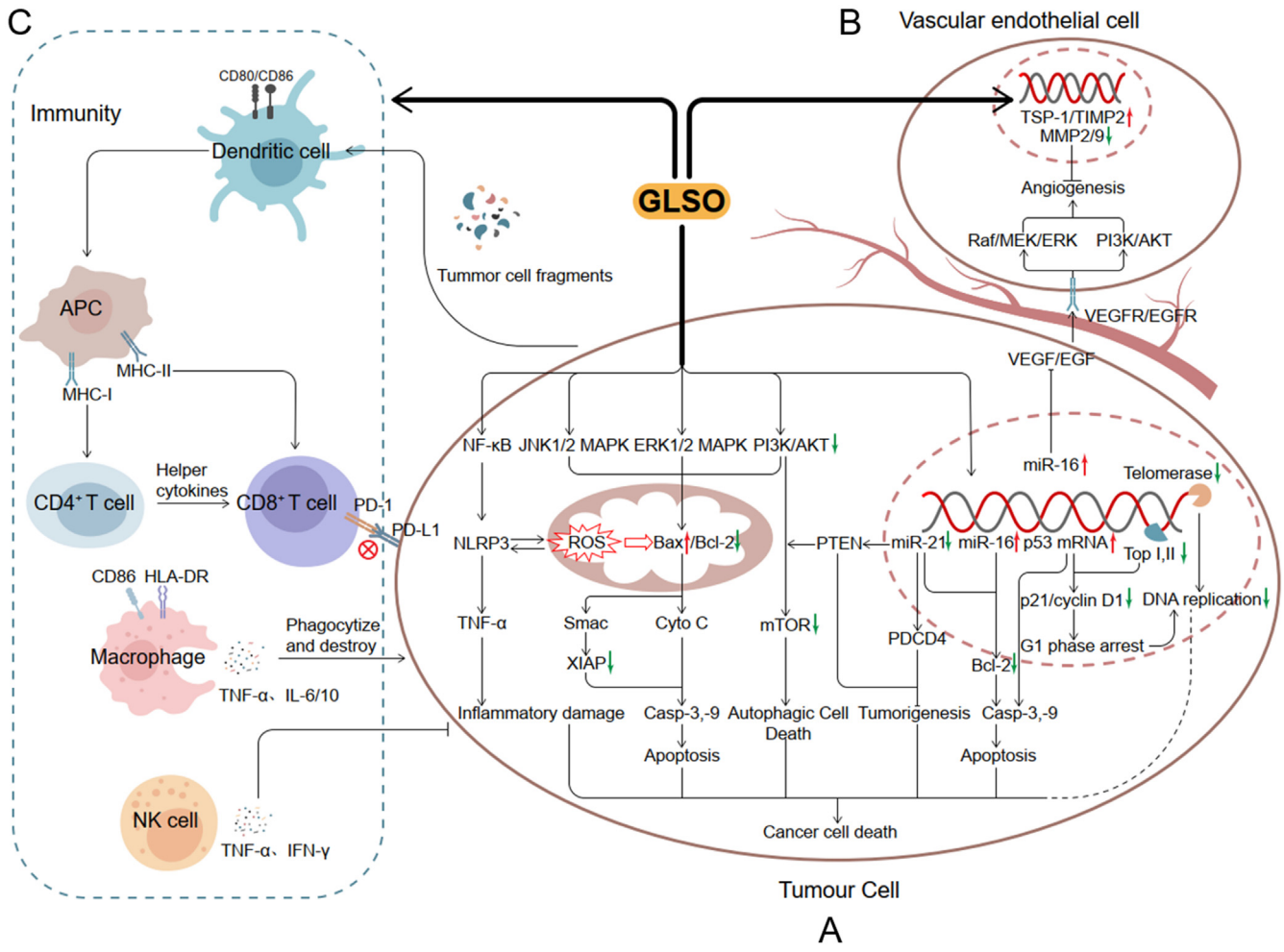

Fig. 5. Antitumor mechanisms of GLSO. (A) GLSO induces cancer cell death through promoting apoptosis pathway, inflammation damage and cell autophagy to inhibit tumor regeneration. (Apoptosis pathway: JNK1/2, ERK1/2, PI3K/AKT, Bax/Bcl-2, Smac, XIAP, miR21, miR-16, p53 mRNA, Caspase-3, -9; Inflammation pathway: NF-κB/NLRP3, TNF-α; Autophagy pathway: PI3K/AKT, mTOR, miR21, PTEN; Tumorigenesis pathway: miR21, PTEN, PDCD4.); GLSO induces cancer cell death through regulating DNA function. (DNA regulation pathway: p53 mRNA, Top I/II, cyclin-dependent kinase inhibitor 1(p21), cell cycle protein D1(Cyclin D1)). (B) GLSO affects tumor cell growth and migration by inhibiting tumor angiogenesis. (C) GLSO exerts its antitumor effects by enhancing immune function.

of GLSO on the rat and mouse models of 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and PD. Based on the behavioral investigation, the team found that GLSO can reduce the involuntary jitter behavior of rats in two model groups; through further experimental data, it is concluded that GLSO increases the content of dopamine (DA) and its metabolites in striatum and reduces the damage of dopaminergic neurons in substantia nigra compacta (Zhu, Liu, Xu, Chu, Ye, Xie, Chen, & Li, 2005; Zhu, Liu, Xu, Xie, Ye, & Chen, 2005). In order to understand the underlying mechanisms, the group has explored them in more depth in several subsequent studies.

By summarizing the subsequent research results, we know that the therapeutic mechanism of GLSO on MPTP model is embodied in the following two aspects (Fig. 6A): Firstly, GLSO can obviously inhibit the production of inflammatory factors such as TNF-α and interleukin-1β (IL-1β) induced by MPTP, reduce the inflammatory reaction of substantia nigra, and make DA neurons continue to survive (Zhu, Liu, Xu, & Ye, 2007); Secondly, MPTP activates the transcription of inducible nitric oxide synthase (iNOS) gene, which produces a large amount of NO and causes oxidative damage to neurons, while GLSO intervention can significantly reduce the expression of iNOS gene (Zhu, Liu, Xu, & Chen, 2007). In another 6-OHDA model, the expression of iNOS mRNA was not significantly altered in either the model or the administered group, but GLSO inhibited the expression of pro-apoptosis gene *Bax* and increased the expression of anti-apoptosis gene *Bcl-2*, which blocked the level of initiation of apoptosis program and effectively prevented the death of dopaminergic neurons in substantia nigra (Zhu, Liu, Xu, & Chen, 2007; Zhu, Liu, Xu, Ye, & Chen, 2007). TNF-RI is mainly expressed on neurons and glial cells. MPTP-induced TNF-α can also bind to its receptor TNF-RI, activating the FADD/Casp-8/Casp-10 complex to promote Casp-8-induced dopaminergic neuron apoptosis and IKK complex (IKKα/IKKβ/IKKγ)-mediated pro-inflammatory signaling cascade (Häcker & Karin, 2006; Mogi et al., 2000; Wang et al., 2008; Zhou et al., 2022). The protective effect of GLSO on small glial cells may also be related to the inhibition of this pathway, which requires further validation by researchers. The entry of 6-OHDA into the nigrostriatal dopaminergic neurons to generate ROS is the key to its pathogenesis, specifically the subsequent decrease in mitochondrial complex I activity and the decrease in

Table 3
In vitro studies of anticancer effects of GLSO.

Cell strains	Phenotyping	Mechanism of actions	Cancer types	References
Human THP-1 cells	Apoptosis rate ↑	ERK1/2 ↓ Akt ↓ JNK1/2 ↑ Caspase-3, -8 and -9 ↑	Leukemia	Wang et al., 2012
Human HepG2 cells Human A549 cells Human HCT116 cells	Cell viability inhibition intensity: A549 cells > HepG2 cells > HCT116 cells	NF-κB ↑ Caspase-3 ↑	Hepatocellular carcinoma Lung adenocarcinoma Colorectal adenocarcinoma	Peng et al., 2019
SPC-A1 cells	Cell proliferation ↓ Cell viability ↓ Cell atrophy Small bubbles appear at the edge of the cell membrane	miR-21 ↓ PTEN ↑ PDCD4 ↑	Lung cancer	Zhao et al., 2011
MDA-MB-231 cells	Cell proliferation ↑ Apoptosis rate ↑	Bcl-2 ↓ XIAP ↓ PARP ↓ FADD ↑ Caspase-3, Caspase-9 ↑ Bax ↑	Breast adenocarcinoma	Jiao et al., 2020
MGC803 cells	Cell survival ratio ↓ Apoptosis rate ↑ Cell migration ratio ↓ Cell invasion ratio ↓ G0/G1 cycling phase accumulation	p53 signaling-mediated cell cycle block ↑ Caspase-3 and -9 ↑ FAK ↑ uPAR ↓ MMP-2/-9 ↓ TIMP-1 ↑	Gastric adenocarcinoma	Dai et al., 2021
Primary tumor cells of lung adenocarcinoma patients	Cell viability ↓ Cell apposition ↓ Cell extension ↓ Apoptosis ratio ↑	Bax ↑ Bcl-2 ↓	Lung adenocarcinoma	Lü et al., 2011
Human LTEP-a2 cells	Cell proliferation ↓ Apoptosis rate ↑ Cell atrophy Small bubbles appear at the edge of the cell membrane	miR-16 ↑ Bcl-2 ↓ VEGF ↓	Lung adenocarcinoma	Wang et al., 2011
Human BGC823 cells	Cell viability ↓ Cell proliferation ↓ Cell migration quantity ↓ Cell atrophy Small bubbles appear at the edge of the cell membrane		Gastric adenocarcinoma	He et al., 2011
Human LNCaP cells	Cell proliferation activity ↓	Protein kinase D phosphorylates ↓ AR ↓	Prostate carcinoma	Zhang et al., 2015
MDA-MB-231 cells	Average tumor weight ↓	EGFR ↓ VEGF ↓ TSP-1 ↑	Breast adenocarcinoma	Zhang et al., 2014
Human HepG2 cells	Cell proliferation ↓ Cell migration speed ↓ Apoptosis rate ↑	VEGF ↓ Bcl-2 ↓ Bax ↓ Toll-like receptor expression ↑	Hepatocellular carcinoma	Sun et al., 2011
Human HL-60 cells Human K562 cells Human SGC7901 cells Murine S180 cells Murine H22 cells	Tumor cell growth ↓	Topoisomerase I and II ↓ Cell cycle protein D1 level ↓ G1 stagnation in cell cycle ↑	Leukemia Gastric adenocarcinoma Hepatocellular carcinoma Sarcoma	Chen et al., 2016

mitochondrial membrane potential and other mitochondrial dysfunction resulting in adenosine triphosphate (ATP) depletion and apoptosis triggered by cytochrome C release (Lu, Kim-Han, Harmon, Sakiyama-Elbert, & O'Malley, 2014). Additionally, ROS produced by 6-OHDA is also the initiator of JNK apoptosis signal system (Choi et al., 1999). GLSO may protect nerve injury by inhibiting the production of ROS, a key product of 6-OHDA, but which downstream pathway to block remains to be determined.

As the population ages, the incidence of Alzheimer's disease (AD) is increasing. Studies have shown that GLSO can alleviate AD in APP/PS-1 transgenic mice. Researchers have explained that GLSO can degrade and reduce senile plaques and neurofibrillary tangles in the brains of AD mice, alleviate amyloid angiopathy, promote the growth of naive nerve cells, and reduce the loss of Purkinje cells. At the ultrastructural level, GLSO can improve neuronal nucleoli disappearance and mitochondrial, Golgi apparatus, and endoplasmic reticulum ultrastructural damage, providing a theoretical basis for the clinical treatment of AD (Fig. 6B) (Qin et al., 2017). Additionally, GLSO has been shown to have an antidepressant effect by reducing excitotoxicity-induced hippocampal neuronal damage and promoting neuron regeneration through reducing glutamic acid (GLU) levels and increasing brain-derived neurotrophic factor (BDNF) content in the brain (Fig. 6C) (Deng et al., 2017). The mechanism by which GLSO increases BDNF levels and achieves its antidepressant effects remains to be determined. Researchers have also observed a protective effect of GLSO against methyl-nitrosourea (MNU)-induced retinal damage. GLSO can effectively inhibit Bax and Caspases-3 expression, promote B-cell lymphoma-xl (Bcl-xl) expression, block MNU-induced apoptosis

Table 4
In vivo studies of anticancer effects of GLSO.

Animal models	Phenotyping	Mechanism of actions	Cancer types	References
4 T1 cells bearing tumor	Tumor growth ↓ Tumor necrosis ↑	Cytochrome *c* ↑ Cystatin-9 ↑ Bax ↓ XIAP ↓	Breast adenocarcinoma	Jiao et al., 2020
H22 cells bearing tumor	Tumor growth ↓	VEGF ↓	Hepatocellular carcinoma	Bian et al., 2007
MDA-MB-231 cells bearing tumor	Average tumor weight ↓	EGFR ↓ VEGF ↓ TSP-1 ↑	Breast adenocarcinoma	Zhang et al., 2014
H22 cells bearing tumor	Spleen enlargement ↓ Survival rate ↑ Tumor growth ↓ $CD4^+$ T cell ratio ↑ $CD4^+/CD8^+$ T cell ratio ↑ CD80 and CD86 ↑	Immune function ↑	Hepatocellular carcinoma	Nie et al., 2010
H22 cells bearing tumor	Tumor growth ↓ Reticuloendothelial cells phagocytosis ↑ Serum hemolytic value (HC50) ↑ Ear swelling ↑ Serum IFN-γ, TNF-α levels ↑	Immune function ↑	Hepatocellular carcinoma	Jin et al., 2011
S180 cells bearing tumor	White blood cells and platelet counts ↑ Tumor growth ↓ Spleen index ↑ Thymus index ↑	Immune function ↑	Sarcoma	Chen et al., 2008
S180 and H22 cells bearing tumor	Tumor volume ↓ Average tumor weight ↓	Topoisomerase I and II activity ↓ Cell cycle protein D1 level ↓ Cell cycle G1 arrest	Sarcoma Hepatocellular carcinoma	Chen et al., 2016
H22 cells bearing tumor	–	Telomerase activity ↓	Hepatocellular carcinoma	Liu, Zhong, Yuan, & Huang, 2000; Yuan, Liu, & Zhong, 2000

in rat photoreceptor cells, and protect retinal function (Fig. 6D) (Deng, Lin, Gao, Sun, & Hong, 2009; Gao, Deng, Li, Luo, & Chung, 2011; Gao, Deng, Sun, & Zhong, 2010). GLSO has also been shown to improve learning ability in mice. In an animal model of $AlCl_3$-injected dementia in mice fed GLSO, significant improvements in learning memory were observed by the Morris water maze method and transmission electron microscopy (Shen et al., 2007). Similarly, another study found that combining GLSO with deep-sea fish oil promoted NOS expression in hippocampal neurons and significantly enhanced learning memory performance in mice (Chen et al., 2007). This may be related to retrograde messenger NO produced by nNOS acting on the presynaptic membrane to release neurotransmitters such as glutamate, generating and maintaining LTP potentials in hippocampal neurons (Fig. 6E). Based on these *in vivo* studies, GLSO can be used as an effective neuroprotective drug for treating nervous system diseases such as AD, PD, depression, and retinal injury.

4.3. Immune regulation

The immune-enhancing effect of GLSO is focused on improving the immune function of mice. GLSO has been shown to increase the phagocytosis of peritoneal macrophages, the killing effect of NK cells and the proliferation and transformation of T cells, as well as the secretion of lymphokines such as TNF-α and IL-2 by the spleen (Liang et al., 2005; Liu et al., 2006). GLSO also stimulates delayed allergic reactions in mice, increases the number of antibody-producing cells and serum hemolysin levels (Liang et al., 2005). In normal and tumor-bearing mice, GLSO injection significantly increased the phagocytosis of the reticuloendothelial system, splenic coefficient and serum IFN-γ levels (Jiang et al., 2013). In an immunocompromised mouse model established by cyclophosphamide treatment, GLSO increased levels of TNF-α and IFN-γ in serum and expressions of interleukin family IL-2/4/10/12, IFN-γ and TNF-α mRNA in the spleen and thymus (Yi et al., 2012). In a model of immune compromise in mice infected with Friend murine leukemia virus, GLSO treatment restored body mass, suppressed splenomegaly and thymic atrophy and significantly increased $CD4^+$, $CD8^+$ and $CD4^+/CD8^+$ values (Huang et al., 2010). These findings suggest that GLSO enhances both specific and non-specific immunity through various mechanisms, with the cellular immune pathway playing a dominant role in improving overall immune function in mice (Fig. 7A).

Some studies have attempted to reveal the immunomodulatory mechanisms of GLSO in the microbial metabolic axis. One study found that GLSO enhanced macrophage phagocytosis and NK cytotoxicity in mice. Further analysis revealed that GLSO caused structural rearrangements in the intestinal microbiota, triggering the regulation of key metabolites highly correlated with enhanced immune function (Fig. 7B) (Wu et al., 2020). Another study demonstrated that GLSO intervention in a mouse burn model promoted skin wound healing by reducing LPS production through modulation of the skin microbiota, reducing Toll-like receptor 4 expression, or directly reducing the expression of T-cells and leukocytes, leading to decreased levels of inflammatory cytokines (Fig. 7C) (Jiao, Xie, Yun, Liang, He, Jiang, Wu, & Yang, 2020). We found that GLSO had different regulatory effects on IL-4, IL-10, TNF-α, and IFN-γ in various studies. IL-4 and IL-10 are important anti-inflammatory cytokines that can synergistically promote B cell proliferation and immunoglobulin differentiation (Franke, Kirchenbaum, Kuerten, & Lehmann, 2020; Gao et al., 2014; Xu et al., 2004; Zhou et al., 2014). Therefore, by promoting the secretion of IL-4 and IL-10 from splenic lymphocytes, GLSO can activate immune cells such as B cells and enhance the secretion of immune mediators, thereby enhancing the body's immunity to external pathogens. However, Jiao et al. found that GLSO reduced the levels of IL-4 and IL-10 during the process of promoting the healing of skin inflammatory wounds (Jiao, Xie, Yun, Liang, He, Jiang, Wu, &

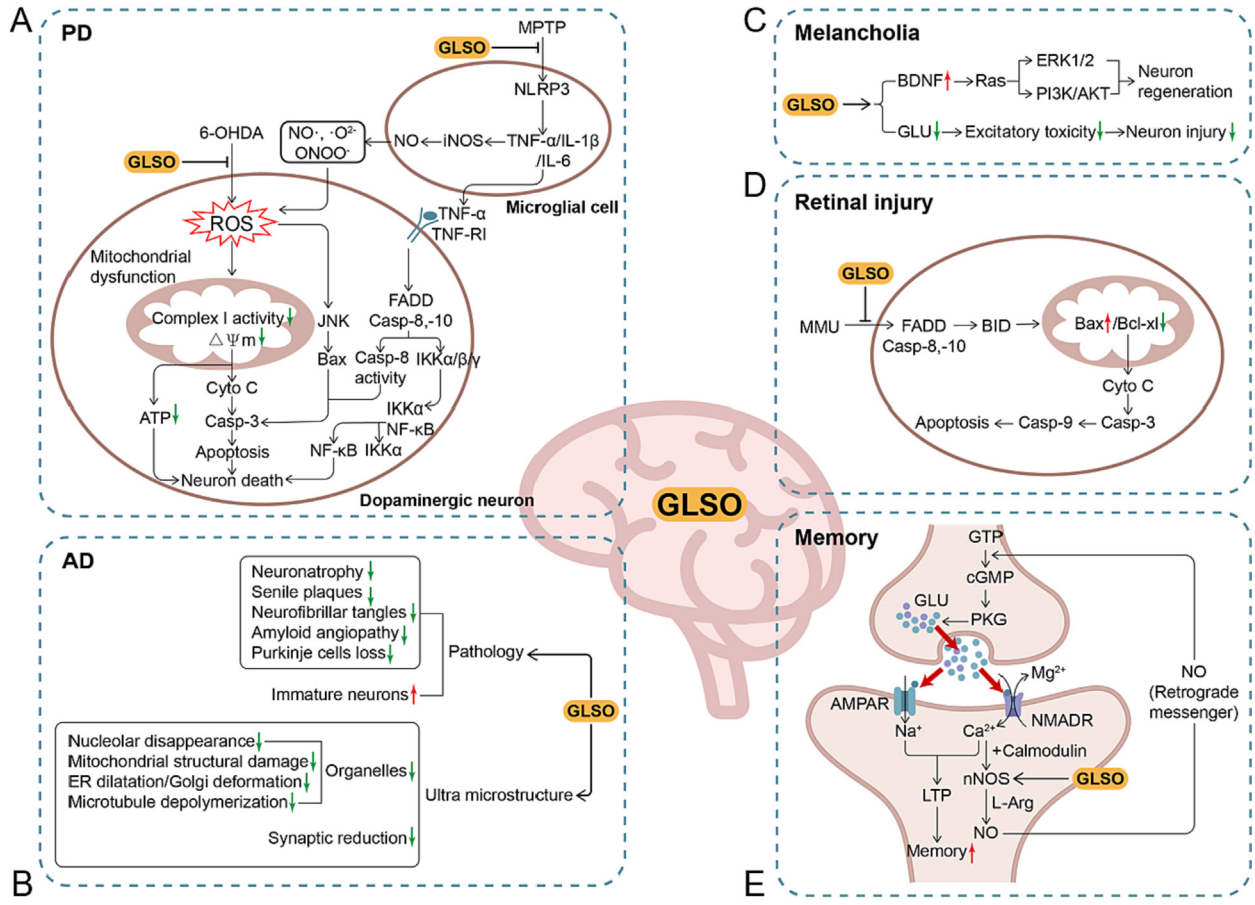

Fig. 6. Protective mechanisms of GLSO in nervous system. (A) GLSO's protection mechanisms against MPTP and 6-OHDA-induced Parkinson's disease. (B) Pathological morphology and ultrastructure changes of Alzheimer's disease by GLSO. (C) GLSO exerts its antidepressant effects by reducing GLU content and increasing BDNF content. (D) GLSO protects retina by blocking MMU-induced photoreceptor apoptosis pathway. (E) Related mechanism of GLSO in improving learning ability by maintaining and enhancing LTP.

Yang, 2020). This seems to contradict the anti-inflammatory effects of IL-4 and IL-10, so the exact role of GLSO in regulating these cytokines during skin wound healing still needs further exploration. The different changes in TNF-α and IFN-γ may involve the different molecular signaling pathways and immune regulatory effects of different active components of GLSO on different sites. IFN-γ not only promotes the activity of antigen-presenting cells but also synergizes with TNF-α to exert a cytotoxic effect on tumor cells (Gu et al., 2023; Ren et al., 2022). GLSO may promote the secretion of TNF-α and IFN-γ from splenic lymphocytes and enhance the cellular immune response through other signaling pathways, such as JAK-STAT pathway, thereby effectively combating tumor cells. TNF-α is a strong pro-inflammatory cytokine and IFN-γ can reduce collagen formation and angiogenesis during wound healing (Ishida et al., 2004). Therefore, one possible mechanism for GLSO's inhibition of TNF-α and IFN-γ secretion from skin wound lymphocytes is that it indirectly inhibits the TLR4/MyD88/NF-κB signaling pathway to reduce their relative levels and accelerate skin wound healing. Other possible mechanisms include the regulation of metabolic products such as reactive oxygen and PGE2, as well as the secretion of inhibitory factors by epithelial cells, which can reduce lymphocyte secretion of TNF-α and IFN-γ to lower the degree of inflammation and promote wound healing. In conclusion, the specific signal pathway through which GLSO differentially regulates TNF-α and IFN-γ remains to be determined.

4.4. Anti-aging

Aging is an inevitable stage in the body's metabolic process and is accompanied by a decline in antioxidant enzyme activity and an excess of free radicals. Superoxide dismutase (SOD) and catalase (CAT) are important indicators of the body's antioxidant capacity and can scavenge peroxide radicals produced by metabolism, reducing damage caused by aging. There is evidence that GLSO can play an anti-aging role through its antioxidant mechanism. In a study using Drosophila as a model, GLSO significantly extended the mean and maximum lifespan of Drosophila under normal and oxidative stress conditions. Mechanistic studies revealed that GLSO treatment promoted Cu/Zn-SOD, Mn-SOD, and CAT enzyme mRNA expression and reduced malondialdehyde (MDA) levels (Zhang et al., 2021). Another study using GLSO on *D*-galactose aging model mice showed that GLSO increased spleen and thymus indices, SOD and CAT enzyme activities, enhanced the C3 complement pathway biological defense response, and inhibited cyclin-dependent kinase inhibitor 1 (p21) expression (Hu et al., 2011). Thus, GLSO can exert anti-aging effects through both antioxidant and immune pathways. GLSO has also been found to be rich in fat-soluble vitamins such as vitamin D2, D3, and E and has strong scavenging ability for 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide anion ($\cdot O_2^-$) radicals (Chen et al., 2012). Radiation can produce large amounts of free radicals in the body, leading to lipid peroxidation and damage. GLSO has been shown

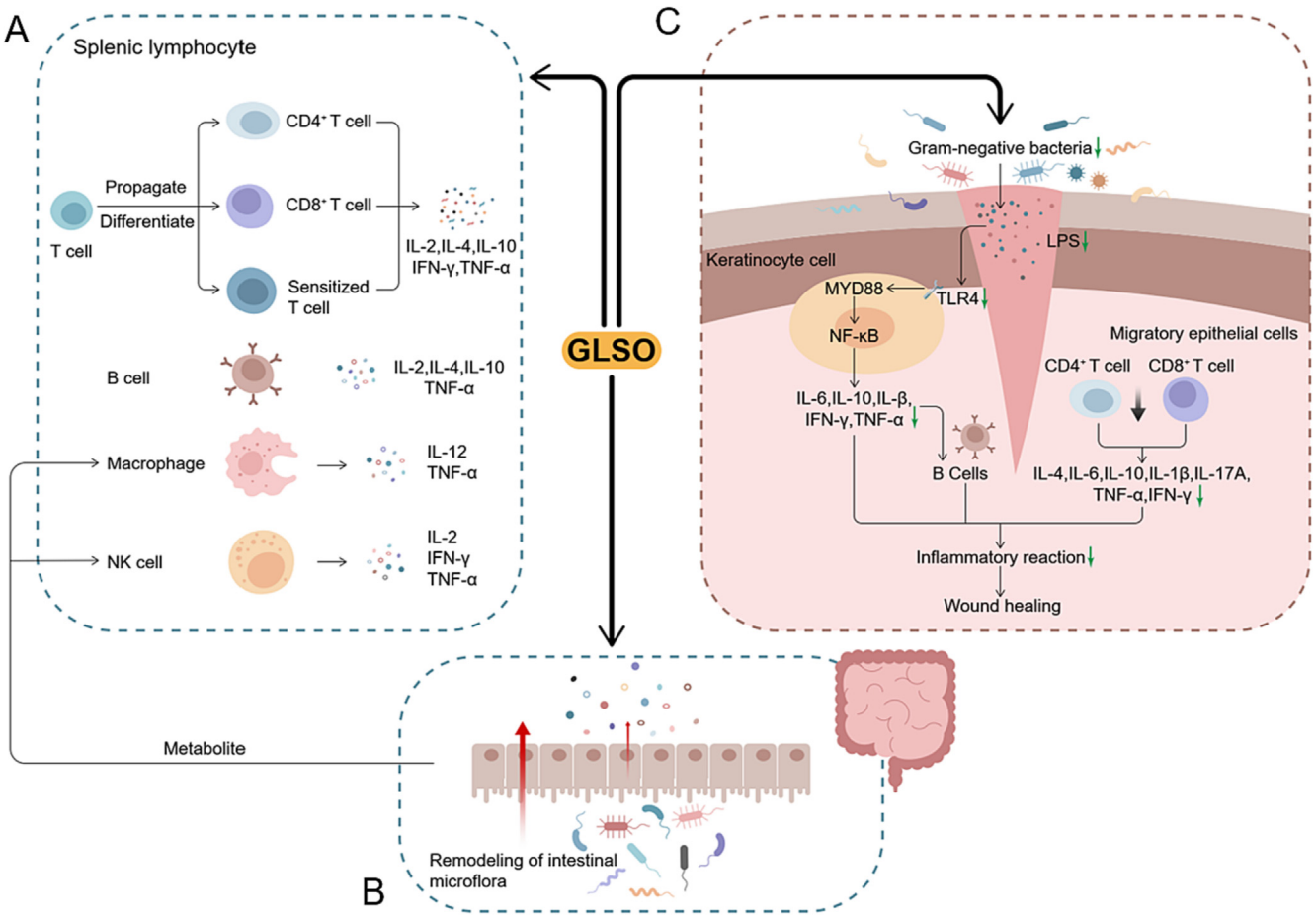

Fig. 7. Immunoregulatory mechanisms of GLSO. (A) GLSO directly enhances immune function of body. (B) GLSO enhances immune function by regulating gut microbiota. (C) GLSO promotes wound healing by regulating skin microbiota.

to have a protective effect on radiation-injured aged mice by increasing thymus/spleen coefficients, peripheral blood leukocyte counts, bone marrow cell DNA content, and liver SOD activity (Jiang et al., 2014). A GLSO@P188/PEG400 nanosystem was designed using modern molecular technology to improve GLSO water solubility and provide protection against X-ray-induced heart disease. The system's effective free radical scavenging ability reduces X-ray-induced ROS production, protects mitochondria, and maintains the body's antioxidant system balance (Dai et al., 2019). In conclusion, GLSO effectively scavenges free radicals in the body by increasing SOD and CAT enzyme activity, minimizing damage caused by ROS-induced lipid peroxide production (Fig. 8). This strongly suggests that GLSO is a promising anti-aging health product.

4.5. Hepatoprotection

The liver is the largest digestive gland in the body and an important metabolic and detoxification organ. Modern lifestyles, such as alcoholism, exposure to chemical toxins like CCl_4, and viruses threaten liver health. GLSO has been shown to have a protective effect on liver diseases caused by various etiologies. GLSO has an auxiliary protective effect on mice with acute alcoholic liver injury induced by ethanol, reducing triglyceride (TG) and MDA production during alcohol metabolism and reducing fat deposition in the damaged liver (Jin, Huang, Wu, Li, & Chen, 2016; Jin, Ruiz Beguerie, Sze, & Chan, 2016; Zhao, Hu, Li, & Chen, 2016). A trial confirmed that GLSO not only had a protective effect against *D*-aminogalactose-induced liver injury in mice but also inhibited transplanted hepatocellular carcinoma (Liu, Zhong, Yuan, & Huang, 2000; Yuan, Liu, & Zhong, 2000). Another study reported that GLSO significantly reduced carbon tetrachloride (CCl_4)-induced increases in serum glutamic alanine aminotransferase (ALT) and glutamic aspartate aminotransferase (AST) levels, suggesting a preventive effect against CCl_4-induced chemical liver injury (Li, Wu, Xi, & Zhao, 2006; Li, Xiao, Mao, & Huang, 2006; Li, Xie, Zhou, & Luo, 2006). GLSO also significantly reduced plasma ALT, AST, MDA, and hyaluronic acid (HA) levels in rats with CCl_4-induced liver fibrosis and reduced liver tissue MDA and hydroxyproline (Hyp) levels (Zhao et al., 2016). In a study of 81 outpatients or inpatients with chronic hepatitis B, the conversion rate of hepatitis B virus DNA was higher in the treatment group receiving interferon-α2b combined with GLSO capsules than in the control group receiving only interferon-α2b (Qian et al., 2005). The antagonistic effect of GLSO capsules on hepatitis may be related to the synergistic immune-enhancing effect of interferon-α2b. While the efficacy of GLSO in treating liver disease is undeniable, the literature is sparse and detailed hepatoprotective mechanisms remain to be elucidated.

4.6. Regulation of metabolic disorders

There is a relative lack of research on the effects of GLSO on metabolic diseases. However, some studies have demonstrated a lipid-modulating effect of GLSO. Li et al. demonstrated that GLSO reduced total cholesterol (TC) and high-density lipoprotein choles-

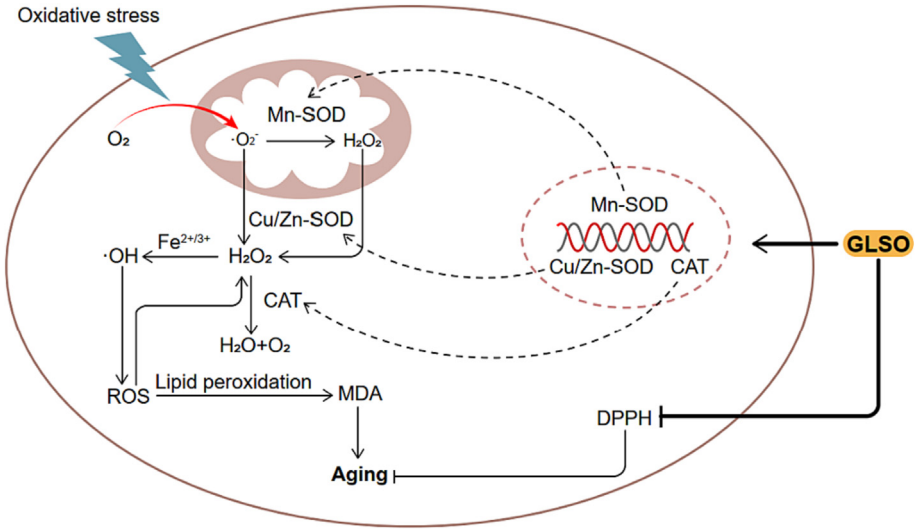

Fig. 8. Antioxidant mechanism of GLSO. GLSO achieves its anti-aging effect by eliminating two types of free radicals, superoxide anion radical ($\cdot O_2^-$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical.

terol (HDL-C) concentrations in the serum of New Zealand rabbits and reduced subintimal lipid deposition in pathological sections, possibly due to its rich content of unsaturated fatty acids and triterpenoids (X. Li, Wu, Xi, & Zhao, 2006; Y. Li, Xiao, Mao, & Huang, 2006; Li, Xie, Zhou, & Luo, 2006). In an *in vivo* trial, Liu's group administered *G. lucidum* spore extract containing lipophilic components by gavage to rats with trimethylamine oxide (TMAO)-induced cardiac insufficiency. Data showed decreases in serum TC, triglyceride (TG), low-density lipoprotein (LDL), and TMAO levels and increases in HDL levels. Further mechanistic studies showed that the lipophilic components of *G. lucidum* extract regulated the expression of lipid metabolism-related proteins such as uncoupling protein 1 (Ucp1), myelin protein zero (Mpz), and fatty acid synthase (Fasn) by modulating the intestinal microbiota, maintaining heart metabolic homeostasis (Liu et al., 2021). While GLSO appears to have a regulatory effect on human metabolism, research on this effect is not yet comprehensive. Further study of GLSO's potential metabolism-related mechanisms is needed.

5. Safety evaluation

G. lucidum is generally considered safe and is listed as a Class 1 drug in the American Herbal Products Association's Plant Safety Manual, with no clinically significant toxic reactions reported. Several researchers have evaluated the safety of GLSO. Li et al. reported that the oral LD_{50} of GLSO was > 10.0 g/kg bw for both male and female mice and that GLSO showed no mutagenic effect on mice by bone marrow polychromatic erythrocyte (PCE) micronucleus assay and sperm deformation assay (Li, Wu, Xi, & Zhao, 2006; Li, Xiao, Mao, & Huang, 2006; Li, Xie, Zhou, & Luo, 2006). Similarly, Xiao et al. reported that the oral LD_{50} of GLSO in mice was > 20.0 g/kg bw with almost no toxicity and that genotoxicity tests were negative (Xiao et al., 2006). The acute toxicity maximum tolerance dose (MTD) of lycopene GLSO soft capsules in mice was > 20.0 g/kg bw and genetic toxicity tests were negative (Sui et al., 2022). A long-term toxicity study of 2036GLSO softgels showed no abnormalities appeared in general conditions such as activity, appearance, food intake, feces, serum biochemical indices, or organ coefficients in rats given the capsules orally for an extended period. No significant pathological changes were observed in organ tissues (Zhao, Jin, Zhong, Chen, Zhong, & Cai, 2011). However, some anomalous indicators have been found during long-term toxicity experiments with spore oil. Rats given 1.67 g/kg, 0.83 g/kg, and 0.42 g/kg for four months and then stopped for one month experienced weight loss and increases in blood alkaline phosphatase (ALP) and TG values in the high dose group and increases in mean corpuscular hemoglobin (MCH) and creatinine (CREA) values in the middle and high dose groups (Chen & Zhang, 2013). These results suggest that GLSO is associated with a small risk of side effects and that long-term high doses can affect blood markers. A safe dose recommendation is no more than 0.42 g/(kg·d). A 30-day subacute toxicity test showed inflammatory cell infiltration in lung and kidney tissues of individual mice and mild to moderate steatosis in some mouse livers (Liu, 2007). These abnormalities may result from long-term high-dose administration and require further investigation. In conclusion, GLSO is safe for long-term oral administration at appropriate doses with little damage to normal mouse organisms. However, attention should be paid to the effects on metabolism and excretion of other drugs to prevent adverse drug interactions.

6. Conclusion and perspectives

Chinese medicine has emerged as a significant source for new drug research. GLSO, a prominent representative of Chinese medicine, contains an abundance of triterpenes, fatty acids, and sterols. These compounds exhibit a wide range of pharmacological effects, including anti-tumor, nervous system protection, immunity improvement, anti-aging, hepatoprotection, and treatment of metabolic diseases. The exploration of GLSO's pharmacological mechanisms not only preserves Chinese medicine culture but also provides new directions and entry points for new drug development. This represents an important breakthrough in the field of innovative new drug research. However, current research on GLSO has its limitations.

Quality control of drugs is essential for accurately evaluating the availability and safety of Chinese medicine. With a variety of GLSO products on the market, their quality can vary significantly. It is necessary to strengthen quality control and establish a robust quality testing mechanism. Additionally, the quality and efficacy of GLSO can be influenced by production and processing technology. Future research should focus on improving production and processing technology to ensure the quality and stability of its active ingredients.

There are various research methods for analyzing the content of GLSO components, but they lack standardization and uniformity, resulting in inconsistent results across different studies. Standard determination techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and UV–Vis/IR spectroscopy can be used to address this issue. However, there are still challenges in extracting triterpenoids from GLSO. The chemical structures of triterpenoids in GLSO contains multiple fatty acid groups or polycyclic structures, making them more lipid soluble. Additionally, the main components of GLSO are fatty acids, which can result in competitive adsorption during the extraction of triterpenoids and low extraction efficiency. Furthermore, due to difficulties in characterizing triterpenoids and the insufficient separation degree of liquid chromatography technology, the purity of triterpenoid compounds in extracted GLSO is low. To address these issues, improvement strategies such as developing mixed extraction techniques and multi-stage continuous extraction techniques to improve extraction efficiency and developing new characterization techniques such as infrared spectroscopy technology to enhance the characterization ability of triterpenoid compounds can be adopted.

Most studies on the specific pharmacological effects of GLSO lack analysis of relevant components. When studying the pharmacological effects of GLSO, it is necessary to adopt a holistic approach and comprehensively analyze the pharmacological effects of its active ingredients and their interactions on human physiological functions. Some pharmacological mechanisms of GLSO remain uncertain, and there is room for further research on its auxiliary treatment for hepatoprotection and metabolic diseases in animals. In the future, multidisciplinary integration can be used to comprehensively analyze the mechanism of action of GLSO. From a molecular biology perspective, further research can be conducted on the molecular biological mechanisms of GLSO in the occurrence and development of diseases, including signal pathways, protein interactions, and gene expression regulation. Network pharmacology methods can be used to model and predict the interactions between target proteins, metabolic pathways, and drug molecules to reveal the pharmacological targets and pathways of GLSO. Preliminary research has shown that GLSO can regulate cardiovascular and immune diseases by directly reshaping the gut microbiota or indirectly affecting its metabolites. However, research is not comprehensive. Methods such as intergroup diversity statistical analysis, OTU construction, species annotation, and functional prediction analysis can be used to further reveal the effect of GLSO on the gut microbiota. A research system combining gut microbiome and metabolome can be established to explore whether GLSO can beneficially affect metabolic diseases such as diabetes, obesity, and liver disease by regulating the production of gut microbiota metabolites such as short-chain fatty acids (SCFAs) or bile acids and specific metabolic pathways such as energy metabolism or sugar, lipid, and amino acid metabolism. In conclusion, multidisciplinary integration can help us better understand the impact and pharmacological mechanisms of GLSO on organisms and improve drug development efficiency while promoting the modernization of Chinese medicine.

Although the pharmacological effects of GLSO have been validated through *in vivo* and *in vitro* studies, clinical trials are scarce and lack rigorous support from double-blind, randomized, and multi-center trials. Therefore, large-scale and more scientifically rigorous clinical trials are needed to validate its efficacy. Despite being a Chinese herbal medicine, the evaluation of key safety factors related to GLSO is still insufficient. More preclinical research is required to conduct comprehensive, detailed, and scientific safety evaluations to ensure its safe use in humans. Clinical studies should investigate the human body's reactions to different dosages of GLSO to develop suitable medication recommendations for different populations. The effectiveness and tolerability of GLSO should be evaluated to develop more insightful and practical guidelines for its application in Chinese medicine.

GLSO is still in a relatively early stage in terms of pharmacokinetic research. The first step should be to strengthen large-scale, multi-center clinical pharmacokinetic studies to determine its pharmacokinetic parameters and study its metabolism, excretion, and pharmacodynamics in different populations to support clinical application and optimize dosage. Research on the main metabolites and metabolic pathways of GLSO should be strengthened to provide a comprehensive and accurate description of its pharmacokinetic characteristics, increasing efficacy and reducing the incidence of adverse reactions. Studies on the interaction between GLSO and other drugs, particularly drug interactions during combined use, need to be further studied based on *in vitro* and *in vivo* experiments to improve clinical safety and efficacy. To improve the bioavailability of active ingredients in GLSO in the human body, appropriate methods of administration can be selected according to individual differences, such as oral, injection, or topical administration, and dosages and frequencies can be adjusted accordingly. Optimizing GLSO's combination with drug delivery systems is an important strategy to improve its therapeutic efficacy. For example, changing particle size, adding absorption enhancers, and using nanotechnology can improve the solubility, stability, and drug release rate of GLSO in the body. These deficiencies in current research need to be addressed urgently. While this may be a long-term and complex challenge, we believe that GLSO has broad application prospects, and that continuous innovation and research will uncover more potential value of GLSO. This is of significant importance for promoting and using GLSO in future clinical treatments.

CRediT authorship contribution statement

Jianying Liu: Conceptualization, Data curation, Validation, Writing – original draft, Writing – review & editing. **Binzhi Zhang:** Data curation, Writing – original draft. **Leqi Wang:** Data curation, Writing – original draft. **Shasha Li:** Supervision, Validation. **Qinqiang Long:** Supervision, Funding acquisition, Investigation, Writing – review & editing. **Xue Xiao:** Supervision, Conceptualization, Funding acquisition, Investigation, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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