Zebrafish as a promising model for investigating biological activities of ginseng species—a review

Meijie Yu[1,2†], Lulu Wang[1,2†], Haiqing Luo[3], Bo Yang[1], Li Li[4], Jiaxin Dong[1], Xianghe Meng[2,5], Mengmeng Sun[1,2*], Min He[1,2*]

[1]Changchun University of Chinese Medicine, Jingyue National High Tech Industrial Development Zone, Changchun, China; [2]The Jilin Province School-Enterprise Cooperation Technology Innovation Laboratory of Herbal Efficacy Evaluation Based on Zebrafish Model Organisms, Changchun University of Chinese Medicine, Jingyue Economic Development District, Changchun, China; [3]Affiliated Hospital of Changchun University of Chinese Medicine, Gongnong Rd. Chaoyang District, Changchun, China; [4]Beijing Institute of Traditional Chinese Medicine, Shuiche Alley Xinjiekou, Xicheng District, Beijing, China; [5]Changchun Wish Biotechnology Co. Ltd., Beihu Science and Technology Park Zone B, Guangji Road, High-Tech North District, Changchun, China

[†]These authors had equal contributions.

[*]**Corresponding Authors**: Mengmeng Sun and Min He, Changchun University of Chinese Medicine, No. 1035 Boshuo Road, Jingyue National High Tech Industrial Development Zone, Changchun 130117, China. Emails: sunmm@ccucm.edu.cn; hemin@ccucm.edu.cn

Received: 7 October 2023; Accepted: 8 December 2023; Published: 31 January 2024

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REVIEW

Abstract

Plants of the ginseng species have a long history with broad traditional applications, as these are recognized as precious tonic herbal medicines since ancient times. More and more multiple chemical constituents and pharmacological activities have been confirmed and discovered recently. Owing to its advantages, such as intuitiveness, cheap, easy to operate, and high-throughput screening, zebrafish has become a very popular model at present. Recently, more and more toxicity tests, bio-activity evaluation, and mechanism studies have been achieved via zebrafish models, many of which were focused on the nature of products, including monomers, extracts, and formulas from ginseng species. This review summarizes the recent pharmacological studies achieved by embryos, larvae, and adult zebrafish. This review provides a theoretical basis for the rational use of ginseng species plants, thus providing guidance for a better rational utilization and the potential innovative product development of ginseng species plants.

Keywords: zebrafish; ginseng species; bioactivity; bioactive compounds; ginsenosides

General background of the ginseng species: their chemical components, processing and pharmacological potentials

The ginseng species belong to the order Umbellifera of the *Araliaceae* family, wildly distributed in East Asia and North America. The most commonly used species are *Panax ginseng* C.A. Meyer (commonly known as "Panax ginseng" or "ginseng"), *Panax quiquefolium* L. (American ginseng), and *Panax notoginseng* (Burkill) F.H. Chen ex C.H (Notoginseng). Since antiquity, ginseng species, including Panax ginseng, is considered to be an emblematic herb of traditional Chinese medicine (TCM) (Attele *et al.*, 1999). According to "*Shennong Herbal Classic*," Panax ginseng is considered to be the most precious and important medicinal herb that draws nutrients from foods and distributes the same to organs, thus benefiting the body systems. Panax ginseng helps to relax not only

ISSN 1757-837X online, DOI 10.15586/qas.v16i1.1393

the body but also the emotional state, including calming down of the overexcited central nervous system, relieving convulsions from shock, strengthening body immunity, relieving unhappiness and depression, improving weight loss, and enhancing longevity (Dharmananda, 2002). As a result, ginseng-based herbal prescriptions, health foods, and cosmetics are popular globally in the health product sector.

Ginseng are perennial herbaceous plants. They have palmately compound leaves and umbel shaped inflorescences of flowers. They are semi-shaded plants and prefer cool and humid climates, avoiding direct sunlight. They are suitable for growing on slopes with light and dense forests. The plants usually require a growth period of 4–6 years to be used for medicinal purposes (Gao *et al.*, 2010).

Various bioactive constituents, including ginsenosides, polysaccharides, alkaloids, glucosides, and phenolic acids, have been identified in ginseng species; of these, ginsenosides are the most prominent components (Yang *et al*., 2021b). The chemical components, purification, and structure identification of these ginseng herbs have been studied in the past several decades and are well known to the researchers. Until now, more than 180 distinct ginsenosides have been identified, most of which belong to the dammarane- and oleanane-type saponins (e.g., Ro etc.). Among the mentioned saponins, the dammarane-type ginsenosides (e.g., Rb1, Rg1, Rc, Rd, Re etc.) are considered as major ginsenosides (or saponins) that are discovered in the ginseng species (Cao *et al*., 2020).

Among the ginseng species, Panax ginseng, American ginseng, and notoginseng contain some general ginsenosides, such as Rg1, Rg2, Re, Rb1 etc., but each species has its unique ginsenosides that are considered as their chemical markers (Jee *et al*., 2014). For instance, notoginseng contains notoginsenoside R1, but without oleanolic acid-type ginsenoside (e.g., Ro). Pseudoginsenoside F11 is considered as a chemical marker of American ginseng, but ginsenoside Rf and RS1 do not exist in American ginseng. The contents of ginsenoside also differ in different growth parts of ginseng (Zhang, 2004). Overall, ginsenoside levels of protopanaxadiol-type saponins were higher in the underground parts of ginseng (except for the main ginseng root); protopanaxatriol-type saponins were higher in the above-ground parts of ginseng; oleanolic acid-type saponins were higher in the rhizome (Lutou) of panax ginseng (Mao *et al*., 2022).

Processing is the most important post-harvesting procedure for herbal materials. According to the color and the processed method, the processed ginseng is mainly composed of the following three ginseng products: (a) White ginseng (fresh ginseng with intact skin are naturally dried under the sun or by the airflow); (b) Red ginseng (fresh ginseng are steamed then dried); and (c) Black ginseng (a revolutionary 9-cycle steaming processing until a dark brown occurs to the ginseng) (Jang *et al*., 2016). Owing to the containing sugar chains on the ginsenoside structures, hydrolysis of the sugar moieties occurs and rare saponins can be derived and obtained from the major ginsenosides during the processing procedure, for instance, heating (boiling/steaming/stir-fried, etc.), sulfur fumigation, or biotransformation by intestinal bacteria *in vivo* (Zheng *et al*., 2017). Generally speaking, the hydrolysis of the sugar moieties from these main ginsenosides can yield the rare ginsenosides, such as Rf, Rk3, Rh4, Rh1, Rh2, compound Y (CY), and compound K (CK) etc. (Cao *et al.*, 2020). In addition, it is reported that the black ginseng contains more rare saponin (Rg3) than red ginseng, which does not existed in the white ginseng (Sun *et al*., 2009a, 2009b).

Although ginsenosides characterizations are identified among or within ginseng species, the application potentials of different ginseng species and different ginsenosides may be diverse and still need to be understood. Although the latest version of Chinese Pharmacopoeia (2020 edition) records ginseng's roots, rhizomes and leaves as medicinal parts (*Pharmacopoeia of the People's Republic of China*, Commission, 2020), flowers (Cui *et al*., 2021) and berries (Lee *et al*., 2017) of Panax ginseng are also used widely as functional foods and cosmetic additives. In recent academic studies, these are reported to have therapeutic potential. Recent research demonstrates that besides the traditional potential regulatory effects, such as anti-aging, memory recovery, cardiovascular protection (Ru *et al.*, 2015), the Panax ginseng has been reported with more pharmacological effects, including anti-inflammation (He *et al*., 2020), antioxidation (An *et al*., 2021), wound repair (Kimura *et al*., 2006), immunity enhancement (Xiao *et al*., 2017) etc. The processing procedure allows a long-term storage of plants to prevent the degradation of certain components; thus, may also allow increase in efficacy and reduce toxicity or undesired side effect etc. (Jang *et al*., 2016). Activity differences among different processing methods are also the flashpoints in recent academic research.

In addition to the most widely used ginseng species, the potential biological functional comparison among these species is valuable to be understood, although they all belong to the same *Araliaceae* family. For instance, the American ginseng has many biological functions similar to Panax ginseng, thus can be alternatively applied in many clinical decoctions/prescriptions, although the action onset and intensity of American ginseng is not as strong as Panax ginseng in boosting life energy (Chen *et al*., 2008). Other ginseng species are also referred to processing procedures for different functional purposes.

For instance, the notoginseng is traditionally utilized for hemostasis and to improve blood circulation whereas steamed notoginseng is used to boost immunity (Ng, 2010).

The emerging frontier hotspot of zebrafish model: its characteristics and advantages for biomedical research

Cellular and mammalian models are broadly used to assess the biological activities of ginsenosides. *In vitro* investigations are often represented by cellular tests. Using cells as a model system successfully studies the bioactivity and mechanism of ginsenoside components. For instance, ginseng exerts their anticancer effects via modulating a variety of signaling pathways, including the control of cell proliferation mediators and growth factors, as demonstrated by research using many tumor cell types (Kwon *et al*., 2021). Nonetheless, it is still a challenge to explore dynamic body response between the compound(s) and the cell-based organism at the systemic level, and cellular-based results have some challenges in validating further functions *in vivo*. Mammalian-based models, such as mice, have been employed to examine the biological activities of ginseng and its components at the system organization level (Hsieh *et al*., 2021), and are frequently complementary to cell-based investigations. Speeding of such investigations is far from the drug discovery road because of its modeling time consumption, cost factors, and frequent demand of stringent ethical assessment and clearance. Therefore, utilizing an *in vivo* animal experimental paradigm to test the bioactivity of herbs belonging to the ginseng species rapidly, conveniently, cost-effectively, visibly, and comprehensively has become a crucial research direction.

Recently, zebrafish has become a new favorite in life sciences research for examining basic challenges, learning the molecular mechanisms involved in embryonic, tissue and organ development, building human disease models, and providing screening and assessment platforms for medicines (Patton *et al*., 2021). Embryo and zebrafish larvae are transparent, making them easy for observing dynamic processes in developing the cardiovascular, nervous and immune systems as well as various parts in real-time (Patton *et al.*, 2021). The small body length of zebrafish larvae allows a large number of samples to be housed in a tiny space (e.g., Petri dish), with small dosage of medications required for testing. Zebrafish can produce hundreds of fertilized eggs each week, which makes it a prolific breeder. A large size of clutches allows the collection of thousands of embryos for testing a wide range of bioactive components (Xie *et al.*, 2021). Furthermore, zebrafish has high homology with humans at the genetic level, thus early development of tissues and organs shares many similarities with humans. The whole genome sequence of zebrafish has been revealed, thus making genetic changes possible. Genetically modified fluorescent zebrafish lines have been well developed in recent years, in which fluorescent protein found in promoter-specific tissues can be marked and observed under the fluorescent microscope (Lin *et al*., 2021). In addition, the advantages of low cost and embryo transparency have led to an increasing interest in zebrafish-based research and testing in laboratories and pharmaceutical companies. These benefits have made zebrafish a prominent *in vivo* model for scientific domains, including studying toxicity and bioactivities of herbal plants and natural products.

Ginseng species research evaluated by zebrafish model

The growing demands for herbal ingredients in pharmaceuticals, functional foods, and cosmetics have resulted in zebrafish to turn into an emerging powerful tool for studying the bioactivities and safety of herbal components. In recent years, natural product extracts or purified compounds have exhibited a broad spectrum of bioactivities, such as antioxidant, anti-inflammatory, antidiabetic, anti-obesity and anticancer properties in a variety of zebrafish models (Lin *et al*., 2021). In this review, we summarized the activity assessment of ginseng species evaluated by using zebrafish models in the past 15 years according the most widely used academic databases, such as Web of Science Core Collection, PubMed, Google Scholar, and Scopus. The most widely used zebrafish models for studying ginseng species, including their detailed operational protocols, are summarized in Figure 1.

Safety and toxicity studies evaluated by zebrafish

During the discovery and development of new drug candidates, adverse side effects are often accompanied with its efficacy. Prior to the screening and activity evaluation of new drug candidates, safety assessment of preclinical pharmacological studies of drug candidates are necessary, which can effectively avoid and reduce their toxic risks. Currently, the safety and toxicity of more traditional Chinese herbals have been studied based on zebrafish model, such as the embryo toxicity of *Andrographis paniculata* (Jayasinghe and Jayawardena, 2019), acute toxicity of Polygonum multiflorum Thunb. (Yang *et al*., 2018), the developmental toxicity of Enicostema axillare (Perumal *et al*., 2021), the reproductive toxicity of Acmella oleracea (Souza *et al*., 2020), the hepatotoxicity of Prunus jamasakura (Komakech *et al*., 2020), and the liver and renal toxicity of Tussilago farfara (Duan *et al*., 2019).

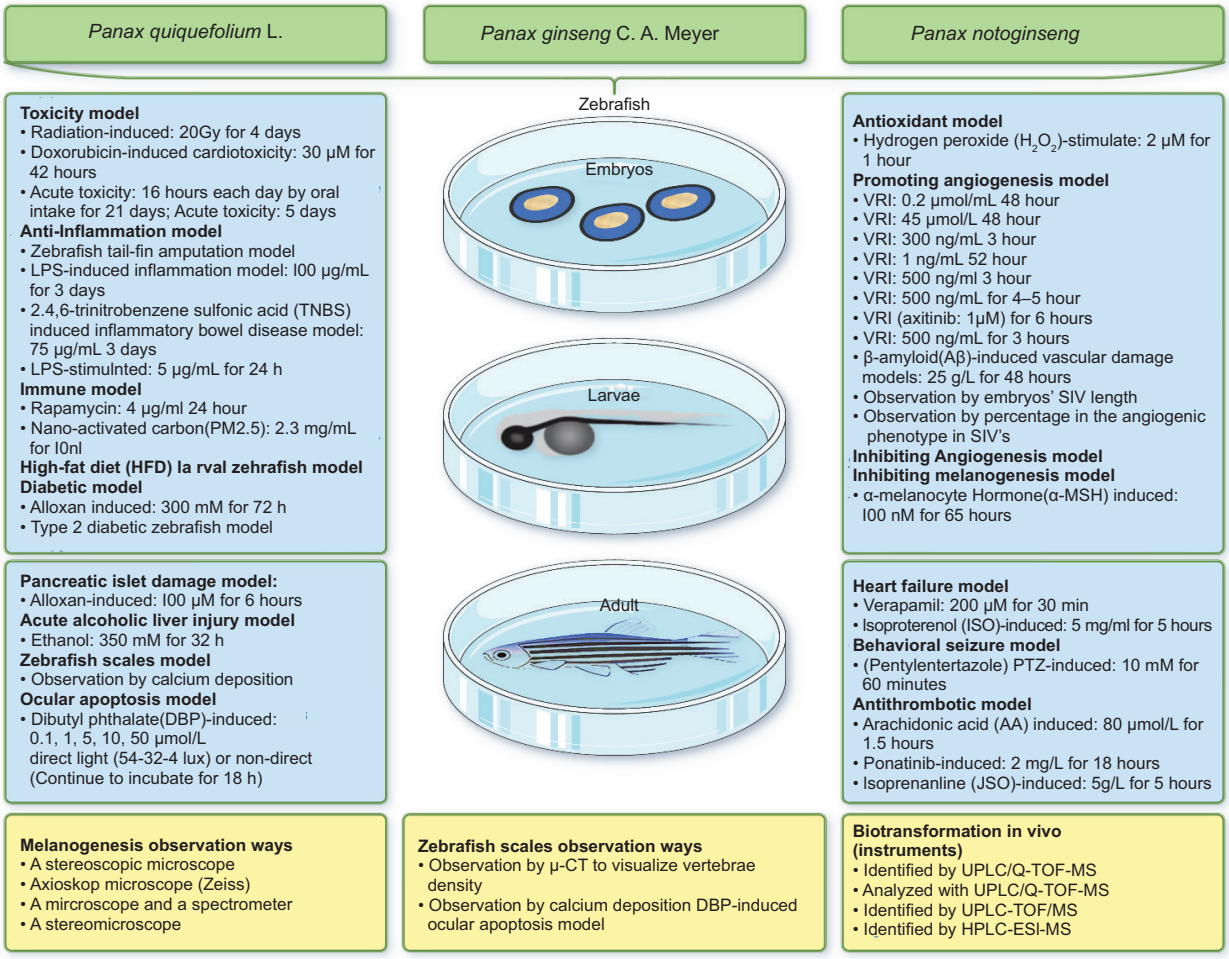

Figure 1. Zebrafish models with a concise summary of modeling protocols applied for evaluating bioactivity of ginseng species. Green box represents ginseng species; blue box represents the modeling method applied to zebrafish; yellow box represents used instrumentations for observation and detection. Zebrafish under various stages of growth are used in these studies and is schematically displayed in the center of the figure.

According to the Chinese Pharmacopoeia, the medicinal limit application dose of ginseng is 3–9 g. This dose range may indicate the relative higher safety of ginseng for oral administration and is considered as medicinal and food homology. However, this does not mean that ginseng can be taken in excess without limitation. Using zebrafish, the toxicity and safety of ginseng species have been studied in several researches; the detailed information is shown in Table 1. The commercially purchased Panax ginseng extracts were reported to be toxic when concentration is higher than 0.039 mg/mL in zebrafish after 3 days of exposure (Nguyen *et al.*, 2017). The decocted extract of Panax notoginseng (dPN) was reported to exert lower acute toxicity than the raw extract of Panax notoginseng (rPN) in inducing death of larval zebrafish, which has relatively higher minimal nonlethal concentration (MNLC) and LC50 (Wang *et al.*, 2023a). Another recently conducted study has reported possible toxicity mechanism of the overdosed administration of Panax notoginseng saponins (PNS) using metabolomics approach based on zebrafish larvae. From the metabolomics point of view, the toxicity mechanism of overdose of PNS could be related to disorders in lipid metabolism, amino acid metabolism and energy metabolism (Fei *et al.*, 2019). In summary, considering that concentration and ratio of saponins have a possible relation to the negative effects on health, it is necessary to raise awareness that the frequency and dosage of applications are crucial and must be prescribed under strict professional supervision to avoid overdose.

Besides the toxicity of ginseng species evaluated using zebrafish, the toxic rescue effects were also reported. For instance, the toxic morphologic abnormalities induced by radiation (including microcephaly, lateral line decrease, and inhibition of yolk sac resorption) can be recovered by red ginseng at a dosage of 30 μg/mL in zebrafish embryos (Chang *et al.*, 2014). The 18 carbon fatty acids (FA)

Table 1. Applications of zebrafish models in toxicity evaluations of ginseng species.

Main effects	Source	Bioactive substances	Zebrafish specifications/models	Results	References
Toxicity evaluation	Panax ginseng	Commercial extracts	2 dpf larvae (AB)	Ginseng extracts caused higher mortality when concentration is higher than 0.039 mg/mL (3 days) in zebrafish larva.	Nguyen *et al.*, 2017
	Notoginseng	Decocted extracts of Panax notoginseng (dPN) vs. raw extracts of Panax notoginseng (rPN)	2 dpf larvae (AB)	Mortality of larval zebrafish after 3 days of oral was observed at doses from 60 to 100 μg/mL rPN and 100 to 200 μg/mL dP.	Wang *et al.*, 2023a
	Notoginseng	Commercial saponins	1 dpf larvae (Germany Tuebingen strain)	The overdosed administration of notoginseng ginsenoside (200 mg/mL, 5 days) can lead changes in 29 endogenous markers and 6 metabolic pathways, including Eicosanoid pathways	Fei *et al.*, 2019
Ability to attenuate toxic damage (including cardiotoxicity)	Red ginseng	Aqueous extraction	6 hpf embryos (AB), radiation-induced toxicity model (20 Gy, 4 days)	Red ginseng significantly attenuates the radiation-induced toxic effects at a concentration of 30 μg/mL (up to 4 days)	Chang *et al.*, 2014
	American ginseng	FA (18:4) from ethanol extraction	30 hpf embryos (AB), doxorubicin-induced cardiotoxicity model (30 μM, 42 h)	The 18 carbon fatty acids with four carbon–carbon double bonds (FA (18:4) at a dose of 0.125 μg/mL (42 h) has ameliorative cardiotoxic effect, by decreasing fluorescence intensity and reducing the production of *reactive oxygen species* (ROS)	Hu *et al.*, 2023

AB: wild-type AB zebrafish; dpf: days post-fertilization.
Most of the studies applied zebrafish larvae as their experimental models.

with four carbon–carbon double bonds FA (18:4) identified from American ginseng inhibit doxorubicin (Dox)-induced cardiotoxicity in zebrafish by maintaining normal ventricle and atrium morphologies, by improving cardiac performance, and by increasing stroke volume, heart rate, and fractional shortening (Hu *et al.*, 2023).

Anti-inflammatory and immune-modulatory activities evaluated by zebrafish larvae

Inflammation is a defense reaction of the body to external stimuli, usually manifested as local tissue discomfort, including redness, swelling, heat and pain. When inflammation occurs, the stimulated tissue releases a series of chemical substances, such as interleukin-1 (IL-1), IL-6, IL-8, tumor necrosis factor-alpha (TNF-α) etc. (Coussens and Werb, 2002). These chemical mediators activate and accumulate immune cells, such as neutrophils and macrophages, toward damaged tissue sites. Through their phagocytic function, neutrophils and macrophages recognize invading microorganisms, dead or damaged cells, and other foreign substances, then release bactericidal enzymes, reactive oxygen species (ROS) and nitric oxide (NO) (Kyritsis *et al.*, 2012). By such ways, these foreign bodies are destroyed and cleared, and immune defense is achieved. Additionally, the production of ROS and NO can also activate NF-kappa B signaling pathway to produce more inflammatory factors (such as cytokines and chemokines), which further stimulate the activation of neutrophils and macrophages and lead to more ROS and NO release as a circuit (Renshaw *et al.*, 2006).

The anti-inflammatory potentials of herbal components can be evaluated by different inflammatory models, such as tailfin amputation model, lipopolysaccharide (LPS)-induced inflamed zebrafish model, 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced inflammatory model, rapamycin-induced neutrophils decreased model, nano-activated carbon ($PM_{2.5}$) phagocytosis model displayed in Table 2. The methanol extracts of black ginseng (up to a concentration of 50 μg/mL) are reported to have anti-inflammatory potential in a H_2O_2-induced inflamed AB strain of zebrafish model by inhibiting ROS production (An *et al.*, 2021). It was previously reported that the root extracts of American ginseng at 10 μg/mL can inhibit both acute inflammation caused by tail-amputation and chronic inflammation induced by LPS and TNBS, which

inhibited the aggregation of leukocytes and inflammatory cytokines (Wang *et al*., 2023b).

The effect on macrophage phagocytosis stimulations between fermented and unfermented white ginseng was compared in a nano-activated carbon (PM2.5)-induced zebrafish model (Xiao *et al*., 2017). The ginsenoside compound K is reported to inhibit significantly the NO production released by LPS induction in wild-type strain zebrafish embryos (Ryu *et al*., 2018). Several transgenic zebrafish lines have been used for modeling and visualizing inflammatory response in zebrafish larvae (Renshaw *et al*., 2006). The transgenic fishline Tg (mpx: EGFP) expresses neutrophils under green fluorescence channel of the microscope, while Tg (mpeg1: mCherry) expresses macrophages under red fluorescence channel of the microscope. Using this fishline for establishing a tail–fin amputated inflammatory model, anti-inflammatory effect of 11 batches of ginsenoside extracts from Panax ginseng were evaluated by comparing ability of immune cell migrations toward the wound edge, paralleled to chemical content tests by high-performance liquid chromatography (HPLC). This research confirmed that the chemical markers of ginsenosides (Rb_1, Rb_2, R_c, R_d, Re, Rg_1, Rg_2, Rh_1 and F_1) have positive correlation to the anti-inflammatory effect, and this bioactivity–chemical quality connection may further help and contribute to the activity–quality control of Panax ginseng (Sun *et al*., 2019). This model was also used for evaluating the anti-inflammatory activity of ginsenoside Rg1 using a tail–fin amputation model. Using such a model, inhibitory migration of neutrophils toward the wound injury by the ginsenoside Rg1 was observed, and several inflammatory indicators as well as matrix metalloproteinases (MMPs) that relate to the tissue impair were detected for their mechanisms by using the polymerase chain reaction (PCR) technique (He *et al*., 2020). Another fishline of Tg (lyz: EGFP) that marks neutrophils as green fluoresce was utilized to conduct rapamycin-induced neutropenia model. Ginsenoside extracts from American ginseng is reported to increase neutrophil counts and increase the IFN-γ level (Lv *et al*., 2020). To summarize, these studies significantly demonstrate that zebrafish is a promising model for evaluating the potential activities of ginseng in regulating immunity and against inflammations.

Angiogenesis regulations evaluated by zebrafish

Angiogenesis is the process through which new blood vessels emanate from preexisting vascular structures. Inadequate vessel maintenance or growth may lead to tissue ischemia, while excessive vascular growth or abnormal remodeling promotes cancer, inflammatory disorders, and retinopathies (Chávez *et al*., 2016).

The transgenic lines of Tg (fli1a: EGFP) and Tg (Flk1: GFP) mark vascular endothelial cell-relevant proteins that drive green fluorescence and are often used to study angiogenesis and vascular regression, as displayed in Table 2. Numerous studies have utilized the vascular endothelial growth factor (VEGF) receptor tyrosine kinase inhibitor II (VRI) to induce arterial damage in these transgenic zebrafish, which significantly attenuate the intensity of fluorescence of blood vessels (Xie *et al*., 2015; Yang *et al*., 2016a). Based on such a model, red ginseng extracts are reported with pro-angiogenic effect on blood vessel sections of zebrafish larvae (Sung *et al*., 2017). Aqueous decoction of both unprocessed Panax ginseng (100 μg/mL) and red ginseng (100 μg/mL) is reported to have pro-angiogenic effect using the Tg (fli1a: EGFP) fishline (Wei *et al* 2017; Shi *et al.* 2021a). The VRI-induced models are also used for evaluating the angiogenic effect activity of other ginseng species. Total saponins from both notoginseng roots and notoginseng flower buds are reported to be pro-angiogenic (Hong *et al*., 2009; Yang *et al*., 2016a), while notoginseng buds have better pronounced protective effect against intersegmental blood vessels (ISVs) and subintestinal vessels (SIVs) than Panax ginseng roots when concentration is 50 g/mL (Zhang *et al*., 2013). In addition, natural compounds, including notoginsenoside R1 and ginsenoside Rb1, Rb3, F1, Re, and Rg1 are reported to increase the intensity of fluorescent blood vessels in the VRI-induced zebrafish, indicating their potential to promote angiogenesis (Xie *et al*., 2015; Yang *et al*., 2016b; Zhang *et al*., 2019; Zhong *et al*., 2020).

β-amyloid (Aβ)-induced vascular damage model is another frequently used model to study the angiogenesis of herbal plants. Both ginsenoside Rg1 (20 μg/mL) and ginsenoside Rb1 (20 μg/mL) increase length and area of blood vessels, and significantly reduce ROS levels. They increase SOD and GSH-Px levels in a Aβ-induced vascular damage model of zebrafish. Further mechanisms were referred to the overexpression of VEGF protein and the inhibition of TGF-β1, Smad2 and Smad3 (Liu *et al.,* 2020b). Interestingly and conversely, ginsenoside Rh2 at concentrations ranging from 42.43 μM to 84.85 μM was reported to inhibit the fluorescence intensity of intersegmental blood vessels (ISV) in 24 hpf zebrafish embryos (Ma *et al.*, 2020).

Anti-melanogenesis evaluated by zebrafish

Zebrafish are transparent in the early stages of development, and melanin develops from the retinal epithelium 24 h post fertilization of embryos. Pigment cells originate from the neural crest cells, a group of cells that differentiate from the dorsal ectoderm, and proliferate, migrate and differentiate into pigmentoblasts (Choi *et al*., 2007).

Table 2. Applications of zebrafish models in bioactivity evaluations of ginseng species.

Main effects	Source	Bioactive substances	Zebrafish specifications/models	Results	References
Anti-inflammation and immuno-modulation	Black ginseng	Methanol extraction	0.75–50 hpf embryos (wild type), H_2O_2-induced model (5 mM, 24 h)	Black ginseng can inhibit ROS production up to 50 μg/mL (1 h)	An *et al.*, 2021
	Ginseng stems and leaves	Ginsenosides	3 dpf larvae (*Tg, mpx:GFPi*^{114}*/mpeg1:m Cherry-FumsF001* and the *grs*^{357} mutant line), tail-fin amputation model (pre-treated for 2 h, treated for 4 h after amputation)	Ginsenosides had (100 and 200 μg/mL) anti-inflammatory effect by inhibiting neutrophil migration	Sun *et al.*, 2019
	American ginseng roots	Ethanol extraction	72 hpf larvae (Tg, zlyz:EGFP), tail-amputation model; LPS-induced inflammation model (100 μg/mL, 3 days) 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced inflammatory bowel disease model (IBD) (75 μg/mL, 3 days)	The American ginseng roots extracts' concentration of 20 μg/mL, significantly decreased the aggregation of leukocytes and fluorescence intensity, increased integrated optical density	Wang *et al.*, 2023b
	Panax ginseng	Commercial ginsenoside Rg1	3 dpf larvae (*Tg, mpx:GFPi*^{114}*/mpeg1:m Cherry-FumsF001* and the *grs*^{357} mutant line), tail-fin amputation model (pretreated for 2 h, treated for 4 h after amputation)	Rg1 (120 μM) inhibits the migration of neutrophils but not that of macrophages. Gene expressions of pro-inflammatory cytokines, their receptors, and matrix metalloproteinases (MMPs) were inhibited by the Rg1	He *et al.*, 2020
	Panax ginseng	Commercial compound K	7~9 hpf embryos (wild type), LPS-induced inflammation model (5μg/mL, 24 h)	LPS-induced NO production was significantly reduced by compound K (40 μM, 1 h)	Ryu *et al.*, 2018
	American ginseng	Saponins (ethanol extraction)	48 hpf embryos (AB & *Tg, lyz:EGFP*), rapamycin-induced neutrophils decreased model (4 μg/mL, 24 h)	American ginseng saponins (10 μg/mL, 24 h) significantly reduced neutrophils number, increased macrophages number, and increased the IFN-γ production	Lv *et al.*, 2020
	White ginseng and fermented ginseng	Total saponins (methanol extraction)	2 dpf embryos (wild type), nano-activated carbon (PM 2.5) phagocytosis model (23 ng/zebrafish)	The fermented ginseng (500 μg/mL) has better effect than that of white ginseng (500 μg/mL) on promoting macrophage phagocytosis	Xiao *et al.*, 2017
Promoting angiogenesis	Red ginseng	Water extraction	24 hpf embryos (wild type)	Red ginseng (<500 μg/mL, 48-h treatment) significantly increased sub-intestinal vessels (SIV) growth	Sung *et al.*, 2017
	Red ginseng	Water extraction	24 hpf embryos *Tg* (Fli1a-EGFP), VEGF receptor kinase inhibitor (VRI)-induced model (0.2 μM, 24 h)	The red ginseng decoction (10 μg/mL, 48-h treatment) increased the diameter of sub-intestinal blood vessels	Shi *et al.*, 2021a
	Panax Ginseng	Water extraction	24 hpf embryos *Tg* (Fli1a-EGFP), VEGF receptor kinase inhibitor (VRI)-induced model (45 μM, 24 h)	The ginseng decoction (30 μg/mL, 48-h treatment) decreased the number of defective blood vessels	Wei *et al.*, 2017
	Notoginseng	Commercial total saponins	1 hpf (1–4-cell stage) embryos (*Tg, fli1: EGFP*), VRI-induced blood vessel formation in SIV model (1 ng/mL, 71 h)	Panax notoginseng saponins (300 ug/mL, 72–120-h treatment) stimulates angiogenesis in the sub-intestinal vessels of zebrafish	Hong *et al.*, 2009
	Notoginseng flowers buds	Total saponins	21 hpf embryos (*Tg, fli-1a:EGFP) y1*, VRI-induced vascular insufficiency model (300 ng/mL, 3 h)	Panax notoginseng flower (>50 μg/mL, 48-h treatment) rescued the VRI-induced vascular insufficiency in intersegmental blood vessels (ISVs)	Yang *et al.*, 2016a
	Notoginseng and notoginseng buds	Saponin (ethanol extraction)	21 hpf embryos (Tg (flila-EGFP) y, VEGF receptor kinase inhibitor II(VRI)-induced model (500 ng/mL, 3 h)	The Panax ginseng buds (50 and 100 μg/mL, >24 h treatment) have better effect on increasing numbers of intersegmental blood vessels (ISVs) and sub-intestinal vessels (SIV) than Panax ginseng	Zhang *et al.*, 2013

	Notoginseng flowers	Ginsenoside Rb1, Rb3; notoginsenoside R1	24 hpf embryos (*Tg, fli-1:EGFP*), VRI-induced chemical loss model (500 ng/mL, 4–5 h)	Notoginsenoside R1, ginsenoside Rb3, and Rb1 (100 µm, 24 h) increased increments in the angiogenesis indexes. R1 showed the highest angiogenic activity	Xie *et al.*, 2015
	Notoginseng	Commercial notoginsenoside R1	24 hpf or 48 hpf embryos (*Tg (fli1: EGFP)y1* & AB), VRI-induced vascular regression model (axitinib, 1 µM, 6 h)	Notoginsenoside R1 (100 µm, >24-h treatment) rescued VRI-induced vessel deficiency	Zhong *et al.*, 2020
	Notoginseng	Commercial ginsenoside F1	30 hpf embryos (*Tg, fli1:EGFP) y1* & wild-type AB, VRI-induced vascular defect model (axitinib, 1 µM, 6 h)	Ginsenoside F1 (40 µM, >24-h treatment) rescued the VRI-induced vascular defect	Zhang *et al.*, 2019
	Notoginseng	Commercial notoginsenoside, R1, ginsenoside, Rg1 and ginsenoside, Re	21 hpf embryos (*Tg, fli-1a:EGFP)y1*, VRI-induced blood vessel loss model (500 ng/mL, 3 h)	Notoginsenoside R1 (300 µM), Rg1 (300 µM) and Re (100 and 300 µM) significantly increased the number of inter-segmental vessels (ISVs), after 48-h treatment	Yang *et al.*, 2016b
	Panax ginseng	Commercial ginsenoside Rg1, Rb1	20 hpf larvae, Tg (Flk, β-amyloid (Aβ)-induced vascular damage models) (25 g/L, 48 h)	Both ginsenoside Rg1 and Rb1 (20 µg/mL) increased in the length of blood vessels	Liu *et al.*, 2020b
	Panax ginseng	Commercial ginsenosides Rg1	24 hpf embryos, Tg (Fli-1a:EGFP), VEGF receptor kinase inhibitor II(VRI)-induced model (0.2 µM, 24 h)	Ginsenoside Rg1 (10 µM, 48-h treatment) increased the intact vessels	Shi *et al.*, 2021b
Inhibiting blood vessel formation	Red ginseng	Commercial ginsenoside Rh2	24 hpf embryos (*Tg, fli1:EGFP* & wild-type AB), angiogenesis model	Ginsenoside Rh2 (42.43–84.85 µM for 24 h) inhibited inter-segmental blood vessels	Ma *et al.*, 2020
Inhibiting melanogenesis	Black ginseng	Methanol extraction	10 hpf embryos (wild type)	Black ginseng extracts (100 µg/mL, 62 h) reduced the intensity of melanin pigment in the eye, head, and yolk sac	Jin *et al.*, 2018
	Ginseng berry	Ginsenoside Rb2 (methanol extraction)	Embryos (wild-type)	Ginsenoside Rb2 (80 µM) reduced the number of melanin pigment spots	Lee *et al.*, 2015b
	Ginseng berry	Floralginsenoside A, ginsenoside Rd, and ginsenoside Re (methanol extraction)	9 hpf embryos (wild-type)	Floralginsenoside A (80 µM, 39 h) gradually decreased the number of melanin pigmentations	Lee *et al.*, 2017
	American ginseng	Ginsenoside C-Y (using 6% PPD ginsenoside substrate in acetate buffer (0.02 M and pH 5.0)	72 hpf embryos (wild type)	Ginsenoside CY (20 µM, 72 h) decreased melanogenesis	Liu *et al.*, 2019
	Ginseng leaves	Ginsenoside Rh23 (methanol extraction)	9 hpf embryos (wild type)	Ginsenoside Rh23 (80 µM, 63-h treatment) inhibited the melanin biosynthesis	Lee *et al.*, 2018
	Ginseng leaves	Ginsenoside Rh6, vina-ginsenoside R4 and vina-ginsenoside R13 (methanol extraction)	9 hpf embryos (wild type)	Vina-ginsenoside R13 (80 µM, 63 h) has the best effect on inhibiting melanin biosynthesis than ginsenoside Rh6 and vina-ginsenoside R4 (80 µM, 63 h).	Lee *et al.*, 2015a

(*continues*)

Table 2. Continued

Main effects	Source	Bioactive substances	Zebrafish specifications/models	Results	References
	White ginseng, red ginseng and black ginseng	Low molecular weight oligosaccharides (methanol extraction)	7 hpf embryos (wild type), α-melanocyte-stimulating hormone (α-MSH)-induced model (100 nM, 65-h exposure)	The low molecular weight oligosaccharides (12.5 μg/mL, 65-h treatment) decreased the melanin content on the surface of the trunk	Dai *et al.*, 2021
	Red ginseng	Isomaltol glycoside (water extraction)	9 hpf embryos (wild type)	Isomaltol glycoside (100 μg/mL, 63-h exposure) decreased the number of melanin pigmentations	Lee *et al.*, 2019
	Ginseng leaves	Picrionoside A methanol extraction)	9 hpf embryos (wild type)	Picrionoside A (80 μM, 63-h exposure) inhibited body's pigmentation biosynthesis	Lee *et al.*, 2015c
	Panax ginseng	Commercial salicylic acid	6-8 hpf embryos (AB), α-MSH-induced model (0.3 μM, 48 h)	Salicylic acid (5 μg/mL, 72 h) significantly suppressed melanogenesis	Liu *et al.*, 2021a
Attenuating body fat accumulation	Red ginseng	Commercial ginsenosides Rg3	5–16 dpf larvae (AB), high-fat diet (HFD) larval model (fed 4% w/w, cholesterol/common twice per day for 40 days)	Rg3 (50 μg/g) reduced the body length and weight to normal levels	Li *et al.*, 2022b
	Commercial vendors	Ginsenosides Rg1 (commercial vendors)	5 dpf larvae (wild type), high-fat diet (HFD)-induced obese model (hard-boiled egg yolk as an HFD once a day for 12–15 days)	Rg1 (10 μM) significantly decreased the accumulation of lipids and triglycerides	Koh *et al.*, 2017
Antidiabetic efficacy/ repairing diabetic sensorineural damage	Red ginseng	Commercial extracts	5 dpf larvae (wild-type *Tg,ins:GFP*), alloxan-induced diabetic neuromast model (300 μM, 72 h)	The red ginseng (50/100 μg/mL, 12 h) decreased pancreatic islet size	Nam *et al.*, 2019
	Panax ginseng	Commercial compound K	5 dpf larvae (wild type), alloxan-induced diabetic model (100 mM, 6 h)	The compound K combined CD inclusion complex (CD:CK=10:1, 1 μM; 5 μM) enhanced the recovery of damaged pancreatic islets and reduced the toxicity of compound K	Nam *et al.*, 2017
	Panax ginseng (mountain-cultivated, red)	APMCG-1 (glycopeptide by ethanol extraction)	5 dpf/1-month-old larvae Tg (kdrl:EGFP&AB), type 2 diabetic zebrafish model (10% high-fat diet for 8 h and 3% glucose solution for 16 h, 4 days; 1.75% glucose and 10% cholesterol diet for 4 weeks)	The APMCG-1 (50 μg/mL) decreased body weight, enhanced swimming endurance, improved the insulin resistance symptoms, recovered the oxidant-induced muscle injury, alleviated the pathological damage of heart and skeletal muscles and muscle fiber breakage, increased the expression of protein kinase B (AKT) in skeletal muscle tissue	Zhou *et al.*, 2023
Protecting alcohol-induced liver injury	Panax ginseng	Commercial ginsenoside Rb1	4 dpf larvae (wild type; *Tg, lfabp10α: EGFP; Tg, MPO:EGFP*), alcoholic liver injury model (350 mM, 32 h)	Ginsenoside Rb1 (12.5 μM, 48 h) alleviated alcohol-induced hepatic steatosis, including decreasing neutrophil migration toward the liver parenchyma, inhibiting expressions of pro-inflammatory cytokines, reducing ROS accumulation, and decreasing fluorescence intensity	Lai *et al.*, 2021
	Shen Quan baijiu (SQJ) (including fermented ginseng)	Ginsenosides	96 hpf larvae (wild type), alcoholic liver disease (ALD) zebrafish model (30% ethanol, 32 h)	Ginsenosides in Shen Quan baijiu (SQJ) (42% alcohol content) with fermented ginseng increased the liver glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) activity and reduced malondialdehyde (MDA) production, thus alleviating liver lipid peroxidation	Tao *et al.*, 2022

Anti-osteoporosis	Panax ginseng	Ginseng water extraction and commercial ginsenoside Re	35 dpf adult (wild-type), osteoporosis scales model	Ginseng aqueous extract (10 μg/mL, 35 days) and ginsenoside Re (5 μM. 35 days) decreased osteoclast differentiation activity	Park *et al.*, 2016
	Panax ginseng	Commercial ginsenoside Re	35 dpf adult (wild type), osteoporosis scales model	Ginsenoside Re (50 μM, 35 days) obviously increased osteoblast mineralization in zebrafish scales	Kim *et al.*, 2016
Inhibiting zebrafish eye cell apoptosis	Panax ginseng	Commercial ginsenosides Rg1	30/54 hpf larvae (AB), dibutyl phthalate (DBP)-induced ocular apoptosis model (10 μM, 18 h)	Ginsenoside Rg1 (50 μM, 18 h) inhibited DBP-induced apoptosis in zebrafish eye cells	Yang *et al.*, 2019
Protecting heart function	Black ginseng	Commercial ginsenoside Rg5	48 hpf embryos (AB), verapamil-induced heart failure model (200 μM, 30 min)	Rg5 (1 μg/mL, 4 h) reduced heart beats, venous congestion, heart dilation, cardiac output, and increased the ejection fraction, and fractional shortening	Liu *et al.*, 2021b
	American ginseng	Ethanol extraction, commercial ginsenosides Rg3, Rg5, Rg6, malic acid, quinic acid, and pseudo ginsenoside F11	48 hpf larvae (AB), verapamil-induced heart failure model (200 μM, 1 h)	Ginsenoside Rg3 (100 μg/mL), ginsenoside Rg5 (0.25 μg/mL), ginsenoside Rg6 (25 μg/mL), malic acid (10 μg/mL), quinic acid (100 μg/mL), and pseudo ginsenoside F11 (50 μg/mL) rescued heart failure, by reducing pericardial area, venous congestion area, and SV–BA distance	Dong *et al.*, 2022
	Notoginseng	Methanol extraction	2 dpf larvae (AB) isoproterenol (ISO)-induced blood stasis models (5 mg/mL)	Panax notoginseng (PN) powder (313 μg/mL, 5 h treatment) increased the blood flow velocity and cardiac output.	Li *et al.*, 2023
Suppressing epileptiform discharges and behavioral seizure	Panax ginseng	Commercial ginsenosides Rg1 and Re	Adult (wild type), pentylentetrazole (PTZ)-induced behavioral seizure model (10 nM, 1 h)	Ginsenoside Rg1 (130 μM, 10 min) showed better effect than Re on reducing the epileptiform discharges in the isolated telenephalon and in delaying the occurrence of behavioral seizures	Lee *et al.*, 2009
Anticancer	Panax ginseng	Commercial ginsenoside Rg1	3 dpf embryos (Tg, *fabp10:rtTA2s-M2; TRE2:EGFP-krasV12*); doxycycline induction (25 μg/mL) enhanced the gene expression of EGFP-krasv12	The 4 days-lapatinib (4 μg/mL) and ginsenoside Rg1 (4 μg/mL) decreased the liver fluorescence intensity	Cui *et al.*, 2023
Inhibiting hematopoietic growth	Notoginseng	Total saponins (ethanol extraction)	8 hpf embryos (tuebingen)	Treated with 100 μg/mL PNS inhibited primitive and definitive hematopoiesis development in zebrafish embryos	Sun *et al.*, 2019
Antithrombotic and resisting myocardial ischemia	Notoginseng	Methanol extraction	5 dpf/2 dpf/3 dpf larvae (AB & Albino), ponatinib, isoprenaline (ISO) and arachidonic acid (AA)-induced model (2 mg/L, 18 h; 5 g/L, 5 h; 80 μM, 3 h)	Notoginseng water extract freeze-dried powder (250–500 mg/L; 7.81, 15.63 mg/L) increased intensity of cardiac erythrocyte staining (SI), 2,000 mg/L group reduced fluorescence intensity of cardiac-modulated cells in ISO-induced model	Li *et al.*, 2022a

AB: wild-type AB zebrafish; dpf: days post-fertilization; hpf: hours post-fertilization. The biological potential of ginseng species evaluated by zebrafish models mainly include the following: anti-Inflammation and immuno-modulation, regulating angiogenesis, inhibiting melanogenesis, suppressing body fat accumulation, antidiabetic properties, alleviation of alcohol-induced liver injury, anti-osteoporosis, anticancer and antithrombotic properties, protecting heart function, and resisting myocardial ischemia. The herbal extracts, chemical monomers as well as herbal formulas containing ginseng species plants were included for this summarization.

This unique characterization is widely utilized for anti-melanogenesis research and whitening tests in the cosmetics industry and experimental laboratories.

The ginseng species have potential to serve as the future precursor molecules for creating novel pharmaceuticals, nutraceuticals, and cosmetics. The extracts of Panax ginseng and its common monomeric saponins, such as Rb1 and Re, possess strong anti-melanin activity in cellular studies (Jiménez-Pérez *et al*., 2017; Wang *et al*., 2014b). Recent studies have reported more anti-melanin activities using zebrafish models (Ferreira *et al*., 2023). For instance, the anti-melanin activity of black ginseng extracts and its component ginsenoside Rb2 by down-regulating the expression of tyrosinase is evaluated and confirmed using 3-dpf zebrafish larvae (Jin *et al*., 2018; Lee *et al*., 2015b).

Ginsenosides as secondary metabolic products are rare saponins that are believed to have potent biological activity, although the current understanding of this is limited. Recently, numerous saponins, including floral ginsenoside A (Lee *et al*., 2017), ginsenoside CY (Liu *et al*., 2019), ginsenoside Rh23 (Lee *et al*., 2018), ginsenoside Rh6, and vina-ginsenoside R4 and R13 (Lee *et al*., 2015a), have shown anti-melanin activities based on zebrafish models. In addition to ginsenosides, several chemical components of ginseng, including oligosaccharides (Dai *et al*., 2021), isomaltol glycoside (Lee *et al*., 2019), picrionoside A (Lee *et al*., 2015c), and salicylic acid (Liu *et al*., 2021a), were reported to inhibit melanin production in zebrafish. These recently published anti-melanin properties of ginseng extracts, as well as ginseng components, including common and rare ginsenosides, are summarized in the Table 2. In summary, these published studies showed the potentials of ginseng species in anti-melanin activities.

Attenuating fat accumulation and antidiabetic activities evaluated by zebrafish

Zebrafish has become an alternative vertebrate model for studying metabolic diseases and lipid perturbations, such as obesity, type 2 diabetes and fatty liver. Feeding of artemia for 8 weeks established a diet-induced obesity model in zebrafish, exhibiting similar disease characteristics as that in mammals, such as increased body mass index (BMI), hyperlipidemia, and fatty liver. Another model with high-fat feeding for a week produced obesity phenotype accompanied by increased fasting blood sugar, impaired glucose tolerance, increased insulin compensatory secretion, decreased insulin sensitivity, and impaired pancreatic β-cell functioning; thus, such models were more inclined for the study of diabetes patients (Lega and Lipscombe, 2020). In addition, over-nutrition model could also produce obesity (Zang *et al*., 2018). The liver plays an important role by maintaining lipid and glucose homeostasis. The excessive accumulation of lipid in the liver may aggravate insulin resistance and worsen metabolic dysfunction, while conversely fatty liver and hyperglycemia could cause hepatocyte injury and increase morbidity in diabetic patients (Xu *et al*., 2020).

Several ancient Chinese medical classics record that classic formulas containing herbs of ginseng species are used to attenuate diabetic manifestations and complications. These records include but not limited to the following: "Shi Quan Da Bu Tang" (recorded in the important prescriptions worth a thousand gold for emergency/in Chinese "Qian Jin Yao Fang"/千金要方, written by Simiao Sun in the Ming Dynasty), "Si Jun Zi Tang" and "Liu Jun Zi Tang" (recorded in the "Shang Han Lun"/伤寒论, written by Zhongjing Zhang in the Han Dynasty), "Huang Qi Gui Zhi Wu Wei Tang" (recorded in the Compendium of Materia Medica/in Chinese "Ben Cao Gang Mu"/本草纲目, written by Shizhen Li in the Ming Dynasty), and ginseng decoction with the formula of "Bu Zhong Yi Qi Wan" (recorded in the "Jin Kui Yao Lue"/金匮要略, written by Zhong jing Zhang in the Han Dynasty).

Current pharmacological studies provide more evidence of ginseng treatment for diabetes and fat accumulation by using cellular or animal models. In animal research, alloxan is a diabetogenic agent that reduces the number of B cells in the pancreatic islets (Ighodaro *et al*., 2017). After alloxan induction, a 12-h exposure treatment with red ginseng extracts to pancreatic islets of zebrafish larvae was confirmed and examined using fluorescence microscopy. This served to study the affect of ginseng species as antidiabetic and against fat accumulation (Table 2). The effect of red ginseng extracts on B cell regeneration was confirmed in the transgenic zebrafish Tg (ins: GFP) by B cell green fluorescence protein labeling (Nam *et al*., 2019). Additionally, the compound K-coupled B-cyclodextrin inclusion complex (CD:CK = 10:1) improved the healing of injured pancreatic islets and decreased the toxicity of compound K in diabetic zebrafish (Nam *et al*., 2017). Besides, the active glycopeptide isolated from mountain-cultivated ginseng (50 μg/mL) is reported to benefit the syndromes related to type 2 diabetes in zebrafish model, including improving the manifestations of insulin resistance, improving the oxidant-induced muscle injury, reducing body weight, enhancing swimming endurance, alleviating pathological damage to the heart, skeletal muscles and muscle fiber breakage as well as increasing the expression of protein kinase B (AKT) in skeletal muscle tissues (Zhou *et al*., 2023).

Additionally, ginsenoside Rg3 (50 μg/g) has been reported to reduce fat formation, inhibit hepatic lipid

droplet accumulation, and ameliorate body length and weight back to normal range in high-fat diet (HFD)-induced zebrafish larvae (Li *et al*., 2022b). Similarly, Koh *et al*. discovered that Rg1 (10 μM) inhibited fat storage in HFD-induced obesity zebrafish model (Koh *et al*., 2017). Lai *et al*. discovered that ginsenoside Rb1 had a protective effect in a zebrafish model of alcohol-induced liver injury because of its resistance to lipid deposition and anti-inflammatory properties (Lai *et al*., 2021). Ginsenoside Rb1 (12.5 μM) is also reported to alleviate alcohol-induced hepatic steatosis in zebrafish larvae by decreasing the intensity of oil-Red staining fluoresced liver, inhibiting neutrophil infiltration into liver parenchyma, inhibiting production of pro-inflammatory cytokines, and reversing ROS accumulation fluorescence (Lai *et al*., 2021). Furthermore, ginsenosides in Shen Quan baijiu (SQJ) with fermented ginseng increased glutathione peroxidase (GSH-PX) and superoxide dismutase (SOD) activity in zebrafish liver and reduced production of malondialdehyde (MDA) (Tao *et al*., 2022).

Other bioactivities of ginseng species evaluated by zebrafish models

Ginseng has a long history of its wide applications in clinical therapies in Asian countries by systematically regulating the body system. Besides the mentioned activities, other numerous pharmacological studies on the efficacy of ginseng and its preparations in zebrafish models have been confirmed.

Bone remodeling requires a proper balance of osteoblasts and osteoclasts, as disturbed bone homeostasis can lead to bone disorders, such as bone fracture, osteoporosis and osteoarthritis (Feng *et al.*, 2011). At present, calcane-related models, such as glucocorticoid-induced osteoporosis model, scoliosis model (Dietrich *et al*., 2021), spinal injury model (Vajn *et al*., 2014) etc., have been established using zebrafish. Although ancient records have seldom mentioned the applications of ginseng species in treating bone disorders, the recent pharmacology research has focused on its potentials. In summary, liquid extracts of Siberian ginseng, ginseng mixture, red ginseng extracts and notoginseng extracts, Rh2, Rb1 and Rd, have been reported to have anti-osteoporotic effect on cellular and rodent models (Siddiqi *et al*., 2013), although such established zebrafish models have limited applications in the research of ginseng species. For instance, the ginsenoside Re has been reported to prevent osteoclast differentiation based on the cellular studies using macrophages generated from mouse bone marrow as well as osteoblast precursor MC3T3-E1 cells. Such potential activity was validated by zebrafish model with adult scales treated with 50 μM for 35 days (Table 2) (Kim *et al*., 2016; Park *et al*., 2016).

Besides the anti-osteoporotic regulative activity, ginseng species have been reported to have more properties. For instance, the verapamil hydrochloride-induced heart failure zebrafish model was used for evaluating 12 batches of ethanol extracts of American ginseng from different regions (Dong *et al*., 2022). Potential bioactive compounds from American ginseng were further screened by metabolomics analysis, including ginsenoside Rg3, ginsenoside Rg5, ginsenoside Rg6, malic acid, quinic acid and pseudo ginsenoside F11. The activity of these monomers in heart failure was validated by such zebrafish models (Dong *et al*., 2022). By using another verapamil-induced heart failure zebrafish model, the Rg5 was reported to be protective against heart failure by reducing heart dilatation, venous congestion, and enhancing cardiac output and heart rate. The potential mechanisms are associated with many metabolic pathway regulations, such as arachidonic acid metabolism, D-glutamine and D-glutamate metabolism, phenylalanine metabolism etc. (Liu *et al*., 2021b). In addition, the zebrafish larvae model of myocardial ischemia was established by isoproterenol (ISO) induction, and Panax notoginseng (PN) powder (313 μg/mL) was reported to increase blood flow velocity and cardiac output of zebrafish (Li *et al*., 2023).

Recently, ginseng species has been established for its antitumor activities. The transgenic fishline Tg (krasv12: EGFP) is fluorescence-labeled with oncogene krasv12 in zebrafish and induction of doxycycline (25 μg/mL, 4 days) was used for oncologic modelling. Using such fishline, the ginsenoside Rg1 was found to have anticancer properties, which were observed in the liver of zebrafish (Cui *et al*., 2023).

The primitive hematopoietic stem cells can be marked and detected by the gene expression of gata1 and hbbe3, while the definitive hematopoietic stem cells can be marked and detected by the gene expression of runx1 and cmyb in zebrafish. The notoginseng total saponins (50 μg/mL) started to inhibit gene expression of both primary and definitive hematopoietic stem cells of zebrafish larvae (Sun *et al.*, 2019).

Arachidonic acid (AA) can affect the development of cardiac erythrocytes, ponatinibi can inhibit the endothelial growth factor receptor, and isoproterenol can lead the apoptosis of cardiac cells; thus, these components are often used for cardiac modeling of zebrafish. The modeled zebrafish can be either stained by o-dianisidine for cardiac erythrocytes observation or by acridine orange staining for cardiac apoptotic cells observation. With a relatively lower dosage (7.81–15.63 mg/L), the lyophilised powder of Panax pseudoginseng aqueous extracts could increase cardiac erythrocytes that were inhibited by the ponatinib induction in zebrafish. When the concentration was 250 mg/L, this type of extracts increased the cardiac

erythrocytes that were inhibited by AA induction in zebrafish. When the concentration reached 2,000 mg/L, it inhibited cardiac apoptotic cells (Li *et al*., 2022a).

In another pentylenetetrazol (PTZ)-induced behavioral seizure model using adult zebrafish, the ginsenoside Rg1 (130 μM) showed better activity than ginsenoside Re to decrease epileptiform discharge in the isolated telenephalon, and delayed the emergence of behavioral seizures (Lee *et al*., 2009). In addition, a dibutyl phthalate (DBP)-induced ocular apoptosis zebrafish model was established and the ginsenoside Rg1 was reported to prevent cell apoptosis in zebrafish eyes (Yang *et al.,* 2019).

Metabolic biotransformation of ginsenosides by zebrafish model

The content of rare ginsenosides in ginseng plants is relatively low, but has potential pharmacological activities, which are worthy to be studied. Biotransformation is an efficient way to obtain rare ginsenosides. The biotransformation of ginsenosides mainly involves the use of microorganisms or enzymes to modify the structure of sugar groups at C3 and C20 positions of panaxdiol-type ginsenosides, and at C6 and C20 positions of panaxtriol-type ginsenosides, so that the sugar groups of ginsenosides with high content could be hydrolyzed. The procedure of biotransformation could effectively improve the utilization efficiency of ginsenosides, optimize clinical effects, and reduce adverse reactions by changing their chemical structures (Chen *et al*., 2022).

Biotransformation of ginsenosides is already applied in cellular and mammal experiments (Wang, 2014a). Using zebrafish, the structures of transformatic ginsenosides could be studied by HPLC–electrospray mass spectrometry (ESI-MS). For instance, the primary constituents of Danshen–Sanqi herbal pair (including the plants of Panax notoginseng and Salvia miltiorrhiza) are salvianolic acid B, Tanshinone II A, ginsenoside Rg1, and Rb1. The metabolic biotransformation of these chemical components was studied in zebrafish larvae. After treating these four monomers and their combinations (monomer: SAB, TIIA, Rg1, Rb1; and combinations: SAB + Rg1, SAB + Rb1, Tanshinone II A + Rg1, and Tanshinone II A + Rb1), four parent components of SAB, TIIA, Rg1, Rb1 and their 18 metabolites were identified. The study also indicated that Rg1 and Rb1 could enhance the metabolism of Tanshinone II A whereas Tanshinone II A and salvianolic acid B could stimulate the metabolism of Rg1 and Rb1; thus, they may explain the potential synergistic effects of the herbal drug pairs (Yin *et al*., 2021).

In addition to the herbal formula containing ginseng species, the metabolic transformation of notoginsenoside R1, ginsenoside Rg1 and ginsenoside Rb1 in adult zebrafish was studied by HPLC–ESI-MS. The metabolic products of R1, Rg1 and Rb1 in zebrafish resulted from de-glycosylation and hydroxylation, which were highly consistent with those from the metabolism of mammals (Wei *et al*., 2011).

In addition, the metabolic processes of rare ginsenosides, F4 and Rg6, Rk1 and Rg5, and Rk3 and Rh4, in zebrafish were analyzed using a similar research methodology. After exposing to adult zebrafish for 24 h, eight metabolites of ginsenosides F4 and and six metabolites of Rg6 were identified. The metabolic study indicated that the primary response of ginsenoside F4 and Rg6 in zebrafish was mainly due to the loss of glucose at C-6 and glucuronidation at C-3 (Shen *et al*., 2018). By using the same experimental protocols, the metabolic processes of ginsenoside Rk1 and ginsenoside Rg5 were studied, and four metabolites of ginsenoside Rk1 and seven metabolites of ginsenoside Rg5 were identified in adult zebrafish. The mechanisms were further deduced to be desugarization, glucuronidation, sulfation and dehydroxymethylation (Shen *et al*., 2017). In another study, five metabolites of ginsenoside Rk3 and six metabolites of Rh4 were identified in adult zebrafish. Rk3 and Rh4 were mainly responsible for the loss of rhamnose at C-6 and glucuronidation at C-3 (Chen *et al*., 2015).

Bioactive effects of TCM formulas containing herbs of ginseng species evaluated by Zebrafish models

In addition to plant extracts and monomeric chemical constituents, some herbal formulas were evaluated using zebrafish models. As shown in Table 3, the biological activities of numerous herbal formulas containing herbs of ginseng species were evaluated using zebrafish models. For instance, besides the transgenic fishline Tg (mpx: EGFP) mentioned previously, the transgenic zebrafish Tg (MPO: GFP) also expressed green fluorescent proteins on neutrophils, and was used for the evaluation of TCM formulas containing ginseng species. The anti-inflammatory efficacy of Sheng-Mai-Yin (SMY) formula was evaluated by three distinct inflammation-induced models (LPS, $CuSO_4$ and tail transection-stimulated models) using transgenic zebrafish Tg (MPO: GFP). In summary, SMY (12.5–50 μg/mL) can significantly inhibit in a dose-dependent manner the recruitment of neutrophils and pro-inflammatory cytokines (including NF-κB, p65, IκBα and STAT3) which were upregulated (Zheng *et al*., 2021). In addition, the formula of Shen Fu Huang (SFH) contains also Panax ginseng. The compound-target network predicted that 18 out of 49 active chemical compounds in SFH are related to ginseng, accounting for approximately one-third of all detected constituents. Then the

Table 3. Application of zebrafish models in bioactivity evaluation of herbal formulas containing ginseng species.

Main effects and mechanisms	Formulas	Main compositions	Models/zebrafish specifications	Results	References
Anti-inflammatory effect	ShengMai Yin (SMY) formula	Panax ginseng., ophiopogonis radix and schisandrae fructus	3 dpf larvae (*Tg,MPO:GFP-1dpf*), LPS-induced inflammation model (0.5 mg/mL, 12 h)	SMY (>12.5–50 μg/mL, 12-h treatment) suppressed the gene expressions of pro-inflammatory cytokines *NF-κB*, *IκBα* and *STAT3*	Zheng *et al.*, 2021
Pro-angiogenic effect	Sailuotong (SLT) formula	Panax ginseng, ginkgo biloba and crocus sativus	Larvae (*Tg, flk1:GFP*), VRI-induced vascular insufficient model	SLT (>10 μg/mL) is pro-angiogenic. SLT (10–50 μg/mL) rescued blood vessel loss in the VRI-induced vascular insufficient Tg (flk1:GFP) zebrafish	Seto *et al.*, 2018
Anti-thrombotic effect	Danshen and Sanqi herbal pair (DS–SQ)	Panax notoginseng and salvia miltiorrhiza	4 dpf larvae (AB), phenylhydrazine (PHZ)-induced thrombosis model (1.5 μM, 12 h)	Rb1 as the most important monomeric chemical constituent (25 μg/mL, 12 h treatment) have better antithrombotic activity than Rg1 (25 μg/mL, 12 h)	Yin *et al.*, 2020
	Shen Fu Huang (SFH) formula	Panax ginseng, aconitum carmichaeli debeaux, and Rheum palmatum L.	3 dpf adult (wild type AB), arachidonic acid (AA)-induced thrombosis model	SFH (1.11 μg/mL, 3-h treatment) has anti-thrombosis properties in the heart by enhancing the generation of red blood cells	Liu *et al.*, 2020a
Promoting the formation of lymphatic thoracic duct	Du Huo Ji ShengTang (DHJST) formula	Panax ginseng, Radix angelicae pubescentis etc.	5 dpf embryos (*Tg, fli1:egfp; gata1:*DsRed), VRI-induced model (30 μM, 6 h)	DHJST (100 μg/mL, 48 h treatment) increased the number and length of the lymphatic thoracic duct (TD) formation	Chen *et al.*, 2016
Cardioprotective effects	Wenxin Keli (WXKL) formula	Panax notoginseng, Codonopsis pilosula etc.	48 hpf larvae (heterozygotes and homozygote transgenic *Cmlc2-GFP*), terfenadine-induced arrhythmia model (6 μM, 24 h)	Ginsenoside Rg1, ginsenoside Re, and notoginsenoside R1 were contributed to cardioprotective effects of WXKL (pre-treated 50 μM, 24 h) by increasing heart rate	Liu *et al.*, 2018
Boosting immunity and reducing fatigue and muscle loss	Renshen rougui ointment (RRO)	Panax ginseng, Cinnamomum cassia (L.) D. Don, Lycii Fructus, Cuscuta chinensis Lam., Schisandra chinensis (Turcz.) Baill., Rubus idaeus L., Plantago asiatica L	2 dpf larvae, AB, ethanol absolute-induced alcoholic muscle injury model (30 h); 4 dpf AB, sodium hydrosulphite-induced fatigue model (24 h); 1 dpf AB, mycophenolate mofetil-induced central nerve injury models (24 h); 6 hpf transgenic peripheral motor neuron fluorescent NBT strain, anhydrous ethanol-induced peripheral nerve injury model (24 h); 4 dpf transgenic red T-cell fluorescent, vincristine tartrate injection-induced T-cell reduction model (24 h); 2 dpf transgenic neutrophils and macrophages fluorescent, vincristine tartrate injection-induced macrophage cytopenia model (24 h)	RRO (0.8 mg/mL) protected muscle fibers and improved locomotor performance by increasing the travel distance. It can also enhance the immune system by enhancing the fluorescence intensity of macrophage and protect against CNS damage by weakening the fluorescence intensity of CNS apoptotic T cells	Dong *et al.*, 2021b
Improving qi and blood health	Ginseng-rose ointment	Panax ginseng, Polygonatum sibiricum Delar. ex Redoute, Polygonatum odoratum (Mill.) Druce, Lycium chinense Miller, Rosa spp., Paeonia suffruticosa Andr	2 dpf larvae, AB, PHZ-induced anemia model (18 h); 4 dpf larvae, AB, ponatinib-induced microcirculatory impairment model (18 h); 6 hpf embryos, AB; 2 dpf larvae, transgenic neutrophils and macrophages fluorescent, vincristine tartrate-induced macrophage cytopenias model; 4 dpf larvae, transgenic red T cell fluorescence, Vinorelbine tartrate injection-induced T-cell reduction models	Ginseng-rose ointment (0.8 mg/mL) can ameliorate anemia and microcirculatory disturbances by increasing the fluorescence intensity of cardiac erythrocyte and mean blood flow velocity. It can also inhibit melanogenesis synthesis by reducing total melanin optical density, and regulate the immune by increasing the fluorescence intensity of macrophages and T cells	Dong *et al.*, 2021a

AB: wild-type AB zebrafish; dpf: days post-fertilization; hpf: hours post-fertilization; LPS: lipopolysaccharide.

anti-inflammatory effect as well as the phagocytic capability of macrophages of SFH was evaluated based on the AA-induced zebrafish model, indicating antithrombotic capabilities of this formula (Liu *et al*., 2020a).

For angiogenesis study of herbal formula, the formula of Sailuotong (SLT) containing Panax ginseng was reported to rescue blood vessel loss in the VRI-induced vascular insufficiency using Tg (flk1:GFP) zebrafish (Seto *et al*., 2018). The herbal pair of Panax notoginseng and Salvia miltiorrhiza was reported to possess potent antithrombotic properties based on a phenylhydrazine (PHZ)-induced zebrafish thrombus model. The up-regulated gene expressions of PKCα, PKCβ, fga, fgb, fgg and vWF (an adhesive multimeric glycoprotein) were contributed to the antithrombotic mechanisms of this herbal pair (Yin *et al*., 2020). Du-Huo-Ji-Sheng-Tang (DHJST) is another wildly used herbal formula containing Panax ginseng as its important component. The DHJST is reported to stimulate the development of lymphatic thoracic duct, based on a transgenic zebrafish Tg (fli1:egfp; gata1:DsRed) in which the blood flow is visible in red (gata1:DsRed), and lymphatic and blood vessels are visible in green (fli1:egfp) (Chen *et al*., 2016).

Many herbal formulas containing ginseng species are wildly used for cardiovascular diseases and can help to regularize heart rhythms. Such formulas were recently evaluated by zebrafish model. Terfenadine can obviously affect heart rhythms, thus is used for establishing the arrhythmia model using transgenic zebrafishline of Tg (Cmlc2: GFP) (myocardium protein was marked and expressed by green fluorescent GFP). Wenxin Keli (WXKL) containing Panax notoginseng is typically used to treat cardiac arrhythmias. By using the terfenadine-induced arrhythmia zebrafish model, the WXKL is reported to steady the heart rhythm. Liquid chromatography combined with high-resolution mass spectrometry (LC-HRMS) identified 71 compounds in WXKL, of which more than one-third were ginsenosides or notoginsenosides. Ginsenosides, Rg1 and Re, and notoginsenoside, R1, were further confirmed as the most active compounds for regulating heart rate in zebrafish (Liu *et al*., 2018).

The formula of Renshen rougui ointment (RRO) also contains Panax ginseng as its main component. It was reported that RRO (0.8 mg/mL) could boost immune system, relief fatigue, protect muscle fibers, and rescue central nervous system (CNS) damage. Various zebrafish models were used in the study, including ethanol absolute-induced alcoholic muscle injury model, sodium hydrosulphite-induced fatigue model, mycophenolate mofetil-induced central nerve injury model, anhydrous ethanol-induced peripheral nerve injury model, vincristine tartrate injection-induced T-cell reduction model, and vincristine tartrate injection-induced macrophage cytopenia model. The results indicated that RRO protected muscle fibers and improved locomotor performance by increasing the travel distance. It could also enhance the immune system by enhancing the fluorescence intensity of macrophage and protecting against CNS damage by weakening the fluorescence intensity of CNS apoptotic T cells (Dong *et al*., 2021).

In addition, PHZ-induced anemia model, ponatinib-induced microcirculatory impairment model, vincristine tartrate-induced macrophage cytopenias model, and ginseng-rose ointment (concentration: 0.8 mg/mL) can ameliorate anemia and microcirculatory disturbances by increasing the fluorescence intensity of cardiac erythrocyte and mean blood flow velocity. It could also inhibit synthesis of melanogenesis by reducing total melanin optical density, and regulate immunity by increasing the fluorescence intensity of macrophages and T cells (Dong *et al*., 2021).

Perspectives

Chemical determinations combined with *in vivo* bioactivity-based assays help in better quality control of plants of ginseng species

The quality control of herbal plants is currently depending on the abundance of chemical components. Generally speaking, content of the most abundant components can to some extent reflect bioactivity of herbal materials. For instance, Sun *et al*. investigated the biological activity of different batches of medicinal Rhei Rhizoma in combination with chemical analysis by using the defecation test and small intestine propelling test in mice (Sun *et al.*, 2020). In another research, 11 batches of ginsenoside extracts were classified into two categories according to the detected nine monomer components, and the batches with higher content of ginsenosides had better inhibition on neutrophil migrations in a tail–fin-amputated zebrafish model (Sun *et al*., 2019).

Apart from these studies, another batch-to-batch consistency of 24 batches of Xuesaitong injection (XST) was evaluated in a zebrafish thrombosis model (Ma *et al*., 2021). Antithrombotic activity of five abnormal batches of XST from 24 normal batches could be efficiently distinguished using a zebrafish thrombosis model, and the inhibition rates of different batches were correlated with the content level of major components (Ma *et al*., 2021). However, chemical biomarkers with abundant concentrations of herbal materials may not always coincide with their good pharmacological activities in applications. Correlations between chemical biomarkers and pharmacological active biomarkers needs to be better understood.

Xie *et al*. studied the decoction of 12 batches of Panax notoginseng flowers and found that four out of 12 batches had a very great effect on promoting recovery of VRI-induced vascular injury in zebrafish (Xie *et al*., 2015). However, these four batches of Panax notoginseng flower extracts did not show significant differences in chemical composition, compared to other batches of extracts based on the most abundantly detected ginsenosides (notoginsenoside R1, ginsenoside Rb3, ginsenoside Rb1 etc.) (Xie *et al*., 2015). These may indicate that the content of the most abundant chemical indicators may not always fully represent the biological quality of herbal plants. Therefore, quality assessment of herbal materials needs integrate chemical component analysis as well as bioactivity studies. This may promote better understanding of herbal quality from more comprehensive perspectives.

Zebrafish *in vivo* model, combined with system biology-based analysis, opens wider window view for visualizing herbal quality evaluation and mechanisms

Zebrafish as an excellent novel *in vivo* model is raising hotspots in evaluating the drug–body interactions. Many studies used zebrafish to validate the activity of ginseng species followed with a cellular model. Bioassays, such as Western blot and PCR, are currently the well-developed and the most popular approaches to explore the underlying mechanisms of pharmacological activities. While considering that herbal plants, including natural products, are always reported with multiple activities by regulating multiple targets and interacting with multiple pathways, single biological pathway with limited indicators is in challenge to reflect the comprehensive working mechanisms of bioactive herbs and their substances. However, studies seldom explored rigorously and systematically the working systems using systems biology-based approaches in zebrafish, especially for ginseng research. More comprehensive and systematic studies are needed for better understanding the interactions between the plant and body systems.

Following the development of genomics, transcriptomics and proteomics, metabolomics, as one of the major omics techniques that are frequently used in the current biomedical academic field, enables researchers to profile entire endogenous metabolic perturbations in biological organisms and organ systems. Recently, based on zebrafish models and applying various biological techniques under the guidance of systems biology, some researchers studied the biological mechanism of natural products. For example, either transcriptome-proteomics, or intestinal microbiota metabolism has been applied to study the potential mechanisms of active natural products (Yang *et al*., 2021c; Xiong *et al*., 2019; Zaynab *et al*., 2018). Network pharmacology combined with metabolomics has been used to study metabolic mechanism of the herbal plant with its anti-arrhythmic activity (Yang *et al*., 2021c).

Moreover, some recent studies have applied metabolomics as a systematic approach to analyze the metabolic regulations of herbs and natural products, including ginseng and ginsenosides. For instance, by the untargeted metabolomics based on the ultra-high performance liquid chromatography–quadrupole time-of-flight mass spectrometry (UHPLC-QTOF-MS) technique, the mechanism of Rg5 against verapamil-induced heart failure zebrafish was related to the regulation of some metabolic pathways, including arachidonic acid metabolism, D-glutamine and D-glutamate metabolism, phenylalanine metabolism, tricarboxylic acid cycle, glycerophospho-lipid metabolism, purine metabolism, steroid biosynthesis, and linoleic acid metabolism (Liu *et al*., 2021b). While the joint applications of multi-omics are more and more in vogue, with in-depth developments and a strong desire for demand in academic areas, metabolomics is used for herbal safety evaluations. For instance, hepatotoxicity mechanism of a natural product called Nux vomica was evaluated in zebrafish larvae by using non-targeted metabolomics, accompanied by histopathology, protein, and gene expression (Zhao *et al*., 2018).

Overall, the zebrafish model *in vivo* combined with the system biology-based analysis opened wider window view for evaluation of herbal quality and its comprehensive mechanisms. Such an approach should be promoted and widely applied in more herbal studies, including ginseng species, because it is helpful to better understand the biological activities and mechanisms from a systematic and holistic perspective.

Conclusion

To sum up, zebrafish is a very popular model because of it being intuitive, cheap, easy to operate, and high-throughput screening. This review summarized the recent toxicity tests, bioactivity evaluations and mechanism studies of ginseng species, achieved by embryos, larvae and adult zebrafish. The summarized ginseng species mainly included *Panax ginseng* C.A. Mey, *Panax notoginseng* (Burkill) F.H. Chen ex C.H. Chow and *Panax quinquefolius* L.; these are wildly used and reported for their therapeutic potentials. Systems biology-based omics technologies combined with a novel zebrafish model have potentials to help in better understanding the multi-functioning and the underlying mechanisms of ginseng. Hopefully, these studies would help to maximize and optimize the potential applications of genus Panax as

a promising herbal medicine, and extend more possible applications of zebrafish model in the research of herbal plants, thereby promoting global health.

Author Contributions

Conceptualization: M. He; writing and original draft preparation: M. Yu and L. Wang; reviewing: H. Luo, B. Yang, L. Li, J. Dong, and X. Meng; and language editing and modifications: M. He and M. Sun.

Funding

Min He appreciated the financial support provided by the National Natural Science Foundation of China (Grant No. 82004030), Scientific and Technological Developing Project of Jilin Province (Grant No. YDZJ202101ZYTS119), Jilin Provincial Development and Reform Commission (Grant No. 2023C028-1), and the Scientific and Technological Developing Project of Changchun City (Grant No. 21ZGM22). Mengmeng Sun appreciated the supported funding provided by Scientific and Technological Developing Project of Jilin Province (Grant No. 20210402042GH) and Scientific and Technological Developing Project of Changchun City (Grant No. 21ZGM21).

Acknowledgment

The authors thank the support of Changchun Wish Technology Co., Ltd.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential competing interests.

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