Hydroxysafflower Yellow A: A Systematical Review on Botanical Resources, Physicochemical Properties, Drug Delivery System, Pharmacokinetics, and Pharmacological Effects

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Hydroxysafflower yellow A (HSYA), as a principal natural ingredient extracted from safflower (Carthamus tinctorius L.), has significant pharmacological activities, such as antioxidant, anti-inflammatory, anticoagulant, and anticancer effects. However, chemical instability and low bioavailability have been severely hampering the clinical applications of HSYA during the treatment of cardiovascular and cerebrovascular disease. Therefore, this present review systematically summarized the materials about HSYA, including acquisition methods, extraction and detection methods, pharmacokinetics, pharmacological effects and molecular mechanism, especially focus on the possible causes and resolutions about the chemical instability and low bioavailability of HSYA, in order to provide relatively comprehensive basic data for the related research of HSYA.

Keywords: hydroxysafflower yellow A, bioavailability, biological activity, chemical stability, delivery systems, botanical resources

INTRODUCTION

Cardiovascular and cerebrovascular disease (CCD), as one of the leading causes of mortality worldwide, has been increased rapidly and presented younger trend, with high mortality all of the world (Collins et al., 2017; Donahue and Hendrikse, 2017). Although many types of therapeutic strategies were used to treat patients with CCD, such as angiotensin converting enzyme inhibitor (ACEI), β-receptor blocker, and statins, the outcome remains not satisfactory due to the inevitable side effects and high treatment expenditure. To address the problem, more and more studies are trying to seek treatment strategy from Traditional Chinese Medicine (TCM). Characterized by minor side effects, TCM has become an important source of natural product, such as Aspirin, Digoxin, Hydroxysafflower yellow A, which exhibit substantially protective effects against CCD (Eichhorn and Gheorghiade, 2002; Dai and Ge, 2012; Desborough and Keeling 2017; Hu et al., 2020).
Hydroxysafflor yellow A (HSYA) is a primary active product derived from safflower (Carthamus tinctorius L.), a plant of the Compositae (Asteraceae) family, which was used to improve blood circulation, eliminate blood stasis, and relieve menstrual pain as early as recorded in Kaibao Bencao. HSYA, as an indicator component to characterize the medical value of safflower recorded in The Pharmacopoeia of the People’s Republic of China from 2005 edition, possesses a broad spectrum of pharmacological activities, such as antioxidant, anti-inflammatory and anticoagulant effects, which play an important role acting on cardiovascular and cerebrovascular disease (Sun et al., 2010; Wu et al., 2012; Ma et al., 2019a; Zhou et al., 2019; Bacchetti et al., 2020). However, chemical instability and low bioavailability of HSYA severely hamper the clinical applications. It can be easily oxidized, hydrolyzed, polymerized by light, high temperature, and alkaline conditions due to its structural characteristics. The high polarity directly leads to difficulty of transmembrane transport, resulting in low bioavailability. To address these problems, new drug delivery systems were developed to improve the therapeutic efficacies of HSYA based on lipid-based carriers, such as microemulsions, self-emulsifying systems, nanoparticles, chitosan, and the combination of HSYA with other drugs, which may have a good application prospect.

The present review systematically summarized the literatures about HSYA, including botanical resources, extraction and detection methods, pharmacokinetics, pharmacological effects and molecular mechanism, especially focus on the possible causes and resolutions about the chemical instability and low bioavailability of HSYA, in order to provide relatively comprehensive basic data for the related research of HSYA.

ACQUISITION OF HYDROXYSAFFLOWER YELLOW A

HSYA is mainly extracted from safflower, but the amount is not enough to support current clinical applications. Therefore, it is urgent find other ways to obtain HSYA. At present, chemical synthesis and biosynthesis are two promising ways to obtain HSYA. The information was detailed as follows.

Acquisition From Safflower

Safflower, as the natural source of HSYA, is widely planted worldwide. It is said that safflower originated in West Asia (Iran, Nicaragua and Turkey), and were later introduced for cultivation on almost every continent except Antarctica, such as America, Australia, China, Ethiopia, India, Italy, Mexico, Spain, and so on (Figure 1). India has developed into the most productive country with the planting areas of over 760,000 hm², and the yield of about 460,000 t, accounting for 50% of the total area and yield in the world (Liang et al., 2015).

Safflower is also widely cultivated in China with planting area of about 30,000–58,000 hm², and dried flowers yield of about 1,500–2000 t every year. Xinjiang province has become the major safflower production area, covering an area of 16,700–40,000 hm², which accounts for more than 3/4 of the total planting areas and provides more than 80% dried flowers and seeds in China. Henan province (Weihonghua, 卫红花), Zhejiang province (Duhonghua, 杜红花) and Sichuan province (Chuanhonghua, 川红花) are described as the authentic product areas of safflower in the history of China.

The content of HSYA containing in safflower were affected by many factors, including geographical origins, color and harvest time. The content of HSYA ranged from 0.05 to 14.99 mg/g by comparing 80 safflower cultivars collected from Africa, America, Asia, and Europe. HSYA in Africa cultivars was higher than that in Asia and Europe, and China cultivars is higher than that in Turkey, India and Kenya. Moreover, color is another factor to influence the content of HSYA. The darker the color of safflower, the higher the content of HSYA (red > orange > yellow > white according to PANEONE) (Tong et al., 2011; Xu et al., 2018). For example, Hebei safflower (red) was richer in HSYA (26.943 mg/g) than that in Wei safflower (white flowers, 0.472 mg/g) in China (Zhao, 2015). The most appropriate time to pick safflower is the...
morning of the third or fourth day after flowering (Tian et al., 2007).

Oxidation Synthesis Pathway

Chemical synthesis is an efficient way to obtain the natural or unnatural products within a short time period. Oxidation synthesis for HSYA is a rapid and highly efficient chemical synthesis method, and the synthetic pathway was shown in Figure 2A.

According to a retrosynthetic analysis of HSYA, di-C-glucosyl chloroacetophenone (1) transformed into di-C-(per-O-acetylglucosyl) phloroacetophenone (4) with BF₃.2AcOH at room temperature for 5 h. A further oxidation afforded the phenolic hydroxyl-free glycoside (5) with the quinol. Moreover, the two enantiomers of 4-(S)-2-acetyl-4,5-dihydroxy-4,6-di-C-β-D-glucosyl-3-methoxycyclohexa-2,5-dienone (6) was obtained after diazomethane added to acetic acid solution of product 4 at 0°C. Finally, compound 6 transformed into HSYA (7). Compared the oxidant synthesis pathway mentioned above, di-C-glucosyl chloroacetophenone oxidation (1) was converted into di-C-glycosylquinol (2) via an oxidation, and then was directly transformed into HSYA (7) with the yield at 18%, suggesting it is worthy promoting in the future (Suzuki et al., 2017).

Biosynthetic Pathway

Biosynthesis is a multi-step enzymatic process, in which a simple product is converted into a more complex desired product in a living organism. Biosynthesis is characterized by continuous and effective production, low-carbon and friendly environment, which provides a great support to the development of natural products (Pang et al., 2015). This production model will be the main source of HSYA in the future. However, the biosynthetic pathway of HSYA in plant remains unclear. According to the vital reaction of chalcone biosynthetic pathway, one molecular of 4-coumaroyl-CoA and three molecules of malonyl-CoA are converted into naringenin chalcone (4,2′,4′,6′-tetrahydroxychalcone) via the intervention of chalcone synthase, and it was transformed into HSYA after glycosylated (Heller and Hahlbrock, 1980; Knogge et al., 1986). The possible biosynthetic pathway of HSYA in phytosomal was shown in Figure 2B.

The content of HSYA is controlled by a nuclear gene with two alleles, HSya and hsya gene. HSya gene dominates completely over hsya gene to promote HSYA biosynthesis (Zhang et al., 2009; Yang et al., 2011). sHSP is a small heat shock protein and encoded by CTL-hsyapr, which might be directly or indirectly disturb HSYA biosynthetic pathway (Tang et al., 2010). When safflower is under external pressure, CT-wpr (TDF-11) was activated, which might arouse sHSP and inhibit the expression of HSya to some extent, finally leading to the inhibition of biosynthetic pathway of HSYA (Li et al., 2010b).

EXTRACTION AND DETECTION

Extraction Methods

HSYA is highly soluble in water, while hardly dissolve in lipophilic solvents such as ethyl-acetate, ether, benzene, and
| No. | Total Sample | Method                          | Pre-treatment                        | Extraction                                                                 | Purification                                                                 | Yield    | Additional Notes                                                                 | References          |
|-----|--------------|---------------------------------|--------------------------------------|-----------------------------------------------------------------------------|------------------------------------------------------------------------------|----------|----------------------------------------------------------------------------------|---------------------|
| 1   | 800 g        | Water immersion                 | NA                                   | Distilled water (10 L, 80°C, 20 min) for 2 cycles                         | The extracts were combined, evaporated and filtered                          | 0.023%   | NA                                                                               | Li et al. (2013)     |
| 2   | 2000 g       | Water immersion                 | The fresh flowers were shade dried and powdered | Distilled water (60°C, 30 min, 20 L) for three times                     | The solvent by evaporation under the reduced pressure, the residue was dissolved in 10% ethanol (1,000 ml), then evaporated to dryness under vacuum to afford a residue | 0.066%   | NA                                                                               | Bai et al. (2012)    |
| 3   | 1 g          | MAS-I microwave extraction system | NA                                   | Distilled water at 70°C for 20 min with solid and liquid ratio 100 for 3 cycles, and then the extracts were filtered | NA                                                                           | 6.96%    | NA                                                                               | Yang et al. (2008)   |
| 4   | 0.5 g        | UAE extraction system           | NA                                   | Ultra-pure water (55°C, 39 min, liquid-to-solid ratio of 16) in ultrasonic (40 kHz, 250 W) for 3 times | The extracts were filtered, and transferred to 100 ml volumetric flask, filtered by a 0.22 μm filter | 1.798%   | Reflow by cold water during ultrasonic procedure                                | Sun et al. (2013)    |
| 5   | 20 g         | Smashing tissue extraction system | NA                                   | Distilled water with liquid-to-solid ratio of 40, at 2.5 min for 90 V, and filtered | NA                                                                           | 1.359%   | NA                                                                               | Wang et al. (2012)   |
| 6   | 500 g        | Alcohol extraction method       | NA                                   | 75% aqueous ethanol (3,000 ml, 12 h) for 10 cycles                         | The extracts were concentrated to dryness in vacuo at 55°C, re-solved with water, and extracted by petroleum ether and ethyl acetate for five times | 0.584%   | RP-MPLC was used to isolate and purify                                         | Zong et al. (2013)   |
| 7   | 2.5 g        | DMSO extraction method          | Stirred 14 times the amount of DMSO at room temperature to avoid light for 30 min, impurity removal, filtered | The filter residue added DMSO to soaking, heating extraction in seal condition at 80°C for 50 min, filtered. Then residue again added 12 times the amount of DMSO, heating extraction in seal condition at 80°C for 50 min, filtered and combined the filtrate | The filtrate added 3 times of the amount of butyl acetate, centrifuged. Washed the precipitate with ethanol, dried | 14.564%  | A comparison of hot, cold and ultrafiltration models                           | Li et al. (2016)     |
| No | Methods                  | Sample source                           | Sample preparation                                                                                       | Chromatographic method                                  | Advantage                                                                 | Reference        |
|----|--------------------------|-----------------------------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------|---------------------------------------------------------------------------|------------------|
| 1  | HPLC-DAD and UPLC-Q-TOF-MS | Plant extracts                          | Dried in the cabinet drier at 35°C for 24 h, crushed and passed through an 80-mesh sieve and stored in a desiccator at room temperature | Waters ACQUITY BEH C18 column (30.0°C), elution solvent: Methanol: Water (1: 3, v: v) And flow rate of 0.8 ml/min | High-speed separation and structural identification of multiple constituents | Hong et al. (2015) |
| 2  | UFLC-Q-TOF/MS             | Bile, urine, plasma and feces samples from SD rat | Mixed sample at same time point, loaded onto a SupelClean™ LC-18 SPE tube | Thermo hypersil gold C18 column (35.0°C), elution solvent: Phase gradient, methanol A and 0.5% acetic acid in water B, flow rate of 0.2 ml/min | NA                                                                         | Jin Y.et al. (2016) |
| 3  | LC-MS/MS                 | Human plasma                            | Mixed with internal standard working solution and vortexed for 30 s | Shim-pack VP-ODS C18 column (30.0°C), isocratic elution system: Methanol and 5 mM ammonium acetate (80:20, v/v), flow rate of 0.4 ml/min | High selectivity, wide linear range, short run time (5.5 min per sample), low LOQ and small injection volume | Wen et al. (2008)  |
| 4  | LC-MS/MS                 | Human plasma                            | Added to an internal standard working solution, vortexed and centrifuged, the supernatant loaded to the activated SPE solid phase cartridge, and then washed with water | Agilent ZORBAX SB C18 column (4.6 mm × 150 mm, 5 μm, 35°C), elution solvent: 0.2 mol L⁻¹ ammonium acetate aqueous solution/methanol (30/70), flow rate of 400 μL/min | NA                                                                         | Li et al. (2014)  |
| 5  | UPLC-MS–MS               | Human urine                             | Freeze-dried, added 10% perchloric acid and 1 ml ethyl acetate, centrifuged, and dried under nitrogen gas blower | UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm), elution solvent: Gradient elution, Acetonitrile-0.5% acetic acid (42:58), flow rate of 0.35 ml/min | NA                                                                         | Zeng et al. (2013) |
| 6  | UPLC-DAD–MS              | Xue fu zhu yu (XFZY)                   | Pills and granules of XFZY ground to fine powder, separated by 50% methanol–water solution extraction. Liquids of XFZY, 1 ml was diluted to 50 ml by 50% methanol–water solution | ZORBAX SB-C18 column (4.6 mm × 100 mm, 1.8 μm); (50.0°C), mobile phase: 0.1% formic acid–water A and acetonitrile B, gradient program, flow rate of 0.5 ml/min | High-speed detection, excellent peak shapes, and less solvent usage       | Zhang et al. (2012) |
| 7  | RP-HP LC-UV              | Xuebiqing injection                    | XBJ injection of 1.0 ml was diluted to 10 ml with millipore water and filtered through 0.45 mm membrane filters | Zorbax SB C18 column, elution solvent: Gradient elution, water with 0.2% phosphoric acid A and acetonitrile B, flow rate of 1.0 ml/min | Better biocompatibility, larger specific surface area, good conduction effect and catalytic activity | Wang et al. (2016) |
| 8  | Novel multilayered porous silicon-based immunoensor | NA                                      | Synthesized the polyclonal anti-HSYA antibody and HSYA artificial antigen by the immediate coupling method | NA                                                                                                      | High surface area, easily preparation, label-free procedures and compatibility with standard microelectronics processing | Lv et al. (2011)  |
chloroform. So, the most general and traditional extraction method to get HSYA is water immersion. However, water immersion has the characteristic of low yield and high consumption of raw materials (e.g., the yield of 0.023%, 800 g of safflower; the yield of 0.066%, 2000 g of safflower) (Bai et al., 2012; Li et al., 2013a). Simultaneously, high temperature, alkaline conditions, and illumination all accelerate the degradation of HSYA in the traditional procedure.

As shown in Table 1, many other extraction systems were developed. Smashing tissue extraction holds an absolute advantage in time consumption compared with other extraction systems, with a yield of 1.359% in two minutes (Wang et al., 2012). MAS-I microwave extraction system with a solid and liquid ratio of 100 maintained at 70°C for 3 cycles in 20 min could obtain HSYA with higher yield of 6.96% (Yang et al., 2008). Hong et al. (2015) compared the effectiveness of MSPD, ultrasound extraction, and Soxhlet extraction methods. MSPD system obtained the highest yield at 14.89% compared with ultrasonic (12.25%) and Soxhlet extraction (13.09%), and also achieved the lowest consumption of raw materials.

Solvents also affect the extraction efficiency. Li et al. (2016) developed the DMSO extraction method as follows: Soak 2.0 g safflower with 14 volumes DMSO and stir for 30 min to remove impurities at room temperature in dark environment. Then soak the filter residue with 14 volumes of DMSO at 80°C for 1 h before heating for 50 min in the same environment. Soak the filter residue with another 12 volumes DMSO and repeat the steps above. Then add 3 volumes butyl acetate to the filtrate and centrifuge to obtain crimson precipitates. Finally, wash the precipitates with an appropriate amount of ethanol before drying to obtain light yellow powder with yield at 14.56%. DMSO is a “universal solvent” in areas of pharmaceutical sciences and cell biology, so it is inevitable to dissolve a lot of impurities during the extraction process, and the toxicity of DMSO is difficult to remove. Therefore, this method is not recommended to be extensively promoted.

Detection Methods
Several detectors coupled with a liquid chromatography (LC) system have already been used for HSYA detection, including diode (DAD) (Qi et al., 2007), electrochemical (ECD), mass spectrometer (MS), and ultraviolet (UV). In addition, the novel multilayer porous silicon-based immunosensor has also been applied for HSYA detection. Detailed information is provided in Table 2.

MS is the most effective detector for qualitative analysis of HSYA with a high detection sensitivity, selectivity and low-interference. HSYA and its metabolites can be accurately identified, based on mass-to-charge ratio (m/z) and fragmentation patterns.

The content of HSYA in human plasma was determined after oral administration of safflower. LC-MS/MS method with isocratic elution system composing of methanol and 5 mM ammonium acetate (80:20, v/v) was proved with a linear range of 1–1,000 ng/ml, a correlation coefficient ≥0.999 (Wen et al., 2008). Li et al. (2014) optimized the sensitivity and selectivity of this method to be more simplified and effective. The researchers replaced the Thermo syncronis C8 with Agilent ZORBAXSB C18, added ammonium acetate to the mobile phase and increased concentration dilution ratio. The modified method reduced the injection volume, improved the response intensity and peak shape, and shortened the retention time.

UPLC-DAD-MS method had the advantages of fast detection speed, good peak shape and less solvent consumption. Twenty-eight compounds were identified only in 30 min, including HSYA from Xue Fu Zhu Yu decoction, a classic prescription of TCM (Zhang et al., 2012).

UPLC-Q-TOF-MS is the most powerful tool with accurate activity measurement and full spectral sensitivity to determine HSYA using gradient elution with acetonitrile and 0.1% (v/v) formic acid aqueous solution in ESI+. This method showed good linearity (r² ≥ 0.9992) and precision (RSD ≤ 3.4%) with the limits of detection (LOD) at 35.2 ng/ml (Hong et al., 2015). Jin et al. (2016b) successfully developed a UFLC-Q-TOF-MS method to detect HSYA and its multiple metabolites in the plasma, bile, urine and feces of SD rats after oral administration with HSYA using the mobile phase consisting of methanol and 0.5% acetic acid in water.

The analysis speed of UV detector for HSYA is very fast, but its sensitivity and selectivity are slightly weaker than that of MS system (Chen et al., 2010). Novel multilayer porous silicon-based immunosensor is an easy way to alter the etching current periodically, which fabricated of pSi photonic crystal. The linear relationship was ranging from 1 to 3 g/ml and detection limit at 0.78 ng/ml for HSYA detection (Lv et al., 2011).

PHYSICOCHEMICAL PROPERTIES

Physical Properties
HSYA, a C-glucosyl quinochalcone, is a yellow amorphous powder with a molecular formula of C_{27}H_{32}O_{16} (Meselhy et al., 1993). It is usually used as a dye owing to its attractive color. It shows maximum absorption at 403 nm due to p-conjugated system coupled with several hydroxyl groups (Ma et al., 2014). The C-glycoside bond, located between the 1,3-diketone on the ring A, is unstable in HSYA. The hydroxyl group located at the C-2 position in the glycoside is easily condensed with the adjacent enol due to the strain effect. The pyranose ring is opened, and then forms an oxyfuran [3,2 days] benzofuran ring by a cyclization reaction (Suzuki et al., 2017). HSYA is easily degraded by light, high temperature, and alkaline conditions. HSYA emits weak fluorescence at 450 nm in aqueous solution owing to the lack of rigid planar configuration in the molecular structure, and borax can significantly increase the HSYA fluorescence intensity by 20 times (Cao et al., 2020). Due to the existence of phenolic hydroxyl groups, HSYA exists in protonated form in natural or alkaline aqueous solutions, which seriously affects its transmembrane ability and leads to low bioavailability.

Chemical Stability
Effect of pH
HSYA is easy to degrade under alkaline conditions. Pu et al. (2017) had illustrated the pH profile (1–14) of HSYA stability in
aqueous solution follows an inverted V curve, and it was most unstable at pH 9. When HSYA was transferred from the aqueous solution to the buffer solution at pH 9.16, the UV absorbance was red-shifted from 404 nm to 426 nm, with the gradually increased absorbance of degradation products at 300 and 380 nm, which indicated that the electron cloud density of the conjugate system increased after rapid ionization under alkaline conditions.

As shown in Figure 3, there were two degradation products proposed in HSYA aqueous solution. The hydroxyl group at C-2’ was ionized under the moderate solution (pH 7–9). Intramolecular nucleophilic attacks Cβ and add O2 to the α, β-unsaturated double bond. The two products are isomers, which obtained after hydrogen migration. There were chalcone, flavones, and carbanion intermediates detected in HSYA acidic neutral and aqueous solution. However, only flavones were found under strong alkaline conditions (pH 13).

**Effect of Temperature**

When HSYA was incubated in boiling water under dark conditions for 0, 0.5, 1, 2, and 4 h, the degradation rate of HSYA was increased gradually detected by HPLC system. The possible mechanism of HSYA degradation was that the colorless glycoside of HSYA bonded with H2O to form conjugate system, which reacted with the adjacent enol to transform the chromophore structure under the high temperature (Yue et al., 2003; Li et al., 2009). HSYA can be directly hydrolyzed and transformed into p-coumaric acid. And the hydroxyl group at the 2-position of C-glycoside of HSYA can be also condensed with the adjacent enol on A ring, and oxidized by O2 at pH 8 under the high-temperature condition, then obtained the degradations (Figure 4). Due to the instability of the degradations as enols, a mixture of the two degradations were obtained (Fan et al., 2011).

**Effect of Light**

HSYA degrades when exposed to light, so it is generally stored in dark conditions. When distilled water solution of HSYA was exposed under sunlight, ultraviolet, incandescent light, and the dark environment respectively, the content of HSYA decreased in turn analyzed by HPLC (Li et al., 2011). The content of HSYA in aqueous solution decreased when stored under natural light at room temperature for 20 days (Wang, 2017).

**Effect of Fe³⁺/Fe²⁺**

When HSYA was incubated with Fe³⁺ (0.1 μg/ml) or Fe²⁺ (0.05 μg/ml) at 60°C for 10 h, the multiple nucleophilic hydroxyl groups and carbonyl groups of HSYA combined with Fe³⁺ and Fe²⁺ to form chelate compounds, leading to accelerate degradation of HSYA. Ethylenediaminetetraacetic acid (EDTA) competes with Fe³⁺ or Fe²⁺ to reduce HSYA degradation (Wang, 2017).

**DRUG DELIVERY SYSTEM**

A drug delivery system is responsible to control the release rate, extend the duration of drug action, and eliminate side effects, which can be divided into 4 types, including lipid-based carriers, polymer nanoparticles, inclusion complexes, and capsulations. At present, the studies on the HYSA drug delivery systems mainly focuses on lipid-based carriers, which can improve the bioavailability by reducing the high-water solubility of HSYA. Moreover, microemulsions, self-emulsifying systems, and nanoparticles can also enhance the transmembrane capacity of HSYA. In this review, we also summarized other drug deliveries, such as chitosan, and the combination of HSYA with other drugs. A detailed description is provided in Ahmed and Aljaeid, 2016, Figure 5.

**FIGURE 3** The pathway of HSYA degradation (Pu et al., 2017).
Microemulsions

Microemulsions are stable liquid solutions consisted of water, oil, surfactant, and co-surfactant, with the characteristic of isotropic and thermodynamics. They are transparent or translucent with small droplet size, typically up to 150 nm (Lopes et al., 2014; Lv et al., 2018). Microemulsions are used to increase the permeability of hydrophilic peptides by enhancing the fluidity of cell membranes and opening tight connections between cells, which is a potential tool for hydrophilic drug molecules. Qi et al. (2011) investigated the bioavailability of HSYA microemulsion by intraduodenally administration. The microemulsion of HSYA was prepared by mixing Cremophor RH40 (surfactant), ethanol (cosurfactant), and PG (oil phase) together. Compared with HSYA aqueous solution, the bioavailability of HSYA microemulsion was increased by almost 1937%. It is worth noting that bile has a significant effect on the absorptive capacity of microemulsions. The microemulsion showed lower enhanced bioavailability of only 181% in bile duct-ligated rats. Microemulsion digested by pancreatic lipase increased 5.56 times permeability than the diluted microemulsion. The lipids and surfactants in the HSYA microemulsion might increase the fluidity of cell membranes and open the tight junctions between cells, thereby improving the permeability of hydrophilic drug molecules.

![FIGURE 4 | Proposed degradation pathways of HSYA in the buffer solution at pH 8.0 at 100°C.](image)

![FIGURE 5 | Scheme of the pharmacokinetics of HSYA.](image)
Self-emulsifying drug delivery system (SDEDDS) is a kind of the microemulsions. It could rapidly and spontaneously form a microemulsion in the gastrointestinal tract, where the peristalsis of the gastrointestinal tract and small intestine provides the necessary agitation for emulsification. SDEDDS have multiple advantages, including 100% drug entrapment capacity, physically stable formulations, no dissolution required, and submicron droplet sizes (Ghasemiyeh and Mohammadi, 2018). SDEDDS of HSYA were synthesized by inner water phase of 0.5% gelatin solution, and the external oil phase of bean phospholipids, medium chain triglycerides, Tween 80, oleic acid, and labrasol (20/65/7.4/2.5/0.1, in wt%). The study demonstrated that SDEDDS significantly improved the permeability of HSYA through Caco-2 cells monolayers, and plasma concentration increased by 2.17 times. The apparent permeability coefficient (Papp) of HSYA and HSYA-SDEDDS were \((3.52 \pm 1.41) \times 10^{-6} \text{ cm/s}\) and \((6.62 \pm 2.61) \times 10^{-6} \text{ cm/s}\) at the same concentrate (0.4 mg/ml), which improved to 1.88-fold by SDEDDS (Lv et al., 2012).

Labrafac lipophilic WL1349 (WL1349) is a medium-chain triglyceride that can be digested, absorbed, and hydrolyzed by pancreatic lipase after being emulsified by an endogenous emulsifier (such as bile). Preparation of HSYA-phospholipid complex increased lipophilicity, and dissolved in WL1349 to form a stable oil solution (a lipid-based preparation). Compared with HSYA aqueous solution, the oral bioavailability of HSYA-phospholipid complex WL 1349 oil solution in rats increased by about 37 times (Cmax of 2.79 μg/ml vs. 0.08 μg/ml within 24 h) and reduced the excretion of the drugs (8.80 ± 2.30% vs. 44.66 ± 8.00% in feces within 24 h) (Wang et al., 2008; Li et al., 2010a).

**Nanoparticles**

Solid lipid nanoparticles (SLN) is the earliest lipid-based nanocarriers formulated from lipids with a submicron size less than 1,000 nm, which are solid at body temperature and stabilized by emulsifiers (Koga et al., 2010). They can protect drugs against harsh environmental and are easy to mass-produce. However, owing to the crystalline structure existed in SLN, the drug-loading efficiency is poor. Some of the main lipids that have been used so far are monostearic acid, stearyl alcohol, stearic acid, glyceryl monostearate, cetyl palmitate, poloxamer 188, Tween 80, and dimethyl octadecyl ammonium bromide (DDAB) (Lee et al., 2016; Tapeinos et al., 2017; Ghasemiyeh and Mohammadi, 2018). As reported by Zhao et al. (2017), 1% Tween 80 was used as an emulsifier. HSYA-SLN with w/o/w structure prepared by micro emulsification procedure significantly improved oral bioavailability. HSYA SLNs is spherical with an average diameter of 174 ± 20 nm, zeta potential of −12.4 ± 1.2 mV, and the encapsulation efficiency is 55%. The SLNs of HSYA were stable within ten days at 4 or 30°C. SLNs of HSYA increased the oral bioavailability of HSYA in rats about 3.97-fold. It also significantly enhanced the Cmax and AUC by 7.76 and 3.99 folds. The pharmacodynamic evaluation showed that HSYA-SLNs had a better therapeutic effect on the cerebral ischemia rats compared to HSYA aqueous solutions (Zhao et al., 2018).

![Proposed metabolic pathways of HSYA](image.png)

**FIGURE 6 | Proposed metabolic pathways of HSYA** (Jin et al., 2016; Wu et al., 2018).
Others Delivery System

The combination of *Ligusticum* chuanxiong volatile oil (CVO) and HSYA also improved the bioavailability in rats. When HSYA co-administered with CVO of 0.02 mg/ml, the bioavailability of HSYA in rats was increased by 6.48 folds. The emulsification of CVO increased Papp of HSYA and the paracellular transport by opening the integral tight junction of Caco-2 cells.

Chitosan, a kind of biological polysaccharide, is a molecule usually obtained by deacetylation of chitin with the carbohydrate backbone structure similar to cellulose, which is composed of two kinds of repeating units, N-acetyl-D-glucosamine and D-glucosamine, combined with (1→4)-β-glucoside linkage. It is characterized by the presence of a lot of amino groups on the chain (Ahmed and Aljaeid, 2016; Hong et al., 2017). HSYA-Chitosan complex effectively improved the oral absorption of HSYA, and the bioavailability increased to 476% (Ma et al., 2015).

Drug delivery system has great potential to improve the bioavailability and chemical instability of HSYA. However, there are also some several challenges, such as high preparation cost and poor promotional effect, which need further improvement.

PHARMACOKINETICS

HSYA is one of the representative chemical compounds of biopharmaceutics classification system (BCS) class III drugs. Pharmacokinetic studies showed that HSYA had low bioavailability. The oral bioavailability of HSYA is only 1.2%, and 48% of the prototype drug is excreted in urine, 2.9% in feces, and only 0.062% ± 0.011% in bile (Zhang, 2006). Similarly, 88.6% was directly excreted through urine after intravenous administration (Sun et al., 2009) (Ahmed and Aljaeid 2016, Figure 6).

Caco-2 cell monolayer model was usually used to study the transmembrane characteristic of HSYA, and the result prompted that the absorption of HSYA is basically in line with the passive diffusion. P-gp inhibitors (verapamil) and energy metabolism inhibitors (sodium azide) failed to block the intake of HSYA, which demonstrated that the absorption of HSYA is irrelevant to the P-gp protein (Zhou et al., 2014). However, this conclusion needed more evidence to verify owing to the opposite result in another study (Wang et al., 2009). The peak concentration of HSYA generally appeared at 10 min after oral administration (Li et al., 2007). A study has shown that healthy volunteers received intravenous injections of 35, 70, and 140 mg of HSYA, the elimination half-life values ($t_{1/2}$) of HSYA was 3.32 h, and the $C_{\text{max}}$ was (2.02 ± 0.18), (7.47 ± 0.67), (14.48 ± 4.70) mg L$^{-1}$, respectively, (Qiao et al., 2009). HSYA has a low plasma protein binding rate (48%–54.6%, 72 h, i. v.), and it has no competitive binding to other drugs. So, HSYA is highly safe in vivo (Chu et al., 2006). Yang et al. (2009)suggested that the therapeutic effect of the single-dose HSYA indicated proportional to the dose ranging from 35 to 140 mg/kg, which conformed to first-order kinetics in

![FIGURE 7 | Schematic representation of the different kinds of colloidal delivery systems.](image-url)
| Study model | Detail | Effective concentration/dose/pattern | Remark |
|-------------|--------|-------------------------------------|--------|
| In vivo: SD rats | LAD of the coronary artery ligation | HSYA combined with NAG | Reduce the myocardial infarct size (MIS), decrease CK-MB, MDA, increase eNOS, SOD, NO |
| In vivo: SD rats | isoproterenol-induced myocardial injury | HSYA and AKBA | Oxygen–glucose deprivation (OGD) model | Apoptosis, increase PGC-1α and Nrf2 |
| In vivo: SD rats | Hypoxia/Reoxygenation (H/R) | HSYA and AKBA | Enter the cardiac myocyte and able to modulate H/R injury |
| In vivo: SD rats | Ischemia–reperfusion (I/R) | HSYA + AKBA (5 µM + 200 µM NA) | Inhibit MPTP opening, enhance nitric oxide production |
| In vivo: Wistar rats, TLR4-knockout C57 mice | Hyperlipidemia combined with MI/R model | HSYA + AKBA | Alleviate myocardial inflammation, decrease inflammatory injury, increase the level of CD31, VEGF-A and nucleolin |
| In vivo: Male C57 mice | MCAO model | HSYA + AKBA | Neuroprotective effect |
| In vivo: SD rats | MCAO/R model | HSYA + AKBA | Increase GFAP, NGF and Bcl-2 expression, suppress the expression of bax, caspase-3 and ICAM-1, IL-1β, TNF-α and NF-κB |
| In vivo: C57BL/6J mice | MCAO model | HSYA + AKBA | Inhibit TLR4, NF-κB, p-p65 expression, ERE1/2, JNK and p38 phosphorylation, suppressed TNF-α, IL-1β, NO production |
| In vivo: SD rats | MCAO/R model | HSYA + AKBA | Increase the expression BDNF, p-PI3K/PI3K, p-ERK1/2, p-p38 and Nrf2 and HO-1 activity |
| In vivo: SD rats | Unilateral 6-OHDA lesion (PD model) | HSYA + NAG | Increase the levels of dopamine and its metabolites, promote learning and memory ability |
| In vitro: PC12 cells | MCAO/R model | HSYA + NAG | Decrease LDH, TNF-β and IL-6, increase SOD, GSH-Px, suppress TLR4 and NF-κB |
| In vitro: BV2 cells | OGD model | HSYA + NAG | Decrease L-1, TNF-α, IL-1β and IL-6, increase SOD, GSH-Px, suppress TLR4 and NF-κB |
| In vitro: H9c2 cells | OGD/R injury | HSYA + NAG | Improve cardiomyocyte viability, maintain mitochondrial function and organelle structure, reduce the activity of caspase-3 and PARP, increase the expression of Bcl-2 and Bcl-xL, protect against cell death |
| In vitro: H9c2 cells | MCAO model | HSYA + NAG | Increase SOD, eNOS and NO, protect against cell death, improve mitotic activity through the AMPK signaling pathway |
| In vivo: SD rats | Hypoxia/Reoxygenation (H/R) | HSYA + NAG | Enhance SOD, eNOS and NO production |
| In vivo: SD rats | MCAO/R model | HSYA + NAG | Increase GFAP, NGF and Bcl-2 expression, suppress the expression of bax, caspase-3 and ICAM-1, IL-1β, TNF-α and NF-κB |
| In vivo: C57BL/6J mice | MCAO model | HSYA + NAG | Inhibit TLR4, NF-κB, p-p65 expression, ERE1/2, JNK and p38 phosphorylation, suppressed TNF-α, IL-1β, NO production |
| In vivo: SD rats | MCAO/R model | HSYA + NAG | Increase the expression BDNF, p-PI3K/PI3K, p-ERK1/2, p-p38 and Nrf2 and HO-1 activity |
| In vivo: SD rats | Unilateral 6-OHDA lesion (PD model) | HSYA + NAG | Increase the levels of dopamine and its metabolites, promote learning and memory ability |
| In vivo: SD rats | Hypoxia/Reoxygenation (H/R) | HSYA + NAG | Enhance SOD, eNOS and NO production |

(Continued on following page)
| Species | Study model | Details | Effective concentration/dose/pattern | Remark | References |
|---------|-------------|---------|---------------------------------------|--------|------------|
| Wistar kyoto (WKY) rats | MCAO model | Increase the ratio of 6-keto-PGF1a and TXB2 | 1.5, 3.0, 6.0 mg/kg via sublingular vein injection | Sun et al. (2012) |
| Wistar rats | Cervical lymphatic blockade model | Alleviate the neurological deficits, attenuated cell apoptosis, prevent the decrease of eNOS mRNA and protein expression | 5 mg/kg, (i.p.) | Pan et al. (2012) |
| SD rats | Isolate brain mitochondria of SD rat | Inhibit Ca²⁺- and H₂O₂-induced swelling of mitochondria, improve mitochondrial energymetabolism, enhance ATP levels and the respiratory control ratio | 10⁻⁸⁻⁰⁻⁴ μmol/L | Tian et al. (2008) |
| SD rats | Vascular dementia (VaD) model | Reduce escape latency in the water maze, enhance the LTP at CA3-CA1 synapses, up-regulated both VEGF and NR1, promote angiogenesis and increasesynaptic plasticity, improve spatial learning and memory | 4 mg/kg (i.v.) | Bie et al. (2010) |
| Pregnant C57BL6 mice | LPS-induced neurotoxicity and neuroinflammation | Decrease the content of IL-1β and NO, attenuate the LPS-induced dopaminergic neurons damage, inhibit the expressions of NF-κB, p65 and iNOS, decrease the content of IL-1β, TNF-α and NO | 0, 10, 20, 40, 80, 160, 320, 640 μg/kg | Yang et al. (2020b) |
| C57BL6 mice | PD model (6-hydroxydopamine) | Reduce iNOS, COX-2 and NF-κB, attenuate neuronal apoptosis, reduce the levels of p-p38 and -JNK and increase that of p-ERK –612 | 2, 4, or 8 mg/kg (i.v.) | Yang et al. (2018) |
| SD rats | MCAO model | Reduce infarct volume, decrease neurological deficit scores, elevate GSK3β phosphorylation and inhibit the activation of iNOS, NF-κB | 2, 4, or 8 mg/kg (i.v.) | Yang et al. (2019a) |
| SD rats | A rat model of vascular dementia | Reduce escape latency in the water maze, promote angiogenesis and increase synaptic plasticity, improve spatial learning and memory | 2.25, 1.13 and 0.57 mg/kg twice per day (i.v.) for 11 days | Ma et al., 2019a |
| SD rats | HCC model | Inhibit the proliferation of liver cancer cells, reduce the extent of tissue damage induced by cisplatin, increase the thymus index of HCC model mice, reduce the expression of Foxp3 and rorγt mRNA, improve the tumor immune microenvironment of HCC model mice | 0, 4, 8, or 16 mg/kg (i.v.) | Ma et al., 2019b |
| Human umbilical vein endothelial cell (HUVEC) | HepG2 cell viability, proliferation, and migration, inhibit apoptosis of HepG2 cells | Suppress JAK2/STAT3 activation, ablation of (E)RAS2, decrease p38MAPK phosphorylation, decrease the viability, proliferation and migration of HepG2 cell viability, proliferation, and migration, inhibit apoptosis of HepG2 cells | 0, 1, 5, 10, 20, 50 μM | Chen et al. (2019) |
| KYSE-30 cells | Human ovarian cancer cell viability and sensitizes cells to chemotherapeutic agents, downregulate WSB1 expression | Suppress p38MAPK phosphorylation, decrease HepG2 cell viability, proliferation, and migration, inhibit apoptosis of HepG2 cells | 0.1, 1, 10, 20, 50, 150 μM | Chen et al. (2020) |
| Kunming mice | HepG2 cell viability, proliferation, and migration, inhibit apoptosis of HepG2 cells | Suppress p38MAPK phosphorylation, decrease HepG2 cell viability, proliferation, and migration, inhibit apoptosis of HepG2 cells | 0, 0.1, 1, 10, 20, 50 μM | Chen et al. (2020) |
| BALB/c mice | In vivo: 80 mg/kg via sublingular vein injection | NA | | Zhang et al., 2019 |
### TABLE 3 | (Continued) Pharmacological effects of HSYA.

| Pharmacological effects | Species | Study model | Detail | Effective concentration/dose/pattern | Remark | References |
|-------------------------|---------|-------------|--------|--------------------------------------|--------|------------|
| **Immunoprotection**    | in vivo: C57BL/6 | Bleomycin-induced mice lung injury model | Attenuate the loss in body weight, alleviate bleomycin-induced increase of mRNA level of TNF-α, IL-1β and TGF-β1 in lung homogenate, inhibited NF-κB and phosphorylation of p38 MAPK in lung tissue | 36.7, 40, 60 mg/kg/d (i.v. for 1 week) | NA | Wu et al., 2012 |
|                         | in vivo: C57BL/6 mice | LPS-induced acute respiratory distress syndrome | Decrease the levels of p65 and phosphorylated ERK1/2 | 14, 28, 56 mg/kg/d for 10 days (i.v.) | NA | Zhang et al., 2017 |
|                         | in vivo: HepG2 liver cancer cell line | NA | Increase LC3-II and beclin 1 expression, decrease the level of p62 and phosphorylated ERK1/2 | 20 mg HYSA solved in 1.02 ml PBS | NA | Liu et al., 2020 |
|                         | in vivo: ICR mice | LPS-induced acute lung injury (ALI) | Attenuate lung vascular permeability and edema, down-regulate myeloperoxidase (MPO), inhibited p38, ERK, JNK, TLR4, MyD88 and TRIF and the phosphorylations of Interferon regulatory factor 3 (IRF3) | 50, 75, 112.5 mg/kg once daily from days 2 (i.v.) | NA | Zheng et al., 2019 |
|                         | in vitro: A549 and H1299 | LPS-mediated inflammatory injury | Suppresses proliferation, migration, invasion, and EMT, inhibit the PI3K/Akt/mTOR and ERK/MAPK signaling pathways | 5, 10, and 20 μM for two weeks | NA | Jang et al., 2019 |
|                         | in vitro: Human bronchial smooth muscle cells (HEBMSc) | NA | Suppress MLC phosphorylation, inhibit the activation, block asthma-relaxed signal transduction pathway, block the binding of PAF to the PAFR on the target cell membrane | 9, 27, 81 μM | NA | Guo et al., 2019 |
|                         | in vitro: E903 human endothelium cell | LPS-mediated endothelial inflammatory injury | Attenuate ICAM-1 and E-selectin mRNA levels elevation, phosphorylation of p38 MAPK, JNK MAPK, inhibit leukocyte adhesion to EC and increase in TNF-α, IFN-γ, IL-2, and IL-13 | 1, 10 and 100 μM | NA | Jin et al., 2016 |
|                         | in vitro: Human small airway epithelial cells (HSAECs) | PAF-induced activation of HSAECs | Inhibit PAF-induced inflammatory activation, inhibit the NF-κB and AP-1 | 9, 27, and 81 μM | NA | Guo et al., 2018 |
|                         | in vivo: Male NIH mice | Cecal ligation and puncture mouse model of sepsis | Improve sepsis induced immunosuppression via inhibiting CDA-positive lymphocytes apoptosis under septic conditions, upregulate the expression of IL-2, IFN-γ, TNF-α, IL-1β, and IL-6 | 120 mg/kg (i.v.) | NA | Wang et al., 2017 |
| **Vascular dementia**   | in vitro: Mouse 3T3-L1 preadipocytes | NA | Inhibit the proliferation and adipogenesis of 3T3-L1 preadipocytes, increase hormone-sensitive lipase (HSL) mRNA expression and promoter activity, increase HSL promoter activity | 1 mg/L | NA | Zhu et al., 2015 |
|                         | in vivo: Wistar rats | PE- (phenylephrine)-induced vascular constriction | Possess vascular relaxation effects, activate the K+ channel in pulmonary vascular smooth muscle cells, increase the cyclic guanosine monophosphate (cGMP) and NO production | 10–5, 10–6 M, 10–7 M, 10–8 M, 10–9 M | NA | Bai et al., 2012 |
|                         | in vitro: C57BL/J mice, in vitro: HUVEC cells | Mouse hindlimb ischemia model | Increase the capillary-like tube formation and migration of HUVEC, increase the phosphorylation of TIE-2, akt, and extracellular signal-regulated kinase 1/2, promote reparation of ischemic hindlimb tissue repair | 6 mg/kg for 11 days via the tail vein, in vitro 0, 1, 10, 50, and 100 μM for 24 h | NA | Chen et al., 2016 |
|                         | in vivo: Water-Hysyo (WH) rats and spontaneously hypertensive rats (SHR), in vitro: H9C2 cells, in vivo: Wistar rats, in vitro: Rat mesangial endothelial cells (primary) | HUVEC cells | Inhibit the expression of HRSF and IQC, block apoptosis, block the binding of PAF to the PAFR on the target cell membrane, reduce intracellular free Ca2+ level | 10–5, 10–6 M, 10–7 M, 10–8 M | NA | Yang et al., 2021 |

(Continued on following page)
| Pharmacological effects | Species | Study model | Detail | Effective concentration/dose/pattern | Remark | References |
|-------------------------|---------|-------------|--------|--------------------------------------|--------|------------|
| Hepatoprotective effects | in vivo: C57BL/6 mice | D-galactose- (D-gal-) induced aging | Increase SOD, CAT, GSH-Px and MDA, decreased the mRNA and protein level of cyclin-dependent kinase inhibitor p16, increase CDK4/6 protein expression and decrease the phosphorylation of retinoblastoma (pRb) | 25 mg/kg HSYA daily (i.v.) for 8 weeks | NA | Min et al. (2020) |
| Pulmonary arterial hypertension | in vivo: male wistar rats | A model of monocrotaline (MCT)-induced pulmonary arterial hypertension (PAH) | Reduce hemodynamic changes, right ventricular hypertrophy and morphometric changes; suppressed inflammation and oxidative stress | 10 mg/kg (i.p.) | NA | Han et al. (2016) |
| Obesity | in vivo: C57BL/6J mice, in vitro: HepG2 cells and 3T3-L1 adipocytes | In vivo: diet-induced obese (DIO) mice, in vitro: H2O2-induced oxidative stress | Increase the expression of Nrf2, GCLC and CAT, improve glucose metabolism and liver function, decrease body weight gain | in vivo: 200 mg/kg/d for 10 weeks (i.v.), in vitro: 10, 50, and 100 mg/L | NA | Yan et al. (2020) |
| Skin photoprotective effect | in vivo female KM mice | Photoaged mouse model | Prevent UV-induced macroscopic skin lesions, promote the ability of the skin to regain its initial shape, elevated the activities of skin anti-oxidant enzymes, increased skin collagen content, maintained the structural integrity of the skin | 50, 100, and 200 l g/mouse following each UV exposure | NA | Kong et al. (2013) |
| Anti-anaphylactoid activity | in vivo: C57BL/6 mice, in vitro: Laboratory of allergic disease 2 (LAD2) human mast cells and mouse peritoneal mast cells | A mouse model of hindpaw extravasation | Attenuate calcium flux, decrease degranulation, attenuated degranulation triggered by endogenous and exogenous substances, decrease the activation of the p38- and c-Jun N-terminal kinase (JNK) signaling pathway regulating calcium fluctuations | in vivo: 0, 2.5 mg/kg, 5 mg/kg, 10 mg/kg (i.v.), in vitro: 0, 50 μM, 100 μM, 200 μM | NA | Liu et al. (2014) |
| Inhibition of hepatic fibrosis | in vivo: SD rats | CCl4-induced fibrogenesis | Decrease fibrogenic, profibrotic expression of α-SMA, and MEK-3C gene expression, decreased expression of Tjβ1, Tjβ2, Mmp9, Mibk5, and phosphorylation of ERK5 | 5 mg/kg/d for 12 weeks | NA | Zhang et al. (2012) |
| Type 1 diabetes | in vivo: SD rats | Human epithelial and keratinocytes (HEKs) | in vivo: Type 1 diabetes mellitus (T1DM) model | Accelerate diabetic wound healing through promoting angiogenesis and reducing inflammatory responses, enhance angiogenesis by upregulation of hypoxia inducible factor-1 alpha (HIF-1α) expression | 20.4 mg/ml of HSYA/DFO hydrogel solution for five weeks | Deferoxamine (DFO) and HSYA | Gao et al. (2018) |
| Type 2 diabetes mellitus | in vivo: Wistar rats | in vivo: HFD feeding-induced T2DM model | Reduce fasting blood glucose and insulin resistance, up regulate of PI3K and AKT, inhibit the apoptosis of β-cells | 120 mg/kg (i.v.) | NA | Lee et al. (2020) |
| Polycystic ovary syndrome (PCOS) | in vivo: ICR female mice | in vivo: Dehydroepiandrosterone-induced PCOS model | Elevate serum E2, P4, LH and AMH levels, reduce FSH level, normalize expression of dermal hormones secretion-related genes Star, Hsd3b1, cyp11a1 and cyp19a1, improve GSH content, enhance the activity of antioxidant enzymes SOD, GSH-Px and CAT | 3.5 mg/kg (i.p.) | HSYA and ginsenoside Rb1 | Luo et al. (2012) |
healthy Chinese female volunteer. The $t_{1/2}$ corresponded to the median of the range from 2.23 to 4.63 h (the average value of 3.17 h) at each dose. But this research had some potential limitations, such as a low-volume sample and only female volunteers, which lack of universality. Moreover, the combination therapy of HSYA and protocatechuic aldehyde greatly promoted the intake of HSYA (Ao et al., 2018).

HYSA is reduced and hydrolyzed, such as hydroxylation, hydration and methylation, dehydration, hydrogenation and hydration, to obtain phase I metabolites under the action of hepatic microsomal drug-metabolizing enzyme in the liver. Phase II metabolism included acetylation and glucuronidation, dehydration, hydrogenation, hydratation, hydroxylation with glucuronidation, deglycosylation, methylation and glucuronic acid conjugation reactions (Jin et al., 2016b; Wu et al., 2018). The possible metabolic pathways were summarized in Ahmed and Aljaeid, 2016, Figure 7. HSYA and its metabolites are distributed in the heart, liver, spleen, lung, kidney, brain and intestines. In addition, it is almost impossible to cross the healthy blood-brain-barrier (BBB), but easily to penetrate damaged BBB (Sheng et al., 2019).

The low bioavailability of HSYA directly blocks its therapeutic efficiency. It is difficult for HSYA to pass through phospholipid bilayer because of its strong polarity and poor membrane permeability, which is also an important reason for its low bioavailability. Therefore, the design of HSYA delivery systems mainly focused on improving its liposolubility. Despite, HSYA exists as an undivided molecule in the strong acid environment of the stomach, it cannot be absorbed by the stomach due to its high molecular weight and strong hydrophilicity (Zhang, 2006). Besides, the small intestine actually is the main absorption site of HSYA. However, gastrointestinal metabolism in a weak alkaline environment is not conducive to the absorption of HSYA, on the contrary, it promotes degradation (Wu et al., 2018).

**PHARMACOLOGICAL EFFECTS AND MOLECULAR MECHANISM**

HSYA shows excellent therapeutic effects on various diseases, such as cardiovascular and cerebrovascular diseases, cancer, and so on. This part provided the biological activity of HSYA in detail (Table 3, Figure 8).

**Cardioprotective Effects**

HSYA has been proved to be a superior agent on the cardioprotective system in vivo and in vitro. HSYA (5 mg/kg, 30 min before ischemia, i. p.) was found to improve ischemia/reperfusion (I/R) injury by reducing the releases of cTnI, IL-6, LDH and the myocardial infarction size (Zhou et al., 2019). Similarly, HSYA (4 or 8 mg/kg) could reduce the expression of MIS, CK-MB, and MDA in experimental acute myocardial ischemic model, which induced by left anterior descending coronary artery (LAD) ligation (Tu et al., 2009). Furthermore, HSYA combined with nitroglycerin showed a better therapeutic action on acute myocardial infarction than HSYA alone, which produced a marked increase in SOD, eNOS, and NO content.

The mechanisms of HSYA on cardioprotective effects are related to antioxidant, free radical scavenging abilities, and anti-inflammatory activity. The nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor responsible for the regulation of cellular redox balance and protective antioxidant and phase II detoxification responses in mammals. On basal condition, Nrf2 binding with the chaperon protein Kelch like-ECH-associated...
protein 1 (Keap1) located in cytoplasm with inhibition abilities. When facing oxidant stress, Nrf2-Keap1 combination is separated. Nrf2 transports to the nucleus and activates the transcription of genes encoding. Heme oxygenase-1 (HO-1), as one of Nrf2-dependent gene, protects against oxidant stress (Loboda et al., 2016; Reuland et al., 2013). Hu et al. (2016) reported that the combination of HSYA and DSS exerted the markedly antioxidant capacity on increasing the expression of HO-1, the phosphorylation of Akt, and the translocation of Nrf2 (Figure 8). Similarly, HSYA attenuated the expression of IL-1β, TNF-α, iNOS, COX-2, MCP-1 on BV2 cells of oxygen and glucose deprivation models (OGD). HSYA also inhibited NF-κB signaling pathway, TLR4 signaling pathway and phosphorylation of p38 (Han et al., 2016; Li et al., 2013). In addition, HSYA decreased the formulation of mitochondrial permeability transition pore in hypoxic cardiac myocytes, thereby inhibiting cardiomyocytes from damage during cardiomyocyte reoxygenation (Huber et al., 2018). In summary, oxidative stress and inflammation related signaling pathways (e.g., Nrf2/OH-1, p38 MAPK, NF-κB signaling pathway and TLR4 signaling pathway all play an important role during the treatment of cardiovascular disease with HSYA.

Neuroprotective Effect

HSYA showed excellent neuroprotective effect. HSYA injection (50 mg/d and 75 mg/d) in patients with acute ischemic stroke of blood stasis syndrome might be to undergo for a phase III clinical trial (Zhu et al., 2020). HSYA (2 mg/kg, tail vein injection) protected the C57BL/6J mice against middle cerebral artery occlusion (MCAO) by decreasing the expression of inflammatory genes factors, including TNF-α, IL-1β, and NO (Lv et al., 2015). HSYA (0.6 mg/100 g, tail vein injection) could also improve spatial learning and memory ability of vascular dementia model rat by promoting angiogenesis and increasing synaptic plasticity (Zhang et al., 2014). Moreover, HSYA (6 mg/kg per day, 2 weeks, i. v.) could reduce the accumulation of amyloid precursor protein, improve synaptic function and reverse homocystine (Hcy) induced cognitive impairment in Alzheimer’s disease mice (Lu et al., 2013).

The neuroprotective effect of HSYA might be related to the regulation of TLR4, NF-κB, p-p65, MAPK, PI3K/Akt and Nrf2/HO-1 signaling pathways (Wei et al., 2005; Lv et al., 2015; Deng et al., 2018). NF-κB is a family of dimeric transcription factors central to regulate immune development, immune responses, inflammation, cancer, and other diseases. HSYA inhibited NF-κB to exert neuroprotective effect (Yu et al., 2020). Similarly, HSYA (20 mg/kg for 28 days) could improve motor dysfunction in C57/BL6 mouse model of Parkinson by promoting the expression of BDNF, p-TrkB/TrkB, DRD3, p-PI3K/PI3K and p-AKT/AKT (Wang et al., 2017).

Anticancer Effect

Some studies have found that HSYA exerted anticancer activity in several cancer cells, such as human umbilical vein endothelial cells (HUVEC), HepG2 cells, Skov3 cells, as well as some in vivo studies. HSYA could eliminate reactive oxygen species (ROS), prevent apoptosis from membrane permeability, and inhibit proliferation and migration (Ma et al., 2019a; Chen et al., 2019; Zhang et al., 2019). HSYA also increased the thymus index, and effectively down-regulate the mRNA levels of forkyhead box P3-positive (Foxp3) and retinoic acid receptor-related orphan receptor-gamma-t (RORet), which contributed to improve the tumor immune microenvironment—the key points of tumor cell proliferation and invasion (Ma et al., 2019b). What is particularly noteworthy is the anticancer abilities of HSYA might related to the excellent antioxidant effect.

Anticoagulant Effect

PAF, as the most effective platelet activator known so far, has a wide range of biological activities, and can be synthesized by a variety of cells such as platelets, leukocytes, endothelial cells. Anticoagulant effect of HSYA plays a significant mediating role in many pathological processes, such as tissue IR injury, coronary heart disease, atherosclerosis, cerebrovascular disease and many other cardiovascular diseases. HSYA dose-dependently inhibited the specific binding between [3H] PAF and washed rabbit platelet, which is considered as a new generation PAF receptor antagonist in the future (Zang et al., 2002). PAF-activated human small airway epithelial cells model was pretreated with HSYA. Guo et al. (2018) proposed that HSYA could attenuate the PAF-induced inflammatory factors, destruct cell-barrier function, and inhibit the expression of protein kinase C, MAPK, activator protein-1, and NF-κB activation to show anticoagulant effect.

Other Pharmacological Effects

In addition to the biological activities discussed above, HSYA also has other diverse pharmacological effects, such as immunodeficiency, anaphylactoid, hepatic fibrosis, pulmonary arterial hypertension, skin photosensitivity, Type 1 diabetes, vascular dementia, and so on.

HSYA is an immunomodulator that regulates the inflammatory response induced by lipopolysaccharide in various cells, including human non-small lung cancer cell line (A549 cells), H1299 and Eahy926 human endothelium cells. It could inhibit many kinase activities related to inflammatory factors, such as TNFa, IL-1β, and IL-6. HSYA could also inhibit the pro-inflammatory proteins expression, such as NF-κB p65, PI3K/Akt/mTOR, MAPK TLR-4, Myd88 and ICAM-1 (Han and Zhao, 2010; Jin et al., 2016; Jiang et al., 2019). HSYA was also proved to relieve certain respiratory conditions by decreasing mRNA levels of ICAM-1 and E-selectin elevation (Liu et al., 2014; Zhang et al., 2017). Moreover, another study implemented by Zheng et al. (2019) showed that HSYA could attenuate ovalbumin-induced allergic asthma in guinea pigs.

HSYA possesses a strong vascular relaxation effect on pulmonary arterial hypertension (PAH). It reduced the vascular tension by activating the Kv channel in pulmonary vascular smooth muscle cells (PVSMCs) (Bai et al., 2012). HSYA (6 mg/kg for 11 days) promoted angiogenesis of ischemic mice. The therapeutic mechanisms of HSYA might be associated to significantly increase the capillary-like tube formation and migration of HUVEC, enhance the expression of angiopoietin 1 and Tie-2, phosphorylations of Tie2, Akt and extracellular signal-regulated kinase 1/2 (Chen T. et al., 2016).
It is also reported that HSYA could exert protection on macroscopic skin lesions induced by ultraviolet rays (UV). It could promote the skin to regain its initial shape, elevate the activities of skin anti-oxidant enzymes, increase skin collagen content and maintain the structural integrity of the skin (Kong et al., 2013).

The combination of HSYA and deferoxamine (DFO) was discovered to improve type 1 diabetes by accelerating diabetic wound healing, promoting angiogenesis, reducing the inflammatory response, and up-regulating the expression of hypoxia-inducible factor-1 α (HIF-1 α) (Gao et al., 2018).

**DISCUSSION AND CONCLUSION**

In the present review, we systematically summarized the materials about HSYA, including acquisition methods, extraction and detection methods, pharmacokinetics, pharmacological effects and molecular mechanism. HSYA is proved to be an excellent antioxidant, anti-inflammatory and anti-coagulant agent, so it plays an exceptional role in the treatment of cardiovascular and cerebrovascular diseases by down-regulating NF-κB signaling pathways, inhibiting MAPK signaling pathways, and attenuating the activation of Nrf-2/HO-1 signaling pathways. However, it is easily to degrade in the process of storage, extraction and separation procedure due to its chemical instability, which brings great challenges to the application of HSYA. Another major challenge is the low bioavailability caused by strong polarity. A large number of studies have been carried out to improve the chemical instability and bioavailability. Microemulsions, self-emulsifying systems, nanoparticles and other drug delivery systems have gradually improved the bioavailability, chemical stability, cellular uptake and biological activity of HSYA, which will be evaluated in clinical trials later.

Although great progress has been made in the research and application of HSYA in the past few decades, there are still many problems and challenges. First of all, chemical instability and low bioavailability of HSYA are still far from being resolved, which is still a key issue of the future research. It is important to continue the development of viable drug delivery systems for HSYA. The crucial aspects will involve in the enhancement of the solubility and bioavailability, as well as methods for selectively targeting these delivery systems to disease sites. Secondly, HSYA has been used to treat cardiovascular and cerebrovascular diseases clinically in China, but the drug target has not been completely revealed at present, which needs in-depth study later. Finally, the approach of obtaining HSYA from plants is cumbersome and complicated, and the yield is unsatisfactory. Chemical synthesis might pollute the environment, so it does not recommend to popularize. Biosynthesis is characterized by high efficiency, energy saving, and environmental protection, and it will transform into the major source of HSYA in the near future.

In short, it is the first time to systematically summarize the basic information about HSYA, which might provide relatively comprehensive basic data for the related research of HSYA.

**AUTHOR CONTRIBUTIONS**

HX, FZ and PW performed the frame design of the manuscript. FZ collected the data and drafted the manuscript. YJ, XZ and DC helped to organize the data. PW revised the manuscript. All authors read and approved the final manuscript.

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Zhao et al. Review of Hydroxysaffor Yellow A

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling Editor declared a past co-authorship with one of the authors HX.

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**GLOSSARY**

AUMC the plasma concentration-time curve  
BBB the blood-brain barrier  
DAD diode  
DDAB dimethyl octadecyl ammonium bromide  
ECD electrochemical  
EDTA ethylenediaminetetraacetic acid  
HIF-1α hypoxia-inducible factor-1α  
HO-1 hemeoxygenase-1  
HSYA Hydroxysafflor yellow A  
I/R ischemia/reperfusion  
LAD left anterior descending coronary artery  
LOD the limits of detection  
LOQ the limit of quantification  
MCAO middle cerebral artery occlusion  
MIS myocardial infarction size  
Nrf2 nuclear factor erythroid 2-related factor 2  
MSPD Matrix solid-phase dispersion  
OGD oxygen and glucose deprivation models  
PAH pulmonary arterial hypertension  
Papp the apparent permeability coefficient  
P-gp P-glycoprotein  
SDEDDS Self-emulsifying drug delivery system  
SLN Solid lipid nanoparticles  
SY safflower yellow  
TBI traumatic brain injury  
UAE ultrasound-assisted treatment.