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Review

A review of the pharmacology and toxicology of aucubin

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ABSTRACT

Aucubin is an iridoid glycoside that is widely prevalent in traditional medicinal herbs, such as *Eucommia ulmoides* Oliv., *Aucuba japonica* Thunb. and *Plantago asiatica* L. This review aims to provide a comprehensive summary of the source, biological activity, pharmacokinetics and toxicology of aucubin with the ultimate objective of providing a guide for future drug development and potential clinical applications of aucubin. Aucubin is a highly active compound possessing extensive biological effects including antioxidant, anti-aging, anti-inflammatory, anti-fibrotic, anti-cancer, hepatoprotective, neuroprotective and osteoprotective properties. Although aucubin has been shown to have poor oral bioavailability in rats, aucubin is widely distributed in multiple organs including kidney, liver, heart, spleen and lung, and there is a sex difference in the absorption of aucubin. Tolerance of aucubin is good and no serious adverse reactions have been observed to date. In short, aucubin is a compound with abundant potential sources, good safety and numerous beneficial biological activities, which exhibits high potential value for use in health care products and pharmaceuticals. In order to accelerate the development and utilization of aucubin-related products, in-depth studies should be focused on the following questions of interest. First, it is necessary to introduce advanced separation and formulation technologies to improve the yield and stability of aucubin products. Second, studies should focus on the specific pharmacological activities of aucubin to determine the structure-activity relationship so as to improve the efficacy and reduce side effects. Finally, clinical studies are needed to confirm the efficacy of aucubin in specific diseases.

1. Introduction

Aucubin (1,4a,5,7a-Tetrahydro-5-hydroxy-7-hydroxymethylcyclopenta (c)pyran-1-yl-beta-D- glucopyranoside, AU, Fig. 1) is an iridoid glycoside that presents in natural medicine. Since the first discovery of AU in *Aucuba japonica* in 1905, scientists have reported that it exists in many natural plants such as *Eucommia ulmoides* Oliv., *Aucuba japonica* Thunb. and *Plantago asiatica* L. [1]. With the development of modern medicine and pharmaceutical science, researchers have demonstrated that AU has a wide range of pharmacological properties including antioxidation, anti-aging, anti-inflammation, anti-fibrosis, anti-tumor, hepatoprotection, neuroprotection, osteoprotection and others. As a result, it has recently received increasing attention. Studies of the pharmacokinetics and safety of AU are increasing year by year. Here we reviewed the sources, physicochemical properties, pharmacodynamics, pharmacokinetics and toxicology of aucubin in order to provide a theoretical reference for the comprehensive development and utilization of AU.

2. Source and physicochemical properties

Aucubin, a member of the iridoid glycosides, is widely distributed in species of the plant kingdom. In 1905, AU was first isolated by Bourquelot and Herissey from the leaves of *Aucuba japonica*, and was successively found in plants such as *Garryaceae*, *Plantaginaceae*, *Orobanchaceae*, *Globulariaceae*, *Scrophulariaceae*, and *Eucommiaceae* [1]. In addition, some species in genera such as *Melampyrum arvense*, *Buddleia globosa, Utricularia* (Lentibulariaceae), *Himatanthus* (Apocynaceae) [2], *Hypericum* (Hypericaceae), *Cotinus* (Anacardiaceae) [3], *Morinda* (Rubiaceae), *Ligustrum*(Oleaceae) [4], *Crescentia*(Bignoniaceae), also contain aucubin. The content of AU is higher in *Eucommia ulmoides*, *Aucuba japonica* and *Plantago asiatica*, and is especially high in the seeds of *Eucommia ulmoides*, reaching 7–10%.

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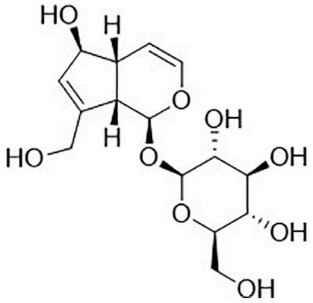

Fig. 1. The chemical structure of aucubin.

Although AU is widely distributed, it is difficult to obtain a large amount of high purity AU for the health care and pharmaceutical industry, not only because its content in plant material is extremely low, but also because its structural instability increases the difficulty of the extraction process. The biosynthesis of iridoid glycosides are all derived from geranyl pyrophosphate [5]. Like all iridoids, AU has a cyclopentan-[C]-pyran skeleton. AU has ten carbons and the stereochemical configurations at C5 and C9 lead to cis fused rings, which are common to all iridoids containing carbocyclic- or seco-skeleton in non-rearranged form. An *O*-linked glucose moiety attached to the C1 gives rise to the structure instability of AU, i.e. the glucosidic bond is readily hydrolyzed by acid. The subsequently-formed hemiacetal structure is so active that can under oxypolymerization. Both the cyclopentane ring (C5-C6-C7-C8-C9) and the pyran ring (C1-O2-C3-C4-C5-C9) of the glucoside adopt an envelope conformation, with alternating positive and negative values of torsion angles. The absolute values range between 1.6(4)° and 56.0(3)° in the pyran ring and 0.3(3)° and 22.9(2)° in the cyclopentane ring [6].

AU is generally considered to be soluble in water. However, in aqueous solutions it spontaneously undergoes oxidation, resulting in the formation of insoluble components. It is also soluble in methanol and ethanol, but is insoluble in organic solvents such as ether, chloroform, benzene and petroleum ether. The concentration of AU in octanol after partitioning from the respective buffers was found to be below the sensitivity of the assay (< 1 μg/ml), indicating that the octanol-water (buffer) partition cofficient of AU is extremely low [7]. The absorption spectra of AU (220, 255, 290 nm) is generally used for the determination of AU isolated from plants [8]. AU contains a glycosidic bond and a diacetal structure, which makes it highly susceptible to degradation and oxidization. The glycosidic bond of AU is cleaved under acidic conditions to produce aglycones, glucose and other products. The hemiacetal structure in the aglycon is spontaneously opened in aqueous solution to form a dialdehyde structure [109]. The aglycon can also undergo intramolecular rearrangement and be converted into a stable isomer (1,10-anhydro-6-deoxy-7,8-dihydro- 7,8-dihydroxyaucubigenin) [110], the possible degradation process of AU is shown in Fig. 2. Chun In Koo analyzed the content of AU with high performance liquid chromatography to evaluate the influence of pH, temperature, ionic strength and metal ions on the stability of AU, and found that AU was rapidly degraded into black substances under acidic conditions. The degradation of AU is dependent on temperature and ionic strength, as evidenced by the degradation rate of AU accelerates with the increase of ionic strength and temperature. Different metal ions can also affect its degradation, and Cu^{2+} has the greatest impact [111]. As mentioned above, the degradation of AU is likely to occur under high temperature, strong acid, and light. At present, AU is mainly extracted from plants. Therefore, it is suggested that AU should be prepared at a low temperature in weak acid and dark conditions to increase the yield and stability of AU.

3. The method for the preparation of AU

Various extraction methods have been developed according to the different physical and chemical characteristics of AU, such as cold-maceration and reflux extraction. In order to increase the permeability of active substances through the cell wall, specific enzymes and ultrasound techniques have introduced to destroy the plant cell wall. The enzymolysis extraction and ultrasonic-assisted extraction aid in the isolation of AU. A microwave extraction method can also be used to extract AU from *Eucommia ulmoides*. Li H compared the extraction efficiency of the supercritical CO_2 and Soxhlet extraction methods for AU from seeds of *Eucommia ulmoides Oliv.* and found that the supercritical CO_2 extraction produced a higher yield with a lower cost for the extraction [112].

Several purification methods have been used to separate and purify AU, including precipitation, crystallization, biphasic extraction, column chromatography, macroporous resin adsorption, membrane separation andpreparative high performance liquid chromatography. The purity of AU obtained by preparative liquid chromatography is as high as 98% or more, but it is expensive and the amount of preparation is relatively small. Therefore, it is suitable for the preparation of AU standards. The macroporous resin adsorption approach has become mainstream for use in the separation of AU due to its simple operation, low-cost, and suitability for large-scale preparations. Xinyu Yang separated and purified AU from the ionic liquid extraction solution of samaras of *Eucommia ulmoides* using a macroporous resin adsorption method, then eluted with ethanol and concentrated to obtain AU with a purity of 79.41% [113]. Our research group added acetone to the ethanol extract of *Eucommia ulmoides Oliv.* for cooling and crystallization and then purified by silica gel column, and concentrated to obtain > 98% of purity AU, providing sufficient material basis for pharmacological studies of AU.

4. Pharmacodynamics

4.1. Antioxidation and anti-aging

Oxygen is essential for various life cellular processes such as signal transduction, regulation of gene expression, cell growth, development and death. The uptake of oxygen is important for oxidative phosphorylation, allowing the aerobic metabolism of glucose to provide the energy required by the body [9]. Meanwhile, reactive oxygen species (ROS) such as oxygen radicals, hydroxyl radical and hydrogen peroxide, are generated by the catalysis of oxidases in the mitochondria and endoplasmic reticulum. Interestingly, in order to adapt to the living environment, the body formed a self-antioxidant defense system including glutathione(GSH), vitamins, coenzyme Q and lipoic acid, which collectively counteract the production of ROS [10]. Moreover, antioxidant enzymes such as superoxide dismutase(SOD), catalase(CAT), glutathione peroxidase(GSH-Px) can eliminate ROS. Under normal physiological conditions, the production and elimination of ROS is in a steady state. However, when the body is exposed to a harmful external stimulus, it can trigger insufficient antioxidants and excessive ROS formation, leading to oxidative stress [11]. Upon oxidative stress, excessive ROS can destroy the structure and function of many macromolecules, such as DNA, proteins and lipids, causing cell necrosis and apoptosis [12]. As reported in the literature, oxidative stress is critical involved in the development of cardiovascular, neurological, and skeletal diseases. Emerging evidences showed that AU is a powerful antioxidant. On one hand, AU could directly reduce the levels of ROS, MDA and 4-hydroxynonenal as well as effectively scavenge oxygen radical, hydroxyl radical and DPPH radical. On the other hand, AU also increases the antioxidative activities of SOD, CAT and GSH-Px. AU enhanced antioxidant capacity to inhibit gastric mucosal injury, endothelium dysfunction, cardiac remodelling and diabetic encephalopathy by correcting the balance between oxidation and the

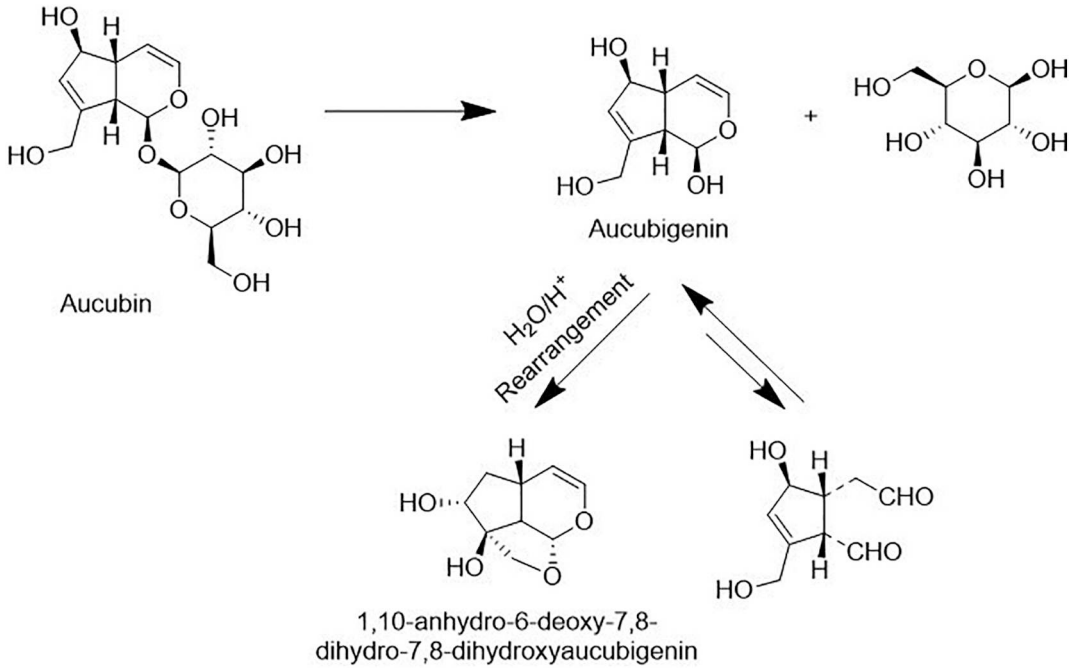

Fig. 2. The degradation of AU.

antioxidant defense system [13–16]. Furthermore, AU could increase the expression of Nrf2-targeted protein including heme oxygenase-1 (HO-1) and quinone oxidoreductase-1 as well as activate the AMPK pathways, which provide protective effects on non-alcoholic fatty liver disease and acute pulmonary injury [17,18]. Reactive nitrogen species including NO and NOS can react directly with the biomolecules of cells to induce oxidative damage. Previous studies have found that AU inhibits the expression of iNOS and the production of NO in IL-1β-treated articular chondrocytes [19]. Finally, AU decreased the formation of ROS and MDA levels and increased the contents of GSH in the Ultraviolet B-irradiated human skin fibroblasts. Because Ultraviolet B can induce cellular senescence and collagen damage which are reflected by an increase in β-galactosidase activity and up-regulation of MMP1, this study further found that the expression of MMP-1 and β-galactosidase activity were markedly decreased after pretreatment with AU [20]. These findings suggest that AU may prevent the photoaging of human skin through its antioxidant effect.

4.2. Anti-inflammation and immunomodulation

AU has showed potent anti-inflammatory activity in carrageenan-induced mouse paw edema and 12-O-tetradecanoylphorbol acetate-induced mouse ear edema with a maximum inhibitory activity of up to 80% [21]. Treatment with AU suppressed the number of neutrophils and macrophages in BALF of mice with pulmonary injury [18,22]. In animal models of neurological diseases, AU also inhibited the activation of glial cells, which are responsible for brain inflammation [23,24].

Arachidonic acid metabolism is an important element in the anti-inflammatory activity of AU. Arachidonic acid is metabolized by cyclooxygenases and lipoxygenases to form leukotrienes, prostaglandins and thromboxanes, which were critically implicated in inflammatory responses. AU inhibited calcium ionophore-stimulated LTC_4 release from mouse peritoneal macrophages and TXA_2 from human platelets [25]. It also alleviated the development of osteoarthritis manifested as AU diminished the levels of iNOS and COX-2 and decreased the production of NO in IL-1β-treated articular chondrocytes [19].

The anti-inflammatory effect of AU is also related to the regulation of inflammatory mediators such as IL-1β, IL-6 and TNF-α. Remarkably, AU reduced the secretion of IL-1β, IL-6 and TNF-αin antigen-stimulated mast cells and in LPS-induced macrophages [26]. AU has been shown to decrease these inflammatory mediators in multiple disease models, such as gastric mucosal injury, osteoarthritis, epilepsy and dry eye disease [27]. Furthermore, AU suppressed the degradation of IκBα and blocked the translocation of the P65 subunit of NF-κB from cytosol into nucleus in antigen-stimulated mast cells [26]. Another study showed that AU hampered TNF-α-induced the atherogenic adipokines in 3 T3-L1 adipocytes by blocking NF-κB activation [28]. Recent study has observed that Nrf2 knockdown or AMPK inhibitor markedly abrogated the suppressive effects of AU on LPS-triggered expression of pro-inflammatory cytokines [18]. Therefore, AU likely exerts its anti-inflammatory effects through the NF-κB, Nrf2 and AMPK pathways.

In LPS-stimulated RAW264.7 cells, a small number of studies showed that hydrolysate of AU restrained the COX-2 activity and suppressed the production of TNF-α and NO [29]. Hydrolysate of AU could also block the IκBα degradation and the translocation of NF-κB into the nucleus, however it had no significant effect on intracellular cAMP levels [30]. Interestingly, AU itself did not change the activity of cyclooxygenases and the expression of TNF-α in LPS-induced RAW264.7 cells. These results suggest that hydrolysate of AU may be an anti-inflammatory mediator in someChinese medicines.

Notably, AU has been shown to increase the proliferation of human peripheral blood mononuclear cells(PBMC) and to augment the secretion of IFN-γ, indicating that AU possesses immune-enhancing activity [31].

4.3. Hepatoprotection and detoxifcation

Aucubin has been reported to be an extraordinary candidate drug for hepatoprotection and detoxifcation. It can dramatically prevent poisoning caused by α-amanitin in mice, intraperitoneal administration of AU after 12 h of α-amanitin induction can still improve the survival rate of mice [32]. The AU-treated Beagle dogs could be also survived after being subjected to mushroom poisoning for 30 min [33]. AU has been shown to dramatically shorten the duration of hexobarbital sodium salt-induced sleep and decreased the activity of serum glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase in the CCl_4 treated mouse, suggesting that AU is effective in preventing CCl_4-induced hepatic damage [34]. AU has also been shown to suppress the

DNA replication of hepatitis B virus [33].

The detoxification effect of AU on α-amanitin is related to the promotion of α-amanitin excretion and binds to calf thymus DNA. Further analysis has showed that hydrolysate of AU may be the real active form of AU for detoxication, because aucubigenin may displace α-amanitin from the binding sites on the plasma albumin molecule while 91% of AU existed in the unbound form. Aucubigenin could also inhibit both DNA polymerase and RNA polymerase activity in HepG2 cells in a dose-dependent manner [35]. In addition, the inhibitory effects of AU on hepatic RNA and protein synthesis as well as the reduction of aucubigenin on CYP450 activity could explain the liver protective effect of AU [34,36].

Nonalcoholic fatty liver disease(NAFLD) is a metabolic syndrome characterized by excessive accumulation of fat in the liver and covers a range of disease states such as steatosis, nonalcoholic steatohepatitis, liver fibrosis, cirrhosis and even hepatocellular carcinoma [37]. NAFLD affects about 30% of the adult population wordwidely [38]. Disturbance of lipid metabolism, endoplasmic reticulum (ER) stress, oxidative stress, inflammatory responses and fibrogenesis are pathogenic stimulators that could cause hepatocellular death and aggravate the deterioration and development of NAFLD [39]. *Eucommoia ulmoides* was reported to exhibit a suppressive activity on hepatic lipid accumulation, hepatic cell death and ER stress in HepG2 cells and high-fat-diet-fed rats. Moreover, as the major active ingredient of *Eucommoia ulmoides,* AU can also exert beneficial effects similar to *Eucommoia ulmoides* on palmitate-induced hepatic lipid accumulation and cell death by enhancing lysosomal activity [40,41]. Another recent study showed that AU increased the expression of Nrf2 and PPAR and promoted the phosphorylation of ACC, AMPKα and AKT, resulting in a reduction of lipid accumulation, oxidative stress and inflammation in a tyloxapol-induced mouse model of NAFLDand in apolipoprotein C-III induced 3T3L1 cells [17]. Interestingly, our study found that AU could block TGF-β1-induced activation of human hepatic stellate cells as well as decrease the expression of collagen protein and the generation of ROS, indicating that AU inhibit liver fibrosis [42]. As mentioned above, AU combats NAFLD via modulating various mechanistic pathways. However, the clinical efficacy of AU in the treatment of patients with NAFLD is yet to be tested.

4.4. Anti-fibrosis

Fibrosis is a common pathological feature in many chronic diseases, defined as excessive deposition of extracellular matrix(ECM) components including collagen and fibronectin. It can occur in most human organs, such as liver, kidney, heart, lung and skin [43]. Fibrosis leads to the malfunction and failure of the organ, which is responsible for up to 45% of deaths in industrialized countries [44]. Although FDA had approved nintedanib and pirfenidone for the treatment of idiopathic pulmonary fibrosis, there is still a large gap in the treatment of pathological fibrosis and new anti-fibrotic drugs need to be introduced quickly [45]. Activation of hepatic stellate cells is the cellular basis of liver fibrosis. Our research group has shown that AU and its hydrolysate-aucubigenin reverses TGF-β1-stimulated the activation of LX-2 cells and reduces the expression of collagen I and collagen III protein. Furthermore, AU and aucubigenin decreased the production of ROS and down-regulated the expression of NOX4 mRNA in human hepatic stellate cells [42]. In addition, we reported that AU inhibited TGF-β1-induced proliferation and differentiation of fibroblasts. In the bleomycin-induced mouse model of pulmonary fibrosis, we observed that AU alleviated bleomycin-induced lung parenchymal fibrotic changes and prevented the increased expression of collagen I, collagen III, TGF-β1, α-SMA and inflammatory injury, improving the pulmonary function [22]. Recent evidence indicates that AU regulates the AMPKα/mTOR signaling to suppress TGF-β1-induced proliferation and activation of cardiac fibroblasts as well as decrease fibrosis-related protein expression [46]. AU also attenuates cardiac hypertrophy, fibrosis, oxidative stress and inflammation to improve cardiac function by regulating neuronal NOS-mediated signaling pathway in myocardial infarction or pressure induced cardiac remodelling in mice [16,47]. In general, AU is a potential therapeutic agent for cardiac remodelling, pulmonary fibrosis and liver fibrosis. However, these beneficial results are only derived from cells and animals. Importently, the abnormal effect of AU on MMP-2 and TIMP-1 expression in human hepatic stellate cells may promote the formation of ECM to exacerbate liver fibrosis [42]. Therefore, the efficacy and safety of AU in treating fibrosis-related diseases remain to be confirmed in the clinic.

4.5. Neuroprotection

The burden of disease caused by neurological disorders accounts for 10.2% of global disability-adjusted life-years(DALYs). Further, neurological disorders were responsible for 16.8% of global deaths in 2015, meaning that neurological disorders were the leading cause of global DALYs and the second leading cause of global deaths. With an increasing and aging population, the burden of neurological disorders has risen rapidly over the past 25 years and the number of patients with neurological disorders will continue to grow in the coming years [48]. Accordingly, the demand for neuroprotective drugs will be also increasing steadily. Natural products are considered to be an important source for the development of disease-modifying drugs because of its ability to block or delay the progression of neurological disease and low toxicity [49,50].

Loss of neurons in the brain is a common pathological feature of neurological diseases, and it can lead to motor, cognitive and behavioral dysfunctions. A growing body of research shows that repairing neuronal loss could improve the symptoms of neurological diseases including traumatic brain injury, Parkinson's disease, Alzheimer's disease, stroke and epilepsy. Oxidative stress results from overproduction of reactive oxygen species (ROS) due to the imbalance between pro-oxidant and endogenous antioxidant system. Excessive ROS oxidized lipids, proteins and nucleic acids of cell, which destroying cellular structures and functions and induced apoptosis or necrosis [51]. H_2O_2 is a major source of ROS and can induce apoptosis of neurons. Xue used AU to pre-treat the H_2O_2-induced PC12 cells and found that AU increased PC12 cell viability and decreased H_2O_2-induced cell damage and necrosis. AU also restored the balance between the expression of Bcl-2 and Bax, inhibited caspase-3 activation and PARP cleavage, as well as reduced the levels of malondialdehyde and enhanced the activity of antioxidant enzymes [52,53], suggesting that AU alleviated P12 cell damage by suppressing mitochondria-mediated necrosis and regulating the endogenous oxidant–antioxidant balance. In addition, their research demonstrated that AU reduced neuronal apoptosis in the CA1 region of hippocampus to improve working memory in rats with diabetic encephalopathy through the same mechanism. This neuroprotective effect was significant both in the short term and in the long term [13,54,55]. Our research in rats with lithium-pilocarpine induced status epilepticus showed that AU alleviated hippocampal neuronal damage manifested as the reduction of apoptotic neurons and an increase in survival of neurons via inducing autophagy and inhibiting necroptosis [56]. Recently, we also found that AU could modulate the levels of GABA and glutamate as well as its receptor and transporter expression to reduce seizure intensity and prolong the latency of seizures in epileptic mice [24]. Another study in the Parkinson's disease model showed that AU obviously mitigated the reduction of dopaminergic neurons and increased dopamine levels in the substantia nigra to ameliorate motor deficits [23]. As mentioned above, AU has the effect of inhibiting neuronal loss and exerts beneficial effects on epilepsy, diabetic encephalopathy and Parkinson's disease. However, its effectiveness and detailed mechanisms of action on other neuronal loss-related brain diseases still need to be further explored.

Neuronal differentiation and neurite outgrowth are the principal processes in the development of the nervous system, and regulation of

these processes to promote nerve regeneration is of great significance for the treatment of nerve injury and neurodegenerative diseases [57]. Early studies in P12h cells showed that hydrolysate of AU could promote neurite outgrowth and neuronal differentiation with the enhancement of the KCl or Carbachol-induced the increase of Ca^{2+} concentration while AU itself performed a weak effect on neurite outgrowth [58,59]. Hippocampal neural stem cells treated with AU displayed largely extended neurite morphology and increased the expression of neuronal markers. In the injury model of the rat sciatic nerve, Kim observed that AU could induce longer and thicker axons as well as re-myelinated at 3 weeks after sciatic nerve injury [60]. Recently, they focused on neuronal differentiation and found that AU facilitates cell survival in differentiated neurons and induces the differentiation of hippocampal precursor cells into GABAergic neurons rather than glutamatergic neurons, suggesting that AU may relieve neurodegenerative diseases caused by the loss of inhibitory GABAergic neurons [61,62].

Microglia and astrocytes are the major effectors of neuroinflammation in the central nervous system. Under pathological stimulation including infectious encephalopathy, brain trauma and neurodegenerative diseases, these two cells are changed from a resting state to an activated state. Overactive glial cells produce numerous pro-inflammatory mediators and cytotoxic factors, such as tumor necrosis factor-α (TNF-α), IL-1β, IL-6, ROS, NO and chemokines [63]. All of those can aggravate inflammatory responses and cause neuronal dysfunction to exacerbate neurological diseases. In epileptic mice, our previous study have showed that AU inhibited the activation of astrocytes and microglia and reduced the expression of TNF-α, IL-1β and HMGB1 [24]. Another study observed that AU alleviated the MPTP-induced the increase of activated glial cells in parkinsonian mice [23], indicating that AU could block neuroinflammation in epilepsy and Parkinson's disease. Nevertheless, how AU regulates the astrocytes and microglia activation, and whether AU alleviates neurological diseases by regulating inflammation remains unclear.

Neurotoxicity is a common dose-limiting side effect in patients with cancer undergoing chemotherapy. It is estimated that about 30–40% of patients are affected by chemotherapy-induced peripheral neuropathy [64]. Today, a lack of drugs are available to treat this condition [65]. As an anti-microtubule drug, paclitaxel is known for causing sensory neuropathy, which affects about one fifth of treated patients. In paclitaxel-induced mechanical allodynia in mice, Andoh found that repeated administration of AU could block the progressive deterioration of paclitaxel-induced mechanical allodynia and inhibit bothspontaneous firing and mechanical stimuli evoked firing in superficial dorsal horn neurons [66]. Moreover, AU reduced paclitaxel-induced endoplasmic reticulum stress in the sciatic nerve of mice and LY-PPB6 cells [67]. Strikingly, as mentioned above, AU promotes axonal growth and myelination in rats with sciatic nerve injury. Therefore, inhibition of endoplasmic reticulum stress and enhancement of axonal formation may explain the improvement of AU on peripheral neuropathy caused by paclitaxel.

4.6. Osteoprotection

Osteoarthritis(OA) is a serious degenerative disease of the joints and is characterized by cartilage destruction, affecting 20–30% of the adult population [68]. Because its pathogenic causes include obesity and aging, the prevalence of OA is rising. Under the injury stimulus, the balance between cartilage matrix synthesis and degradation is broken to chondrocyte cell death, which leads to cartilage damage, matrix depletion and loss of cartilage cellularity [69]. The inflammatory mediators and oxidative stress play a crucial role in the pathogenesis and progression of OA. Inflammatory cytokines, especially IL-1β and TNF-α, not only block the synthesis of ECM components but also upregulate the expression of metalloproteinases and a disintegrin and metalloproteinase with thrombospondin motifs, such as MMP-1, MMP-3, MMP-13 and ADAMTS-4, which degrade the components of cartilage [70]. Similarly, oxidative stress contributes to cartilage degradation during OA by directly degrading matrix components, activating MMPs and inducing cell death [71]. Moreover, inflammation interacts with oxidative stress to form a vicious cycle that aggravates cartilage destruction. An early experiment in a rat model of arthritis observed that *Eucommia* obviously reduced the levels of MMP-1, −3 and − 13 as well as improved the morphology of articular cartilage, suggesting that *Eucommia* inhibited the degradation of the extracellular matrix of the articular cartilage to create a cartilage-protecting effect [72]. As the major bioactive components of *Eucommia*, AU was reported to prevent the up-regulation of MMP-3, MMP-9, MMP-13 and the inflammatory mediators in IL-1β-treated rat chondrocytes. AU could also retard phosphorylation and nuclear translocation of NF-κB caused by IL-1β in the chondrocytes [19]. These results suggested that cartilage protection of AU in OA was associated with its anti-inflammatory and anti-catabolic effect through regulation of the NF-κB signaling pathway. Another study in H_2O_2 and compression induced OA-like chondrocyte models showed that AU decreased ROS production and caspase-3 activity to inhibit H_2O_2-induced chondrocytic apoptosis and necrosis. AU increased the gene expression of ACAN and COL2A1 but lowered the levels of MMP13 and IL-6. It also enhanced sulfated-glycosaminoglycan (sGAG) production and content in chondrocytes after compressive stress [73]. Interestingly, AU itself showed beneficial protection against IL-1β-induced human articular chondrocytes, and it can also enhance the anti-catabolic and anti-inflammatory effects of hyaluronic acid on OA chondrocytes. This was evident from a reduction in the expression of inflammatory factors and chondrocyte ECM-related proteins, an increase in the tissue inhibitor of metalloproteinase as well as the restoration of antioxidant capacity after treatment with the combination of AU and hyaluronic acid [74]. In short, AU has a protective effect against OA-like cartilage damage, and its function is related to anti-inflammatory effects, regulation of chondrocyte matrix metabolism and anti-oxidation. AU also enhanced the efficacy of HA on osteoarthritis. Although AU has achieved beneficial results in osteoarthritis, its effects need to be further confirmed by other cell and animal models.

Osteoporosis is a chronic bone disease characterized by low bone mass and microarchitectural deterioration which leads to increase bone fragility and the risk of fractures. It affects about 200 million people worldwide and its prevalence is increasing due to an agingpopulation [75]. The balance between osteoclast-mediated bone resorption and osteoblast-mediated bone remodelling is disrupted by estrogen deficiency, aging, glucocorticoid use and unhealthy lifestyle, resulting in osteoporosis [76,77]. In spite of with the impressive efficacy of current anti-osteoporosis drugs, their side effects limited its long-term application [78]. *Eucommia ulmoides* has been recorded in classical medicine exhibited the functions of nourishing liver and kidney, strengthening muscles and bones [79]. It has been used alone or in combination with other traditional Chinese medicines for the treatment of osteoporosis for a long time in China. Modern medical research has demonstrated that crude extracts, total glycosides and lignans of *Eucommia ulmoides* could increase biomechanical quality of the femur, bone mineral density and bone microarchitecture to prevent postmenopausal osteoporosis [80]. A previous study found that AU, one of the iridoid glycosides of *Eucommia ulmoides*, suppressed the activity of osteoclasts in a concentration-dependent manner while it enhanced the proliferation of osteoblast-like cells, and these effects of AU was stronger than that of $1,25(OH)_2D_3$ and E_2 [81]. Further, AU boosted the activity of ALP and elevated the expression of collagen I, osteocalcin, osteopontin, integrin β1 and Osterix in MG63 cells, which suggested that AU could facilitate osteoblast differentiation. BMP2 was a key protein for bone formation and reconstruction, and AU was reported to significantly promote the expression of BMP2 and the activation of its downstream pathway in MG63 cells after incubation including MAPKs, Smads and Akt/mTOR/p70s6k [82]. Moreover, in Titanium particles-treated MC3T3-E1 cells, AU not only inhibited the apoptosis of MC3T3-E1 cells by reducing the oxidative stress and regulating the expression of Bax and Bcl-2 but also

facilitated osteogenesis through enhancing the activity of ALP, increasing the osteogenesis-related genes expression and upregulating the BMP2/Smads/RunX2 pathway [83]. In general, based on its ability to inhibit osteoclast activity and enhance bone formation, AU may be a potential agent for the treatment of osteoporosis.

4.7. Anti-cancer

Early studies reported that AU had a weak antileukemic activity against chronic myelogenous leukemia K562 cells [84]. Further research revealed that AU could promote the formation of DNA cleavage complex to induce ring opening and cleavage of DNA by suppressing DNA-topoisomerase I instead of topoisomerase II [85]. AU was able to block the proliferation of human non-small cell lung cancer A549 cells via upregulating the expression of p53 and p21 protein to block cell cycle progression in the G0/G1 phase. Meanwhile, AU also facilitated the apoptosis of A549 cells through inducing the activation of the Fas/FasL system [86]. A recent study in human myeloid leukemia cells reported that the hydrolysate of AU significantly inhibited the viability of K562 cells whereas the effect of AU on these tumor cells was not significant. Treatment with hydrolysate of AU induced apoptosis through activation of caspase-3. It also inhibited the proliferation of tumor cells to cause the accumulation of the cells in the sub-G1 phase of the cell cycle. Moreover, hydrolysate of AU reduced BCR–ABL phosphorylation and inhibited constitutive STAT3 activation via suppressing JAK2 and c-Src activation, leading to apoptosis of human myeloid leukemia cells [87]. As mentioned above, AU was more effective against non-small cell lung cancer, and its hydrolysate has a better anti-leukemia effect than AU itself.

4.8. Others

Prior work has reported the minimum inhibitory concentration of AU and aucubigenin [88], but subsequent study has observed that AU showed no antibacterial activity while aucubigenin exhibited the significant inhibitory activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella enterica*, especially the strongest effect against *Staphylococcus aureus* with the MIC value of 0.0313 mg/ml and the MBC value of 0.0625 mg/ml [89], suggesting that aucubigenin may be the antibacterial activity. Fortunately, AU has been reported to cause a dose-dependent reduction on the total growth, biofilm formation, metabolic activity, and cell surface hydrophobicity of *Candida albicans* [90], indicating an antifungal activity of AU.

Furthermore, AU has been demonstrated to possess other biological activities, such as antinociceptive activity via inhibiting the p-benzoquinone-induced writhing [91]; antispasmodic activity expressed as the inhibition of the ACh-induced contractions of the guinea-pig ileum [92]; promotion of dermal, oral and gastric mucosal wound healing [14,93]; antidiabetic effect by increasing the number of beta cells in the pancreas [94]; the promotion of angiogenesis in a mouse model of hindlimb ischemia through the ERβ pathway [95].

5. Pharmacokinetic properties

Due to the limited research focus on the pharmacokinetic characteristics of AU, a complete overview of the absorption, distribution, metabolism and excretion profiles of AU is still relatively lacking. To date, there have been only three reports on pharmacokinetic studies of AU in rats and rabbits. The pharmacokinetic profiles of AU showed bioexponential decay, and the pharmacokinetic behavior were linear in rats after intravenous administration of AU at the dose of 40-400 mg/kg [7]. The pharmacokinetic model of AU was consistent with the two-compartment open model in New Zealand rabbits were administered intravenously or intragastrically with AU [106], the pharmacokinetic parameters were showed in Table 1. According to the pharmacokinetic parameters of rats and rabbits, it can be seen that there is no species difference in pharmacokinetics. In other studies, the plasma concentration and pharmacokinetic characteristics of AU in animals were analyzed after administration of *Eucommia ulmoides Oliver* extracts. Among these studies, it was found that the concentration-time curve showed a bimodal phenomenon, after administration of *Eucommiae cortex* extract, the first peak sharply appeared at 10–25 min while the second peak occurred at 6–10 h post-dose [96]. Another study showed that the maximum concentration appeared at about 1st h and 10th h after taking Du-zhong tea extract [97]. Although the mechanism responsible for this phenomenon is still not clear, it is speculated that the bimodal concentration of AU might be due to the drug reaching the small intestine is divided into two parts, multi-sites absorption or enterohepatic circulation. Compared with taking *Eucommia ulmoides* extract, the double peak did not occur in the concentration-time curve after taking AU alone, so it is probably due to the interaction between the components of *Eucommia ulmoides* extract and AU, but this statement needs to be verified by experiments. Recently, researchers uncovered that there are sex differences in the pharmacokinetics of AU. For instance, the absorption of AU was increased while the distribution and elimination processes were decreased in male group in contrast with female group after the administration of *Eucommia ulmoides* extract [96]. Previous studies have demonstrated that AU exhibits an estrogen-like effect through activation of estrogen receptor-dependent transcription of target genes [95,98], AU may regulate sex-specific activity and expression of drug metabolism enzymes and transport systems, which may explain sex differences in its pharmacokinetics. Disease conditions also cause changes in the pharmacokinetic features of AU because the plasma concentration of AU used to describe the concentration-time curve could not be detected in ovariectomized mice compared to normal mice [99]. The combined extracts of *Eucommia ulmoides* and *Dipsacus asperoides* reduced the absorption rate and extent of AU and accelerated the elimination of AU in rats [100].

The absorption of AU was rapid in animals with T_{max} of 0.20–1.58 h, Table 1. The bioavailability of AU was 19.3% after oral administration of AU which was lower than after intraperitoneal and hepatoportal administration, indicating the absorption of AU is poor after oral administration [7]. In vitro research further showed that the degradation of AU was highly acid specific, and the degradation half-life were 5.02, 5.78 and 14.84 h at pH 1.2, 1.6 and 2.0, respectively [7]. The concentration of AU in octanol after partitioning from the respective buffers was below 1 μg/ml, indicating extremely low octanol/water partition coefficients [7]. Moreover, AU could be rapidly degraded by β-glucosidase in the mucosa of the gastrointestinal tract. Therefore, the low oral bioavailability of AU was probably due to its pH-instability, low partition coefficient, enzymatic hydrolysis by β-glucosidase. Further research needs to confirm the absorption sites and the absorptive mechanisms of AU in vitro and in vivo.

AU was mixed with whole blood from rats and incubated at 37 °C for 30 min, the plasma-to-blood partition was analyzed, found that the fraction of AU in the blood cells was about 18.5%. The plasma protein binding was determined by equilibrium dialysis, and showed that 91% of AU exists in unbound form [7]. Rat serum albumin incubated with [^{3}H]aucubin revealed that [^{3}H]aucubin in itself did not result in binding to protein while it covalently bound to rat serum albumin in the presence of b-glucosidase, indicating that the open-chain aglycone of AU can irreversibly bind to the active site of serum albumin [101]. Early research reported that AU was weakly distributed in body tissues. However, the radioactivity appeared in the liver and kidney of rats after administration of [^{3}H]aucubin [101]. Another study found that AU was widely distributed to tissues including blood-abundant tissue and blood-rare tissue. The concentration of AU in the kidney was the highest, followed by the liver, lung, heart, spleen and testis [102]. Recently, AU could be detected in rat brain with the peak concentration of 587.42 ± 212.31 ng/g at 5 min post dose, indicating that AU could pass through the blood-brain barrier in rats [103].

The $t_{1/2}$ of AU was reported to be 0.83–10.18 h, Table 1. The large

Table 1
Pharmacokinetic profiles of aucubin after adminstration of drugs.

Drugs	Administration	Objects	Methods	T_{max} (h)	$t_{1/2,\alpha}$ (h)	$t_{1/2,\beta}$ (h)	$t_{1/2}$ (h)
Aucubin, 100 mg/kg	*po*	Male Wistar rats, *n* = 3	HPLC	0.91 ± 0.18			
Aucubin, 100 mg/kg	*ip*	Male Wistar rats, n = 3	HPLC	0.33 ± 0.06			
Aucubin, 100 mg/kg	*pv*	Male Wistar rats, n = 3	HPLC				
Aucubin, 100 mg/kg	*iv*	Male Wistar rats, n = 3	HPLC			0.85 ± 0.17	
Aucubin, 50 mg/kg	*iv*	Male Wistar rats, n = 3	HPLC			0.81 ± 0.14	
Aucubin, 50 mg/kg	*po*	Male SD rats, *n* = 6	LC-MS/MS	1.08 ± 0.50			7.38 ± 2.90
Aucubin, 500 mg/kg	*po*	New Zealand white rabbits, n = 6	HPLC		0.46 ± 0.18	0.87 ± 0.24	
Aucubin, 100 mg/kg	*iv*	New Zealand white rabbits, *n* = 6	HPLC		0.17 ± 0.06	0.67 ± 0.05	
Do-zhong tea extract, 2 g/kg	*po*	Male Wistar rats, n = 6	HPLC-MS/MS	0.46 ± 0.19			6.60 ± 0.28
Dipsacus asperoides extract (1140 mg/kg)	*po*	Female SD rats, n = 6	LC-MS/MS	0.88 ± 0.31			2.68 ± 1.17
Eucommia ulmoides extract (500 mg/kg) and Dipsacus asperoides extract (1140 mg/kg)	*po*	Female SD rats, n = 6	LC-MS/MS	2.50 ± 1.10			2.26 ± 2.12
Eucommiae cortex extract, 50 g/kg	*po*	Male SD rats, n = 6	UPLC-MS/MS	0.41 ± 0.11			2.92 ± 1.09
Eucommiae cortex extract, 50 g/kg	*po*	Female SD rats, n = 6	UPLC-MS/MS	0.25 ± 0.05			5.76 ± 1.28
The injection containing 2.0 mg/kg Aucubin, 2.0 mg/kg Ajugol and 10.0 mg/kg Catalpol	*iv*	SD rats, n = 6	LC–MS/MS				1.07 ± 0.24
Eucommiae cortex extract, 50 g/kg	*po*	Female KM mice, n = 6	UHPLC-MS/MS	0.30			1.92

Drugs	MRT (h)	C_{max} (μg/ml)	$AUC_{(0-t)}$ (μg·h/ml)	$AUC_{(0-\infty)}$ (μg·h/ml)	Vd (ml/kg)	CL (ml/h/kg)	Bioavailability (%)	References
Aucubin, 100 mg/kg		17.4 ± 6.6		43.50 ± 13.23			19.3 ± 5.9	[7]
Aucubin, 100 mg/kg		111.2 ± 6.4		173.33 ± 40.83			76.8 ± 18.1	
Aucubin, 100 mg/kg				188.67 ± 22.17			83.5 ± 9.8	
Aucubin, 100 mg/kg				225.83 ± 53.67	383 ± 46	0.12 ± 0.03		
Aucubin, 50 mg/kg				117.33 ± 12.12	296 ± 73	0.12 ± 0.01		
Aucubin, 50 mg/kg	4.08 ± 0.90	4.02 ± 2.39	11.03 ± 4.67	11.31 ± 4.63				[104]
Aucubin, 500 mg/kg			144.33 ± 13.19	147.17 ± 14.47	1691 ± 1158	0.95 ± 0.08		[106]
Aucubin, 100 mg/kg			256.47 ± 24.59	292.36 ± 25.34	215 ± 16	0.10 ± 0.02		[106]
Do-zhong tea extract, 2 g/kg	7.88 ± 0.26	0.03 ± 0.00	0.23 ± 0.02	0.23 ± 0.01				[97]
Dipsacus asperoides extract (1140 mg/kg)		0.08 ± 0.02	0.12 ± 0.05	0.15 ± 0.09				[100]
Eucommia ulmoides extract (500 mg/kg) and Dipsacus asperoides extract (1140 mg/kg)		0.04 ± 0.02	0.13 ± 0.03	0.13 ± 0.03				
Eucommiae cortex extract, 50 g/kg	8.80 ± 0.26	3.90 ± 0.63	35.18 ± 5.69	36.17 ± 5.63	1800 ± 260	420 ± 0		[96]
Eucommiae cortex extract, 50 g/kg	9.42 ± 0.55	1.77 ± 0.16	12.87 ± 1.67	13.03 ± 1.65	8650 ± 140	1070 ± 10		
The injection containing 2.0 mg/kg Aucubin, 2.0 mg/kg Ajugol and 10.0 mg/kg Catalpol	0.58 ± 0.10		1.95 ± 0.24	1.95 ± 0.24	1621 ± 528	1035 ± 122		[103]
Eucommiae cortex extract, 50 g/kg	3.60	0.65	2.90	3.18				[99]

Note: *po*: oral; *pv*: hetatoportal; *ip*: intraperitoneal; *iv*: intravenous; T_{max}, time to reach C_{max}; $t_{1/2,\beta}$ & $t_{1/2}$, elimination half-life; C_{max}, peak plasma concentration; AUC, area under the curve, Vd, apparent volume of distribution; CL, clearance; N/A: Not applicable; SD: Sprague Dawley. All data represented as the mean ± SD.

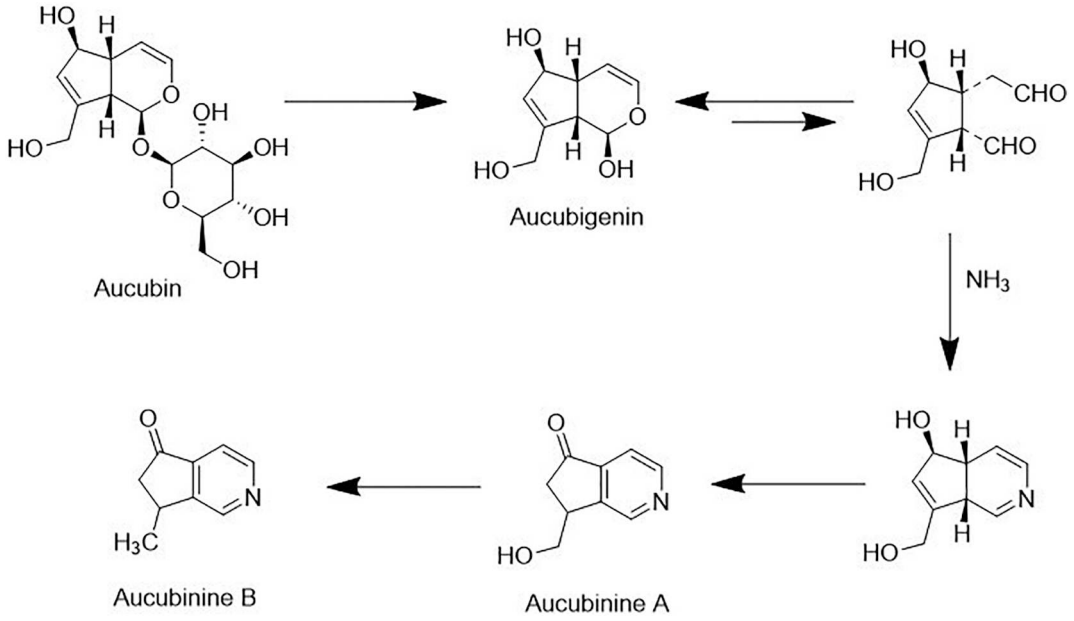

Fig. 3. Possible metabolic processes of aucubin by human intestinal bacteria.

variation is caused by different administration methods, detection methods and pharmacokinetic parameter calculation methods. So far, there have been few studies on the metabolism of AU. In the rat liver microsomes and hepatocytes, AU could not be transformed into its aglycone, and it also failed to inhibit the activity of 7-ethoxycoumarin o-deethylase, which indicated that AU was not metabolized by liver microsomes and AU had no effect on the activity of hepatic CYP450 [36]. Intestinal microorganisms also play an important role in drug metabolism. β-glucosidase is widely distributed in microorganisms, plants, animals and can degrade the glycosidic bond of AU into its aglycone. Anaerobic incubation of AU with fecal flora and bacterial strains isolated from human feces, AU was transformed to aucubigenin, aucubinine A and aucubinine B. AU was significantly promoted to produce Aucubinines A and B in the presence of ammonium ions [105].The possible metabolic processes leading to the production of aucubinines A and B was showed in Fig. 3.

Previous literature reported that AU was the most abundant in the kidney after intravenous administration of AU, indicating that AU might be rapidly cleared by the kidneys [102]. High levels of AU were detected in urine while the presence of AU was not found in the fecal excretion in New Zealand rabbits after intravenous and intragastric administration of AU [106]. However, aucubigenin was detected in urine and fecal excretion. These findings suggest that AU may be primarily excreted through the kidney, but the mechanism of its excretion is still not clear.

6. Safety and tolerability

No mice died within 24 h after intraperitoneal injection of 100–900 mg/kg of AU, but the activities of serum GOT and alkaline phosphatase were slightly decreased as well as the contents of triglyceride had an increasing tendency in the AU group above the dose of 300 mg/kg [107]. Mice were intraperitoneal administrated with AU at the doses of 20, 40 and 80 mg/kg on day1, 3, 5 and 7 to further explore the toxicity of AU, and found that the activities of GPT, GOT and alkaline phosphatase were not significantly changed in the AU treated group [108]. The contents of glucose, triglyceride, urea nitrogen and total proteins were comparable to that in normal rats. Notably, abnormal liver histology did not appear in mice liver biopsy specimens after AU treatment. All Wistar rats survived after receiving a single intraperitoneal injection of AU at 1–100 mg/kg, but 100 mg/kg of AU could cause paralysis in rats [55]. Recently, an acute toxicity test was conducted on mice administered by intragastric administration of AU at the dose of 10, 20, and 40 g/kg. Mice given 40 g/kg of AU had a slight decrease in free movement and food intake, and appeared the fat feces or soft stools. But these phenomena gradually returned to normal on the 2-3rd day. No symptoms of poisoning or death were observed in the animals within 14 days after AU treatment [106]. No abnormal pathological changes were found in the main organs, such as heart, liver, spleen, kidney, stomach and intestine. They also performed a 6-month long-term toxicity study in rats and observed that intragastric administration of 200-800 mg/kg of AU had no significant effect on weight, hair, locomotor activity, diet and general of rats [106]. Hematology and blood biochemical indicators were within the normal physiological range. Similarly, no animal death or abnormal histopathology of major organs were observed. During the recovery period, no chronic toxicity or delayed toxicity occurred in rats. Therefore, AU appears to be a low toxic compound. In intraperitoneal administration, the minimum lethal dose may be > 900 mg/kg in mice. In intragastric administration, the maximum tolerable dose of AU in mice may be 40 g/kg. Long-term intragastric administration of AU in the dosage range of 200–800 mg/kg for rats is safe.

7. Conclusion

Aucubin is an iridoid compound widely distributed in natural plants and is especially abundant in *Eucommia ulmoides*, *Aucuba japonica* and *Plantago asiatica*. To date, AU has only been extracted from plants. Because of the unstable structure of AU, few pure products can be obtained. Of interest, AU exhibits impressive pharmacological effects such as anti-oxidation, anti-inflammatory, anti-cancer and liver protection. In recent years, it has also been explored for its roles in anti-fibrosis, neuroprotection and bone protection. The first-pass effect of AU in absorption results in poor oral bioavailability. AU is widely distributed in the kidney, liver, lung, heart, spleen, brain and testis. Sex and disease status appear to affect the pharmacokinetic characteristics of AU. AU is a low toxic compound with a minimum lethal dose > 900 mg/kg in mice injected intraperitoneally. The maximum tolerated dose was 40 g/kg in mice after intragastric administration. Therefore, AU is a compound with abundant sources, good safety and various biological activities, which exhibits high value in health care products and pharmaceuticals and deserves further research and development.

At present, the preparation and pharmacological research of AU is still limited. In order to accelerate the development and utilization of AU-related products, in-depth studies should be focused on the following aspects in the future. First, due to the instability of AU and the low content of AU in plants, it is necessary to introduce advanced separation technology to improve the yield of AU pure products, and advanced pharmaceutical formulation may help improve the bioavailability of AU. Second, although AU has many pharmacological activities, it requires significant further study. Further work should study the structure-activity relationship so as to determine strategies to improve efficacy and reduce side effects. Finally, the current data on AU comes exclusively from the study of cells and animals, and it is impossible to predict the safety and effectiveness of AU in humans. Therefore, based on existing safety and efficacy results, clinical studies should be considered to assess the efficacy of AU in specific diseases.

Declaration of Competing Interest

The authors have declared that there are no conflicts of interest.

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