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REVIEW

Comprehensive Review on the Toxicity of Five Main AQ Constituents from Rhubarb: Mechanisms, Challenges and Future Perspectives

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Background: Emodin, rhein, aloe-emodin, physcion, and chrysophanol are five representative anthraquinones (AQs) in rhubarb. They exhibit diverse pharmacological activities, including antitumor, anti-inflammatory, antibacterial, and antioxidant effects, and are widely used in traditional Chinese medicines (TCMs), dietary supplements, and functional foods. However, with their increasing application, the potential toxicity of AQs has become increasingly prominent. Moreover, their toxic mechanisms and metabolism-related toxicities remain incompletely elucidated, which has limited their clinical development and safe application to a certain extent.

Objective: This paper systematically reviews the toxicological characteristics, toxic mechanisms, and metabolism-related toxicity of the five major AQs in rhubarb, and explores research strategies for AQs toxicity based on advanced technologies, aiming to provide new insights and references for their safe application and further studies.

Methods: A comprehensive search was conducted in PubMed, Google Scholar, Web of Science, and CNKI for peer-reviewed research articles and reviews published in the past 15 years. The research progress of the five major rhubarb AQs and the applications of cutting-edge technologies in their toxicity studies were summarized.

Results: The toxicities of AQs mainly target the liver, kidney, heart, reproductive system, and nervous system. Their toxic mechanisms involve multiple pathways, including mitochondrial apoptosis, oxidative stress, death receptor pathway, endoplasmic reticulum (ER)-related apoptosis, caspase-dependent apoptosis, autophagy, inflammation, DNA damage, and bilirubin metabolism. The in vivo metabolism of AQs is complicated, and both Phase I and Phase II metabolites are linked to toxicity. The diverse metabolites can interconvert and undergo various reactions in vivo, posing great challenges for toxicity research. In the future, the application of advanced technologies, such as multi-omics and single-cell sequencing, artificial intelligence (AI) and computer simulation, microfluidics, and mass spectrometry imaging (MSI) will provide strong support for accurately elucidating toxic mechanisms, enabling toxicity prediction, and optimizing detoxification strategies, and will become effective approaches to advance toxicity studies of rhubarb AQs.

Conclusion: The intrinsic toxicity and complex metabolic processes of rhubarb AQs restrict their clinical application. Current studies on the toxic mechanisms, metabolism–toxicity relationships, and multifactorial regulation of AQs still require further in-depth investigation. Future research should focus on the metabolism-toxicity relationship, key toxic targets, and detoxification strategies of AQs, and establish an integrated toxicity research system combined with advanced technologies, so as to provide robust support for the safe application and clinical development of rhubarb AQs.

Keywords: rhubarb AQs, toxicity, toxicity mechanism, metabolism, perspectives

Introduction

Rhubarb is the dried root and rhizome of plants of the genus Rheum in the family Polygonaceae. It has been widely used in clinical practice since its use for constipation, jaundice and other conditions was first documented in Shennong's Classic of Materia Medica.[1,2] The Pharmacopoeia of the People's Republic of China (2020 Edition) specifies emodin,

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rhein, aloe-emodin, physcion, and chrysophanol as the five indicator components for the quality control of rhubarb. As the core constituents of anthraquinones (AQs), these five components are abundant in rhubarb and possess the most potent biological activities.[3,4] Modern pharmacological studies have demonstrated that these five AQs, as representative active components of rhubarb, exhibit a broad spectrum of biological activities, including improving gastrointestinal function, exerting antitumor effects, regulating gut microbiota, displaying antibacterial activity, protecting the liver, and producing anti-inflammatory effects.[5,10] In addition, our previous research revealed that these five AQs possess anti--hepatocellular carcinoma activity.[11]

However, the clinical development and application of these five AQs are limited by their potential toxicities, including hepatotoxicity, nephrotoxicity, reproductive toxicity and cardiotoxicity.[12] Furthermore, key issues such as their toxic doses, toxic mechanisms, and in vivo metabolic profiles remain unclear, posing major challenges to the toxicological research of rhubarb AQs. Therefore, this review comprehensively discusses toxicity-related studies on AQs published before 2025 by searching PubMed, Web of Science, and CNKI databases. It aims to clarify their toxic mechanisms, explore toxicity-attenuating strategies from the perspective of drug metabolism and other aspects, and propose future directions in the toxicological research of AQs combined with cutting-edge technologies, so as to provide guidance and support for the clinical research and development of rhubarb AQs.

The Content of AQS

The 2020 edition of the Chinese Pharmacopoeia stipulates that the total content of five compounds (emodin, rhein, chrysophanol, aloe-emodin, and physcion) in qualified rhubarb should not be less than 1.5%. The molecular structures and relative content ratios of five AQs in rhubarb are presented in Table 1. Specifically, the content ranges for emodin, rhein, chrysophanol, aloe-emodin, and physcion are as follows: 0.018–1.15%, 0.009–1.072%, 0.007–1.465%, 0.102–2.864%, and 0.065–3.385%, respectively.

Pharmacological Effects of AQS

The modern pharmacological effects of AQs have been widely studied, mainly including anti-tumor, anti-inflammatory, anti-bacterial, and antioxidant.[6,16,18] The pharmacological effects and mechanisms involved are shown in Table 2 and Figure 1.

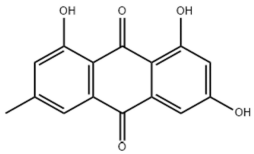
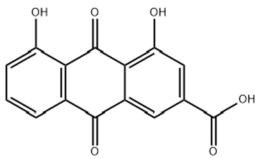
Anti-Tumor

AQs exhibit efficacy in combating a diverse range of cancers, including gynecological cancer, liver cancer, colorectal cancer, gastric cancer, lung cancer, breast cancer, etc. Anti-cancer activity is associated with promoting tumor cell apoptosis and cell cycle arrest, and inhibiting tumor cell growth, migration and invasion. The related mechanisms involve apoptosis, autophagy, inflammation and energy metabolism pathways.

Research findings indicated that emodin possessed the capability to trigger apoptotic processes in gynecological cancer cells and significantly reduced mitochondrial membrane potential (MMP) and adenosine triphosphate (ATP) release, and also induced cell cycle arrest in the G0/G1 phase. Further mechanism studies have shown that emodin may regulate multiple targets to induce apoptosis (caspase-9 and cleaved-caspase-3) and autophagy (MAP LC3, Beclin-1 and Atg12-Atg5), cell cycle arrest (Cyclin D and Cyclin E) and angiogenesis inhibition (VEGF, VEGFR-2).[19] The apoptosis mechanism involving the cysteinyl aspartate specific proteinase(caspase) family might be the main anti-cancer pathway of emodin. Xiong Simin et al[20] found that emodin caused the accumulation of reactive oxygen species (ROS) in HepG2 cells, which subsequently triggered the opening of mitochondrial permeability transition pores, and resulted in the outflow of calcium ions and cytochrome C, thereby activating caspase and leading to cell apoptosis. In addition, the inflammatory mechanism involving Mitogen-activated protein kinase (MAPK) is also an important anti-cancer pathway of emodin. Emodin significantly inhibited the migration and invasion of MHCC-97H human hepatocellular carcinoma cells, which might be related to the activation of the p38 MAPK signaling pathway and the inhibition of the extracellular signal-regulated protein kinases (ERK)/MAPK and phosphatidylinositol 3-kinase/protein kinase B(PI3K/AKT) signaling pathways.[21]

Rhein and chrysophanol can also participate in anti-tumor effects by mediating apoptosis and autophagy pathways. Yang et al discovered that rhein was able to promote the expression of the apoptotic protein Bcl-2-associated X protein (Bax) by inhibiting the Signal transducer and activator of transcription 3 (STAT3) pathway. Additionally, it downregulated the

Table 1 Basic Information of Five AQs

Compound	Structural Formula	Chemical Name	Source	Origin	Content (mg/g)	Citation
Emodin		1,3,8-Trihydroxy-6-methylanthraqulnone	*R. tanguticum*	/	1.14 ± 0.84	[13]
			R. palmatum	/	1.91 ± 0.83	
			R. officinale	/	0.35 ± 0.31	
			R. undulatum	/	0.49 ± 0.37	
			R. crispus	/	1.20 ± 0.22	
			Rhubarb	Chengdu, Sichung	0.46±0.002	[14]
			Rhubarb	Longnanyuangdong, Gansu	0.69±0.036	
			Rhubarb	Xihe, Gansu	0.74±0.065	
			Rhubarb	Songxian, Henan	0.80±0.001	
			Rhubarb	DatongQghai	0.30±0.003	
			Rhubarb	Fenyang, Shaanxi	0.95±0.008	
			Rhubarb	Taibai, Shaanxi	0.18±0.001	
			Rhubarb	Zhangye, Shaanxi	1.30±0.007	
			Rhubarb	Danba, Sichuang	0.22±0.008	
			Rpalmatum L.	Gansu	11.5±0.22	[15]
			R.Tanguticum Maxim.Ex Reg.	Qinghai	7.64±0.12	
			R. officinale BailL	Sichuan	5.52±0.13	
Rhein		4,5 dihydroxy-9,10-dioxoanthracene-2-carboxylie acid	*Rhubarb*	Chengdu, Sichung	0.42±0.005	[14]
			Rhubarb	Longnanyuangdong,Gansu	0.82±0.018	
			Rhubarb	Xihe, Gansu	0.85±0.005	
			Rhubarb	Songxian, Henan	0.09±0.000	
			Rhubarb	DatongQghai	0.88±0.009	
			Rhubarb	Fenyang, Shaanxi	1.25±0.028	
			Rhubarb	Taibai. Shaanxi	0.23±0.001	
			Rhubarb	Zhangye, Shaanxi	0.21±0.002	
			Rhubarb	Danba, Sichuang	0.43±0.021	
			Rpalmatum L.	Gansu	10.72±0.27	[15]
			R.Tanguticum Maxim.Ex Reg.	Qinghai	2.51±0.07	
			R. officinale BailL	Sichuan	7.63±0.14	

(*Continued*)

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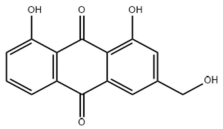
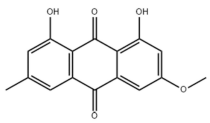
Table 1 (Continued).

Compound	Structural Formula	Chemical Name	Source	Origin	Content (mg/g)	Citation
Aloe-emodin		1,8-dihydroxy-3-(hydroxymethyl) anthracene-9,10-dione	*Rhubarb*	Chengdu, Sichung	0.54±0.001	[14]
			Rhubarb	Longnanyuangdong, Gansu	0.85±0.002	
			Rhubarb	Xihe, Gansu	1.14±0.01	
			Rhubarb	Songxian, Henan	0.07±0.000	
			Rhubarb	DatongQghai	0.25±0.006	
			Rhubarb	Fenyang, Shaanxi	0.66±0.009	
			Rhubarb	Taibai. Shaanxi	0.31±0.006	
			Rhubarb	Zhangye, Shaanxi	1.01±0.001	
			Rhubarb	Danba, Sichuang	0.20±0.003	
			Rpalmatum L.	Gansu	11.42 ±0.27	[15]
			R.Tanguticum Maxim.Ex Reg.	Qinghai	14.65 ±0.3	
			R. officinale BailL	Sichuan	5.56±0.14	
Physcion		1,8-Dihydroxy-3-methoxy-6-methyl-9,10-AQ	*Rhubarb*	Chengdu, Sichung	6.26±0.026	[14]
			Rhubarb	Longnanyuangdong, Gansu	4.94±0.006	
			Rhubarb	Xihe, Gansu	28.64±0.096	
			Rhubarb	Songxian, Henan	5.12±0.071	
			Rhubarb	DatongQghai	2.76±0.017	
			Rhubarb	Fenyang, Shaanxi	5.29±0.021	
			Rhubarb	Taibai. Shaanxi	1.02 ±0.029	
			Rhubarb	Zhangye, Shaanxi	34.09 ±0.163	
			Rhubarb	Danba, Sichuang	1.82±0.010	
			Rpalmatum L.	Gansu	6.54|±0.12	[15]
			R.Tanguticum Maxim.Ex Reg.	Qinghai	12.49±0.25	
			R. officinale BailL	Sichuan	3.07±0.08	

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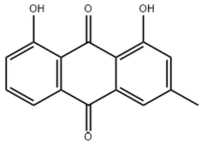
Chrysophanol		1,8-Dihydroxy-3-methyl-9,10-AQ	*R. tanguticum*	/	3.79 ± 3.24	[13]
			R. palmatum	/	6.35 ± 3.58	
			R. officinale	/	2.13 ± 1.66	
			R. undulatum	/	4.64 ± 3.20	
			R. crispus	/	3.90 ± 0.54	
			Rhubarb	Chengdu, Sichung	2.27±0.010	[14]
			Rhubarb	Longnanyuangdong, Gansu	1.70±0.017	
			Rhubarb	Xihe, Gansu	10.58±0.097	
			Rhubarb	Songxian, Henan	2.51±0.007	
			Rhubarb	DatongQghai	0.74±0.011	
			Rhubarb	Fenyang, Shaanxi	2.82±0.049	
			Rhubarb	Taibai. Shaanxi	0.71±0.018	
			Rhubarb	Zhangye, Shaanxi	12.1±0.028	
			Rhubarb	Danba, Sichuang	0.65±0.008	
			Rpalmatum L.	Gansu	24.53±052	[15]
			R.Tanguticum Maxim.Ex Reg.	Qinghai	33.85±0.79	
			R. officinale BailL	Sichuan	12.81±0.2	

Notes: "/" Geographical origins of the samples were not documented in detail.

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Table 2 Pharmacological Effects and Mechanisms of Five AQs

Pharmacological Effects	Toxicity	Models	Molecular Mechanisms	References
Anti-tumor	Emodin	Gynecological cancer-derived cells	(1) Promotes tumor cell apoptosis by regulating caspase proteins; (2) Induces tumor cell cycle arrest by downregulating key cell cycle regulators Cyclin D and Cyclin E; (3) Causes autophagic cell death by regulating Beclin-1 and Atg12-Atg5; (4) Inhibits angiogenesis by regulating VEGF and VEGFR-2;	[19]
		HepG2 cells	Activates caspase and induce cell apoptosis	[20]
		MHCC-97H human hepatocellular carcinoma cells	Promotes cell apoptosis by activating the p38 MAPK signaling pathway and inhibits the ERK/MAPK and PI3K/Akt signaling pathways	[21]
	Rhein	Human NSCLC cell lines	Promotes cell apoptosis by inhibiting the STAT3 pathway	[22]
		HepG2 cells	Regulates p53 and CD95/CD95L apoptosis system	[23]
		Human CRC cell lines	Inhibits cancer cell growth by inhibiting the mTOR pathway	[24]
	Chrysophanol	Human colorectal cancer HCT-116 cell lines	Promotes cancer cell apoptosis by regulating the expression of Bax, p53 and Bcl-2	[25]
		Gastric cancer cells	Regulates the mTOR signaling pathway to induce cancer cell apoptosis	[26]
	Aloe-emodin	Non-small cell lung cancer cells	Induces ROS-dependent autophagy	[27]
		Hela and SiHa cells	Inhibits glucose metabolism by reducing glucose transporter-1 expression and promotes cancer cell apoptosis	[28]
	Physcion	HeLa human cervical adenocarcinoma cells	Promotes cancer cell apoptosis by regulating the caspase 3/7 pathway and Bcl-2 protein expression	[29]
		Human Huh7 and Bel7402 cells	Induces apoptosis of human HCC cells by activating the AMPK signaling pathway	[30]
		Human breast cancer cell MCF-7	Promots cancer cell apoptosis by regulating oxidative stress-mediated mitochondrial apoptosis and immune response	[31]
Anti-inflammatory	Emodin	Mice with acute radiation proctitis	Alleviates proctitis by regulating AKT/ERK/NF-κB/VEGF and JNK/p38 pathways	[32]
	Rhein	BV2 microglia stimulated with LPS	Inhibits neuroinflammation by regulating PI3K/Akt/mTOR, TLR4/NF-κB, ERK1/2, and P38 signaling pathways	[33]
	Aloe-emodin	DSS-induced colitis in mice	Alleviates inflammatory bowel disease by activating the IL-4/IL-13 pathway	[34]

(*Continued*)

Table 2 (Continued).

Pharmacological Effects	Toxicity	Models	Molecular Mechanisms	References
	Physcion	An oxygen-glucose deprivation/reperfusion (OGD/R) model in SH-SY5Y cells	Attenuates neuronal inflammation by inhibiting the TLR4/NF-κB pathway	[35]
	Chrysophanol	Paraquat-induced lung injury in mice	Alleviates lung injury and inflammation by activating the PPAR-γ pathway and inhibiting the NF-κB pathway	[36]
Antibacterial	Emodin	Aeromonas hydrophila	Binds to cellular DNA and inhibits the cellular function of Bacillus hydrophila	[37]
	Aloe-emodin	Gram-positive bacteria	Binds to the peptidoglycan layer of the cell outer membrane and disrupts cell membrane permeability	[38]
Antioxidant	Emodin	H2O2- or 6-OHDA-induced apoptosis in PC12 cells	Protects against mitochondrial fission and apoptosis in neuronal cells by activating the PKM2/Nrf2/ARE pathway	[39]
	Chrysophanol	Aβ1-42 oligomers induce mitochondrial oxidative stress in primary neurons	Activates the SIRT1/PGC-1α pathway to counteract Aβ1-42 oligomer-induced oxidative stress	[40]
	Physcion	An oxygen-glucose deprivation/reperfusion (OGD/R) model in SH-SY5Y cells	Inhibits ROS/MDA production and enhances GSH/SOD activity	[35]

expression of the anti-apoptotic protein B-cell lymphoma/Leukemia-2 (Bcl-2), thereby inducing apoptosis in non-small cell lung cancer cells.[22] In addition, rhein also promoted apoptosis in human HepG2 cells by regulating the tumor protein p53 (P53) and CD95/CD95L apoptotic systems.[23] The mammalian target of rapamycin (mTOR) is a major regulator of cell growth and metabolism.[41] Rhein inhibited the growth of colorectal cancer cells by inhibiting the mTOR pathway.[24] Similarly, with regard to inducing tumor cell apoptosis, chrysophanol primarily induced apoptosis in colorectal cancer cell line HCT-116 by upregulating the expression of Bax and p53 and downregulating the expression of Bcl-2.[25] In terms of inhibiting tumor cell proliferation, chrysophanol also induced ferroptosis in tumor cells by inhibiting the solute carrier family 7 member 11/glutathione peroxidase 4 (SLC7A11/GPX4) signaling pathway, thereby inhibiting cell proliferation, migration, and invasion. This also might be related to the ability of chrysophanol to regulate the mTOR signaling pathway.[26]

Aloe-emodin participates in anti-tumor effects mainly by mediating inflammatory and energy metabolism signaling pathways. Shen et al[27] found that aloe-emodin induced autophagy in non-small cell lung cancer cells by activating the MAPK signaling pathway and inhibiting the Akt/mTOR pathway. Furthermore, aloe-emodin exerted an inhibitory effect on glucose metabolism through downregulating the expression level of glucose transporter-1, thereby promoting apoptosis of cervical cancer cells and reducing the expression of human papillomavirus-related proteins E6 and E7.[28] Modern research has found that glucose transporter-1 may play an important role in the progression of cancer by mediating glycolysis and proliferation.[42]

The anticancer effect of physcion is mainly related to promoting tumor cell apoptosis. Physcion promoted apoptosis of cervical cancer cells by activating caspases 3/7 and reducing the expression of Bcl-2.[29] AMPK and Nuclear factor erythroidderived 2-like 2/nuclear factor kappa-B (Nrf2/NF-κB) signaling pathways were also involved in the apoptosis pathway mediated by physcion. For example, physcion stimulated endoplasmic reticulum stress (ERS) by activating the AMPK signaling pathway, thereby causing mitochondrial dysfunction and inducing apoptosis of human HCC cells.[30] In addition, physcion regulated oxidative stress-mediated mitochondrial apoptosis and immune response by affecting the transduction of Nrf2/NF-κB signaling, ultimately causing apoptosis of breast cancer cells.[31]

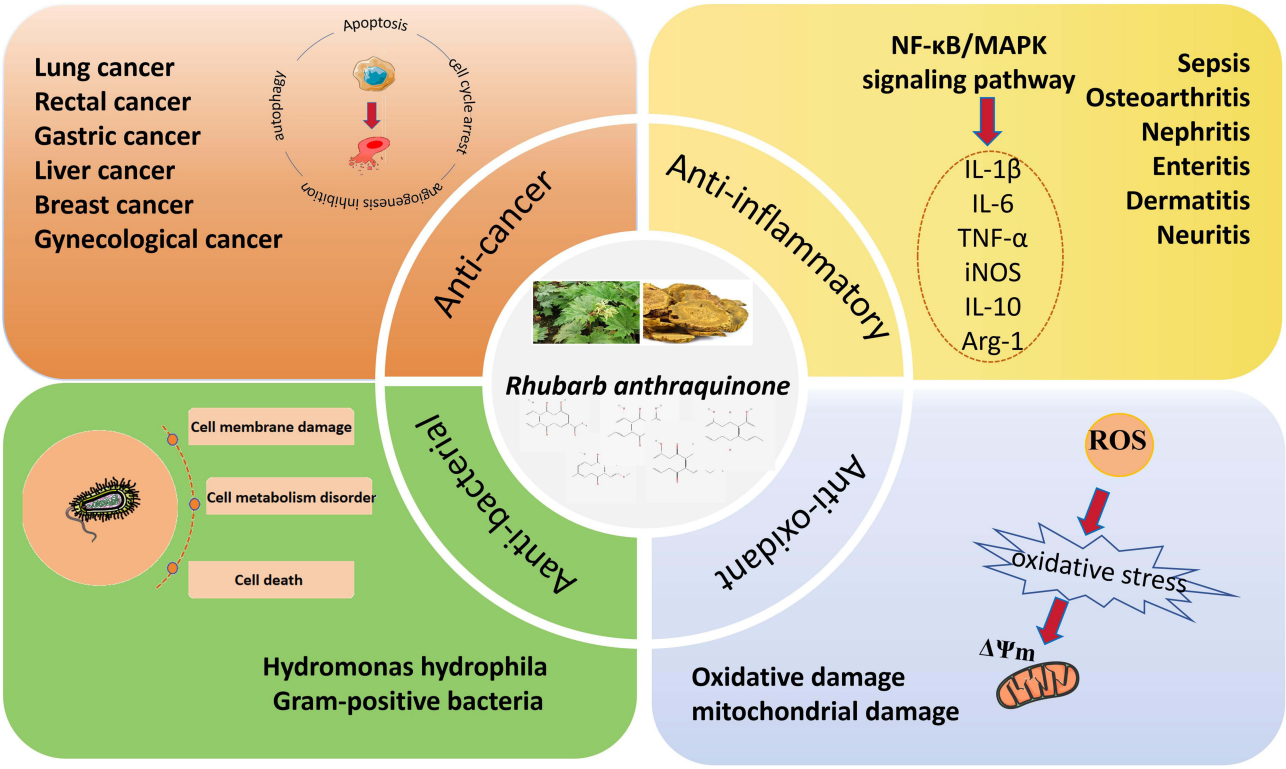

Figure 1 Pharmacological effects and mechanisms of AQs.
Note: Red arrows indicate the gradual relationship between processes.

Anti-Inflammatory

AQs exhibit significant anti-inflammatory effects. Studies have found that AQs exert anti-inflammatory effects by regulating inflammatory factors such as IL-1β, TNF-α, IL-6, iNOS, IL-10 and Arg-1. The signaling pathway involved is mainly NF-κB/MAPK, which has a therapeutic effect on a variety of inflammatory diseases such as sepsis, osteoarthritis, kidney inflammation, brain inflammation, dermatitis, intestinal inflammation and neuroinflammation by regulating inflammatory factors.[35,43,46] NF-κB is a recognized classic molecular target for the treatment of inflammatory diseases and is particularly important in the anti-inflammatory effects of AQs.[47] Lipopolysaccharide (LPS), recognized as a classic activator of the NF-κB signaling pathway, elicits a pronounced upregulation in the expression of pro-inflammatory cytokines, thereby exacerbating systemic inflammatory responses and ultimately precipitating tissue damage in the host organism.[47] Rhein inhibited neuroinflammation in LPS-stimulated mouse microglia by inhibiting the PI3K/Akt/mTOR and p38 signaling pathways, activating the expression of ERK1/2 and inhibiting the Toll-like receptor 4 (TLR4)/NF-κB signaling pathways.[33] Emodin alleviated acute radiation proctitis in mice by inhibiting the AKT/ERK/NF-κB/VEGF signaling pathway and inducing c-Jun N-terminal kinase (JNK) and p38-mediated apoptosis.[32] Aloe-emodin reduced the secretion of inflammatory cytokines and alleviated intestinal immune cell infiltration through the IL-4/IL-13 pathway, thereby treating inflammatory bowel disease.[34] Dong X et al found that physcion attenuated the oxygen glucose deprivation/reoxygenation (OGD/R)-induced inflammatory response in rat neurons by inhibiting the TLR4/NF-κB pathway and significantly reduced the expression of proinflammatory factors TNF-α, IL-1β, IL-6, and IL-10.[35] The study on the anti-inflammatory effect of chrysophanol on the lung injury mouse model found that it reduced the levels of malondialdehyde (MDA), myeloperoxidase and inflammatory cytokines, and increased the activity of superoxide dismutase (SOD). Moreover, chrysophanol increased the expression of Peroxisome proliferator-activated receptor gamma (PPARγ) and inhibited the activation of NF-κB pathway after lung injury inflammation.[36] In addition, emodin, rhein, and aloe-emodin exerted at least dual-target (NF-κB, iNOS) inhibitory effects on the inflammatory response of LPS-induced RAW264.7 macrophages.[48] However, compared with rhein and emodin, aloe-emodin exhibits a significantly stronger anti-inflammatory effect, with its inhibitory potency on iNOS protein expression being approximately twice that of NF-κB phosphorylation.[35]

Other

In addition to the anti-tumor and anti-inflammatory pharmacological effects mentioned above, AQs also has multiple pharmacological effects such as anti-bacterial and antioxidant. Studies have demonstrated that emodin permeates bacterial cell membranes and inhibits cellular functions of Aeromonas hydrophila through DNA binding.[37] Aloe-emodin exhibits anti-bacterial activity against clinically prevalent Gram-positive pathogens, including multidrug-resistant strains. The anti-bacterial mechanism might involve binding to the peptidoglycan layer of the cell outer membrane and the destruction of cell membrane permeability, which led to cell metabolic disorders and ultimately cell death.[38] Under normal physiological conditions, the oxidative and antioxidant systems of the organism is in balance. When the body is stimulated, the balance between ROS and antioxidant enzymes is broken, and antioxidant enzymes cannot remove excess reactive oxygen and free radicals in time, leading to oxidative damage.[49] AQs have the effect of resisting free radical damage, which is beneficial to cell anti-aging and body repair.[50] Emodin significantly protected against mitochondrial fission and apoptosis in PC12 neuron-like cells from oxidative damage by activating the Pyruvate kinase M2 (PKM2)/Nrf2/antioxidant response element (ARE) pathway.[39] Rhein significantly reduced mitochondrial ROS levels, reversed the depletion of MMP, and protected neurons from oxidative stress-related apoptosis, all of which were attributed to its excellent antioxidant activity. Rhein regulated mitochondrial biogenesis by activating the Sirtuin1/Peroxisome proliferator-activated receptor-γ coactivator-1α (SIRT1/PGC-1α) pathway. PGC-1α in the nucleus activates the downstream transcription factor NRF1, which produces healthy mitochondria by promoting mitochondrial biogenesis, thereby counteracting the oxidative stress induced by Aβ1-42 oligomers.[40] Physcion reduced OGD/R-induced oxidative stress in SH-SY5Y cells, inhibited ROS/MDA production, and increased glutathione (GSH)/SOD activity, suggesting that physcion has a protective effect on neuronal oxidative damage.[35]

In summary, AQs exhibits multiple physiological effects and demonstrates potential for therapeutic development. However, its inherent toxicity significantly restricts clinical development and application.

The Main Toxicity and Toxicity Mechanism of AQs

Although AQs exhibit a wide range of pharmacological activities, recent studies have revealed that AQs exhibits discernible toxic effects, with toxicity predominantly localized to target organs including the gastrointestinal tract, liver, heart, and kidneys.[51,53] The toxicity mechanism of AQs involves multiple pathways, including mitochondrial apoptosis, oxidative stress, death receptors, ER-associated apoptosis, caspase-dependent apoptosis, autophagy, inflammation, DNA toxicity, and bilirubin metabolism. Its toxicity and related mechanism are shown in Table 3.

Toxicity and Mechanism of Emodin

The toxicity profile of emodin primarily encompasses hepatotoxicity, nephrotoxicity, and reproductive toxicity, as evidenced by extensive in vivo and in vitro investigations. The toxicity of emodin is the most extensively studied of the AQs.

Hepatotoxicity of Emodin

According to some experimental and clinical studies, high-dose and long-term use of emodin or herbal medicine containing emodin was associated with liver cell damage and abnormal liver function.[92,95,98] Apoptosis is the main pathway of emodin-induced hepatotoxicity. For example, Lin et al demonstrated that L02 cells exhibited apoptosis following 48-hour incubation with emodin (50 μM), and emodin suppressed the activity of all respiratory chain complexes in hepatic mitochondrial, thereby disrupting oxidative phosphorylation, which subsequently reduced MMP, elevated ROS levels, impaired ATP synthesis, and ultimately induced mitochondrial dysfunction and hepatocellular apoptosis. These effects may be associated with caspase pathway activation.[54] Similarly, emodin (20–80 μM) arrested HepaRG cells at the S and G2/M phases and increased ROS production in HepaRG cells through the mitochondrial apoptosis pathway, thereby inducing apoptosis. The pathways involved included the mitochondrial apoptosis pathway, and molecular regulation was demonstrated by up-regulating the expression of Bax, cleaved caspase-3, 8, and 9, and cleaved poly (ADP-ribose) polymerase (PARP).[55] Yang et al also demonstrated that oral administration of emodin (150 mg/kg) to SD rats for 4 weeks could result in certain damage to the rat liver. Emodin inhibited flavin adenine dinucleotide/nicotinamide adenine dinucleotide phosphate (FADH/NADPH) proton transport, activated the mitochondrial apoptosis pathway, and induced the expression of caspase-9 and caspase-3.[56] In addition, oxidative stress is also

Table 3 Toxicity and Related Mechanisms of AQs

Compound	Toxicity	Models	Dosing Method	Dosage of Administration	Duration of Medication	Molecular Mechanisms	References
Emodin	Hepatotoxicity	L02 cells	Incubate	50 μM	48 h	Inhibits the function of mitochondrial respiratory chain complexes, leading to mitochondrial damage and cell apoptosis	[54]
		HepaRG cells	Incubate	20–80 μM	24 h	Induces mitochondrial apoptosis pathway to initiate cell apoptosis	[55]
		Rats	Oral	150 mg/kg	4 weeks	Inhibits FADH/NADPH proton transport and activates mitochondrial apoptosis pathway	[56]
		Rats	Oral	150 mg/kg	4 weeks	Targeted binding with acadvl induces oxidative stress damage in hepatocytes	[57]
		Grass carp hepatocytes	Incubate	1–25 μg/mL	24 h	Promotes MMP disruption and ROS production	[58]
		HepG2 cells	Incubate	20–80μM	24 h	Inhibits PI3K/Akt and ERK signaling pathways and activates p38 protein	[59]
		HepG2 cells	Incubate	30 μM	24 h	Activates IRE1α-XBP1 signaling pathway	[60]
		L02 cells	Incubate	25–50 μM	24 h	Activates the BiP/IRE1α/CHOP signaling pathway and generates ROS	[61]
		Primary human hepatocytes	Incubate	10–100 μM	48 h	Activates CYP3A and depletes GSH	[62]
		Mice	Oral	360 mg/kg	30 days	Promotes ROS generation and ER stress	[63]
		Rats	Oral	150 mg/kg	28 days	Inhibits the expression of UGT2A1, UGT9B2 and MRP7 protein	[64]
	Nephrotoxicity	Mice	ip	0.4–0.8 mg/kg	14 days	Inhibits Notch1/Nrf2/GPX4 antioxidant system	[65]
		HK-2 cells	Incubate	40 μM	24 h	Activates PPARγ and induces apoptosis	[66]
		HK-2 cells	Incubate	40 μM	24 h	Activates CB protease and induces cell apoptosis	[67]
	Reproductive toxicity	Human sperm	Incubate	50–400 μM	4 h	Reduces sperm [Ca(2+)]i and inhibits tyrosine phosphorylation	[68]
		Mice	Oral	1000 mg/kg	5 days	Activates IGF-1 receptor signaling pathway	[69]
		Mice	Oral	20–40 μM	24 h	Caspase-dependent apoptosis	[70]
		Mice blastocyst cells	Incubate	25–75 μM	24 h	Activates apoptosis signaling pathway	[71]
		Zebrafish embryos	Incubate	0.25 μM	7–72 hpf	Increases CYP3A and MDR1 gene expression	[72]

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Rhein	Hepatotoxicity	HepG2 cells	Incubate	75–100 μM	24 h	Promotes Fas and mitochondrial apoptosis pathways to induce cell death	[73]
		Mice	ig	4000 mg/kg	24 h	Promotes oxidative stress pathways and mitochondrial dysfunction	[74]
		HL-7702 cells	Incubate	50−100 μM	12 h	Induces hepatocyte apoptosis through a mitochondrial-mediated pathway and oxidative stress mechanisms	[75]
		Rat primary hepatocytes	Incubate	50 μM	24 h	Promotes cell apoptosis through the mitochondrial apoptosis pathway and the depletion of intracellular GSH	[76]
		HL-7702 cells	Incubate	50–100 μM	24 h	Induces cell apoptosis through the ERS pathway	[77]
		Rat hepatocytes	Incubate	50 μM	16 h	Induces apoptosis by depleting intracellular GSH and ATP and accumulating lipid peroxidation products	[78]
		L02 cells	Incubate	50-100 μM	24 h	Induces apoptosis through the Fas death receptor pathway and mitochondrial pathway	[79]
	Nephrotoxicity	HK-2 cells	Incubate	50, 100 μM	24 h	Induces cell apoptosis through the UCP2-related mitochondrial pathway	[80]
		HK-2 cells	Incubate	50, 100 μM	24 h	Induces mitochondrial apoptosis pathway	[81]
	Cardiotoxicity	Mice	Oral	350, 175, 87.5 mg/kg	45 days	Promotes Fas-induced apoptosis pathway	[82]
	Reproductive toxicity	Rat	Oral	175 and 350 mg/kg	10 days	Causes skeletal deformities	[83]
		Mouse embryos	Incubate	5–20 μM	24 h	Induces oxidative stress and immunotoxicity	[84]
Aloe-emodin	Hepatotoxicity	mice	Oral	0.8 and 1.6 g/kg	11 weeks	Inhibits the transport activity of MRP2	[85]
		zebrafish	Incubate	0.01505 mg/mL	72 h	Activates the NF-κB inflammatory pathway and the P53 apoptosis pathway	[86]
		HL-7702 cells	Incubate	20 UM, 40 UM	48 h	Involved in Fas death pathway, mitochondrial pathway, and caspase-dependent apoptosis	[87]
		L02 cells	Incubate	20–40 μmol/L	48 h	Involved in mitochondrial damage and oxidative stress imbalance	[88]
	Nephrotoxicity	HK-2 cells	Incubate	100 UM	48 h	Activates caspase 3 and ER stress	[89]
		Mice	ig	0.8, 1.6 g·kg−1	11 weeks	Regulates the expression of TGF-β1 protein and participates in oxidative stress and cell apoptosis	[90]
		Mice	Oral	2000, 1000 and 500 mg/kg/day	24 h	Promotes DNA strand breaks	[91]
Physcion	Hepatotoxicity	Mice	Oral	100 mg/kg	14 days	Promotes downregulation of Bsep and Mrp2 and induces bile acid accumulation	[92]
		L02 cells	Incubate	10, 20, 40 and 80 μmol/L	24 h	Blocks bilirubin metabolism	[93]
	Erythrocyte toxicity	Red blood cells	Incubate	50 and 100 μM	24 h	Induces hemolysis and programmed cell death	[94]

associated with the apoptosis pathway implicated in the effects of emodin. When emodin was administered at a dose of 150 mg/kg, it induced oxidative stress damage in rat hepatocytes. This damage might be related to the binding of emodin to very long-chain acyl-CoA dehydrogenase and the consequent inhibition of fatty acid β-oxidation, the citric acid cycle, and oxidative phosphorylation in mitochondria, thereby triggering hepatic oxidative stress.[57] Cui et al incubated grass carp hepatocytes with emodin (1–25 μg/mL) for 24 h and found that emodin showed severe hepatotoxicity, and destroyed ΔΨm and generated ROS in grass carp hepatocytes, and significantly reduced the SOD activity and total antioxidant capacity, thereby inducing hepatocyte apoptosis.[58] In addition, p38-activated mitochondrial or death receptor pathways are also worthy of attention. Emodin (20–80 μM for 24 h) induced apoptosis in HepG2 cells via mitochondrial and death receptor-mediated pathways. The emodin-mediated death pathway was manifested by the simultaneous activation of the cell-surface fas receptor (Fas), Fas-ligand (Fas-L), caspase 8, and tBid. In addition, emodin also induced apoptosis in HepG2 cells by inhibiting the PI3K/Akt and ERK pathways and activating the p38 protein.[59] ERS-induced apoptosis served as the primary pathway of hepatotoxicity caused by emodin, and the signaling pathway involved was mainly Inositol-requiring enzyme type 1-X-box binding protein 1 (IRE1α-XBP1). ERS induces the formation of large, microscopically visible IRE1 clusters or foci in IRE1 during its activation.[99] Emodin (30 μM, 24 h) induced ERS by activating the IRE1α-XBP1 pathway and reduced the metabolic activity of HepG2 cells. The molecular mechanism was reflected in the increased expression of ERS marker proteins (such as BiP, IRE1α, and CHOP). In addition, HepG2 cells treated with emodin also increased the Bax/Bcl-2 ratio and the relative protein expression of cleaved caspase-3 in a dose-dependent manner.[60] Qiu et al used emodin (25–50 μM) to induce hepatotoxicity in L02 cells and found that emodin (25–50 μM, 24h) promoted excessive ROS generation and redox imbalance in human hepatocytes, thereby triggering BiP/IRE1α/CHOP signaling-mediated ERS and unfolded protein response and intracellular Ca^{2+} overload, leading to ER-related cell apoptosis rather than mitochondria-dependent cell apoptosis.[61] ERS induced by emodin metabolites is also one of the factors that induce hepatotoxicity. Jiang L L et al found that when human primary hepatocytes were incubated with emodin (10–100 μM), the metabolic enzyme CYP3A was activated and GSH was depleted, leading to toxicity in hepatocytes.[62] Studies have found that emodin (360 mg/kg in mice, 50 μM in L02 cells) can activate the aryl hydrocarbon receptor (AHR), inducing CYP1A1 expression, leading to a decrease in GSH and an increase in ROS in hepatocytes, thereby triggering ERS and producing hepatotoxicity.[63] The hepatotoxicity resulting from combined medication should not be overlooked. When emodin is combined with hepatotoxic drugs, an increase in hepatotoxicity will ensue. For example, Wu et al found that the combined use of emodin (150 mg/kg) and probenecid exacerbated the hepatotoxicity of emodin in rats. Therefore, it is suggested that gout patients should avoid long-term use of emodin-containing preparations combined with probenecid.[64] Additionally, Tu et al demonstrated that neither emodin (20, 40, 80 mg/kg) nor LPS (2.8 mg/kg) alone could induce liver damage in rats. However, when rats were fed a non-toxic dose of LPS in combination with emodin for 10 hours, acute liver damage occurred in the rats.[64]

Nephrotoxicity of Emodin

Emodin has the potential to induce nephrotoxicity when administered in large doses over an extended period. The renal tubules represent the primary site where emodin-induced nephrotoxicity occurs.[100] Ferroptosis has been confirmed as a pathway through which emodin induces renal injury. Xing et al reported that severe and extensive renal tubular lesions were observed in mice following intraperitoneal injection of emodin (0.4–0.8 mg/kg for 14days). Moreover, in vitro studies showed that the activity of renal tubular epithelial cells in rat treated with emodin (40–160 μM) was greatly reduced, which might be related to emodin inhibiting the Nrf2/GPX4 antioxidant system and inducing renal damage through ROS-mediated iron allergy.[65] In addition, apoptosis is also an important pathway of renal injury induced by emodin. When administered at a concentration of 40 μM for a duration of 24 h, emodin was observed to induce a reduction in the viability of human renal proximal tubular epithelial cells, caspase-3 cleavage and activation, loss of ΔΨm, and release of Cyt-C from mitochondria to the cytosol, leading to HK-2 cell apoptosis. This complex mechanism might be related to the activation of proliferator-activated receptor γ (PPARγ).[66] Furthermore, emodin (40 μM, 24 h) increased the expression of cathepsin B protein, thereby activating caspase-3, leading to the decreased viability of HK-2 cell.[67] In addition, mesangial cells are also regarded as the main target of renal toxicity induced by emodin,[101] which is associated with emodin-induced cell cycle arrest at the G1 phase.[102]

Reproductive Toxicity of Emodin

It has been reported that the toxicity of emodin also manifests in the reproductive system. Emodin (50–400 μM) has been shown to reduce the overall motility, forward-progressive motility, and linear velocity parameters of human sperm. It alters the sperm's penetration ability in viscous media, along with progesterone-initiated capacitation and the acrosome reaction. The underlying mechanism primarily involves reducing sperm intracellular calcium ion concentration ([Ca(2+)] i) and inhibiting tyrosine phosphorylation.[68] Emodin has been identified as an inhibitor of casein kinase 2. Male mice with the casein kinase 2 gene knocked out were found to be infertile. It was found that mice fed with emodin (1000 mg/kg) could cause decreased sperm production, increased eosinophils and germ cell apoptosis. The reproductive toxicity of emodin might be related to the insulin-like growth factor 1 (IGF-1) receptor signaling pathway, and the differentially expressed genes indicated that emodin affected the expression of casein kinase 2 and spermatogenesis.[68] In addition, Mouse oocytes treated with emodin (20–40 μM for 24h) showed reduced maturation and fertilization rates, accompanied by impairment of early embryonic development. Pretreatment with a caspase-3 specific inhibitor effectively reduced the series of effects triggered by emodin, suggesting that the reproductive toxicity of emodin might occur through a caspase-dependent apoptotic process.[70] Treatment of mouse blastocysts with emodin (25–75 μM for 24 hours) resulted in inhibition of early embryonic development to the blastocyst stage. Emodin triggered damage in mouse blastocysts via the activation of intrinsic apoptotic signaling pathways, but the specific mechanism was unclear.[71] Emodin (0.25 μg/mL 7–72 hpf) caused edema, trunk bending, and morphological abnormalities in zebrafish embryos, which reduced the survival and hatching success rate of zebrafish embryos. The main mechanism was related to increased expression of CYP3A and multidrug resistance 1 (MDR1) proteins.[72] Generally, the reproductive toxicity of emodin is evident in sperm, oocytes, and embryos. However, the specific toxicity mechanism remains to be further investigated.

In summary, hepatotoxicity has been studied relatively extensively, and its mechanisms mainly involve mitochondrial apoptosis, ERS and enzyme activity regulation. Nephrotoxicity primarily occurs in the renal tubules, involving ferroptosis and mitochondrial apoptosis pathways. The mechanisms of reproductive toxicity require further investigation. Indeed, current research on the nephrotoxicity and reproductive toxicity of emodin is limited, suggesting that the liver may be the primary site of toxicity. The toxic mechanism of emodin is shown in Figure 2.

Toxicity and Mechanism of Rhein

Rhein serves as a crucial component of AQs. The toxicities of rhein primarily encompass hepatotoxicity, nephrotoxicity, reproductive toxicity, and cardiotoxicity. It is widely acknowledged that its toxicity is associated with both dose and duration.[103]

Hepatotoxicity of Rhein

Mitochondrial-mediated apoptosis and oxidative stress are the main pathways of rhein-induced hepatotoxicity. For example, studies have found that rhein (75–100 μM for 24 h) promoted the production of ROS, the loss of MMP and S phase cell cycle arrest, thereby inhibiting the viability of HepaRG cells and inducing cell death. The apoptosis mechanism involved was mainly the mitochondrial-mediated apoptosis pathway, which was reflected in the up-regulation of the expression of p53, p21, Bax, caspases-3 and PARP, and the down-regulation of the expression of Bcl-2, cyclin A and cyclin-dependent kinase 2 proteins.[73] Similarly, Yang et al also found that rhein (4g/kg for 24 h) caused mitochondrial dysfunction through the oxidative stress pathway, activated the mitochondrial apoptosis pathway, and caused apoptosis of mouse liver cells, showing increased levels of ROS, NRF-2 and MDA, and decreased levels of SOD.[74] In addition, Guy-Armel Bounda et al found that the hepatotoxicity caused by rhein was related to mitochondrial apoptosis pathway, oxidative stress, and lipid imbalance.The study revealed that rhein (50–100 μM for 12h) mediated the excessive production of ROS, decreased MMP, and increased synthesis of triglycerides (TG) and total cholesterol (TC). Rhein significantly up-regulated the expression of caspase-3, caspase-9, caspase-8, p53, p53-up-regulated modulator of apoptosis (PUMA), and Apaf-1, and participated in cell apoptosis. In addition, it also increased the expression of three key enzymes involved in lipid metabolism: 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HGM-CoAR), glycerol-3-phosphate acyltransferase (GPAT), and Acetyl-CoA carboxylase (ACoAC). Immunoblotting showed that cyt-c was released from mitochondria into the cytosol in large quantities.[75] Studies have also shown that hepatotoxicity caused by rhein is a very complex process involving multiple signaling pathways. Gati Krushna

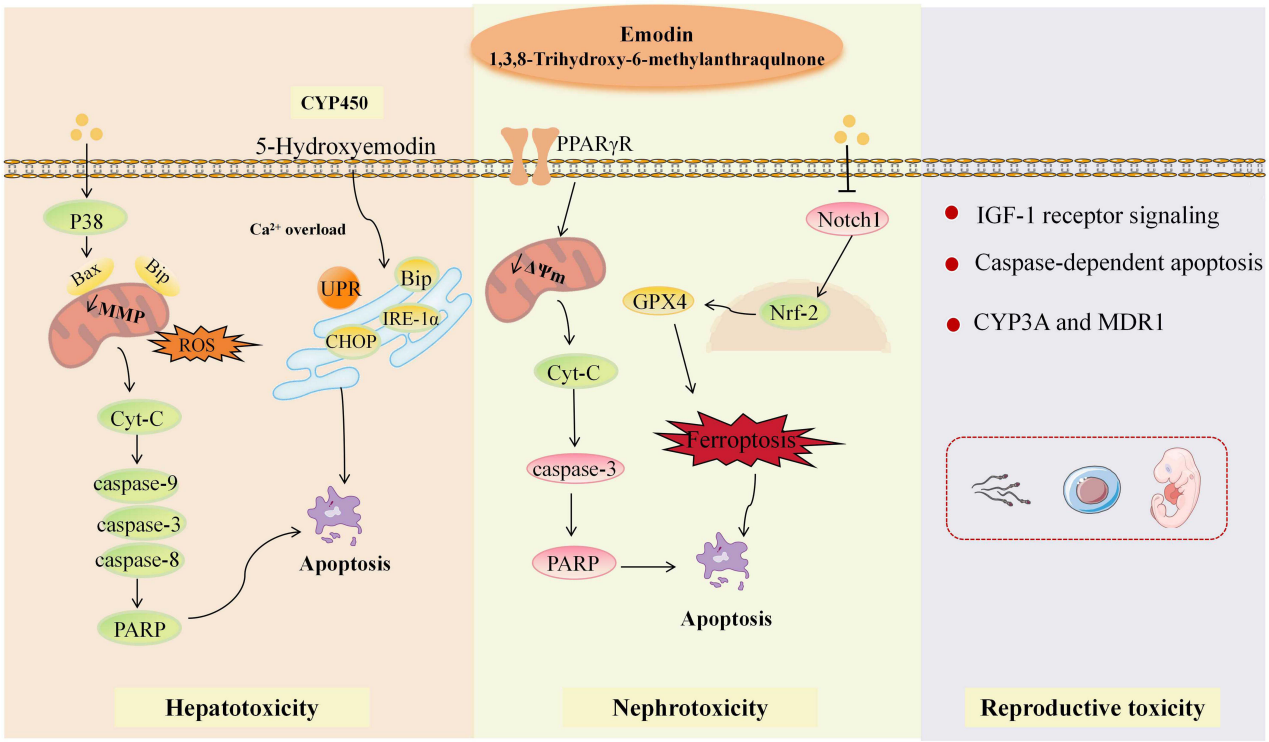

Figure 2 The toxic mechanism of emodin.
Note: →stands for activation, ⊣ stands for inhibition.

Panigrahi et al discovered that rhein (50 μM for 24 hours) caused apoptosis in rat primary hepatocytes by generating ROS, increasing intracellular Ca(2+), reducing MMP, and consuming intracellular GSH content. This was mainly related to the DNA damage, thereby increasing the expression of p53 and p21, and ultimately activating the endogenous apoptosis pathway mediated by Bax, Bcl2, cyt-c, caspase-3, caspase-9, and PARP, which involved multiple pathways such as p53, MAPK, and mitochondrial.[76] In addition, ERS and increased intracellular calcium ion levels were the main reasons for rhein-induced hepatocyte apoptosis. The ERS pathway involved in rhein was reflected in the up-regulation of the expression of glucose-regulated protein 78 (GRP 78), protein kinase receptor-like ER kinase (PERK), JNK, and CCAAT/enhancer binding protein homologous protein (CHOP). At the same time, the ERS marker caspase-4 was also activated, followed by caspase-3. In addition, rhein could also promote the increase of intracellular calcium ion concentration, thereby inducing cell apoptosis, which might be related to JNK activation.[77] Kågedal et al incubated rat hepatocytes with rhein (50μM for 16 hours) and also found that rhein increased the $Ca2^{+}$ concentration in hepatocytes, thereby inducing hepatocyte apoptosis.[78] Studies have shown that treatment with rhein can increase intracellular free Ca2+ concentration by nearly 10 times, which is related to mitochondrial dysfunction.[104] TNFα-mediated apoptosis and autophagy have also been shown to be pathways for rhein-induced hepatotoxicity. Rhein (50–100 μM for 24 h)-induced hepatocyte apoptosis might be related to the increase of intracellular ROS, and induced hepatocyte L02 apoptosis through the TNF-α apoptosis pathway and the mitochondrial pathway, which was manifested by up-regulating the expression of TNF-α, Tumor Necrosis Factor Receptor 1 (TNFR1), tumor necrosis factor receptor-associated death domain protein (TRADD) and cleaved caspase-3, and down-regulating the expression of procaspase-8. At the same time, rhein up-regulated the expression of Bax and down-regulated the expression of procaspase-9 and procaspase-3, indicating that the mitochondrial pathway was activated. In addition, after treatment with rhein, the expression of autophagy-related protein light chain 3 (LC3)-II and beclin-1 was significantly reduced, while the expression of Sequestosome 1(P62) was significantly increased. It is speculated that rhein may inhibit L02 cell autophagy, reduce the cell self-clearance ability, and promote cell apoptosis.[79]

Nephrotoxicity of Rhein

There are few studies on the nephrotoxicity of rhein.[103] Zhu Yanna et al proposed that rhein primarily induces renal injury by triggering apoptosis, and this apoptotic process may involve multiple signaling pathways.[103] Rhein (50μM, 100μM for 24h) induced apoptosis in HK-2 cells through the mitochondrial-mediated pathway, including destruction of MMP, reduction of ATP levels, release of cyt-c from mitochondria to cytoplasm, and up-regulation of Bcl-2 and Bax levels. In addition, rhein significantly increased ROS levels and inhibited the expression of mitochondrial uncoupling protein 2 (UCP2). The inhibition of UCP2 significantly enhanced oxidative stress and exacerbated rhein-induced cell apoptosis.[80] Rhein (50 μM and 100 μM, 24 hours) inhibited HK-2 cell proliferation and induced apoptosis and S/M accumulation in a concentration-dependent manner. The apoptotic mechanism was associated with caspase 3 activation, loss of ΔΨm, and expression of Bcl-2 and Bax.[81]

Reproductive Toxicity of Rhein

Most studies on the reproductive toxicity of rhein have focused on embryotoxicity. Studies have confirmed that rhein (175 and 350 mg/kg) exhibits teratogenic effects on fetal development in rats. Rats received rhein gavage for 10 successive days from pregnancy day 6 to day 15. The fetuses would develop various skeletal deformities such as sternal cross-section loss, incomplete skull ossification, and thoracic vertebrae separation or deformation.[83] The developmental potential of mouse blastocysts implanted into the uterus after incubation with 5–20 μM rhein for 24 hours was impaired. In vivo tests confirmed that intravenous injection of rhein (1, 3, and 5 mg/kg body weight per day) into maternal mice for four days would lead to embryonic apoptosis, early embryonic development damage, and fetal weight loss. Rhein-induced embryotoxicity was related to the induction of oxidative stress and immunotoxicity, which induced the production of ROS and down-regulated the transcriptional levels of innate immunity-related genes chemokine (CXC motif) ligand 1(CXCL1), IL-1β, and IL-8 in maternal mouse fetuses.[84]

Cardiotoxicity of Rhein

Rhein is the only one of the five AQs that has been shown to be cardiotoxic. The study found that after 48 hours of exposure to rhein (106, 124, and 132 μmol/L), H9c2 cells showed atrophy, rounding, cell wall shedding, and cell proliferation inhibition. The mechanism involved rhein-induced microscopic changes in cardiomyocyte membrane potential and mitochondria. In addition, mice treated with rhein (350, 175, and 87.5 mg/kg) for 45 days showed a significant reduction in left ventricular ejection fraction and left ventricular fractional shortening. Furthermore, serum Ca^{2+}, cTnT, CK, and LDH levels were significantly increased. Rhein-induced cardiotoxicity might be related to mitochondrial-mediated and Fas-induced apoptosis, as evidenced by up-regulation of Bax, Caspase-3, −8, and −9, Fas, and P53, while the expression of Bcl-2 showed a downward trend.[82]

In summary, the hepatotoxicity mechanisms of rhein involves mitochondrial apoptosis, oxidative stress, abnormal lipid metabolism, and complex endogenous apoptosis. The nephrotoxicity, reproductive toxicity, and cardiotoxicity of rhein have been less studied. Nephrotoxicity mainly involves the mitochondrial apoptosis pathway, reproductive toxicity mainly involves oxidative stress and immunotoxicity, and cardiotoxicity is mainly related to mitochondrial and Fas-mediated apoptosis pathways. This also suggests that the liver may be the main toxic organ of rhein. As the heart is a vital organ, the cardiotoxicity of rhein needs to be taken seriously. The toxic mechanism of rhein is shown in Figure 3.

Toxicity and Mechanism of Aloe-Emodin

The liver and kidney are definite target organs of aloe-emodin toxicity, whereas its genotoxicity remains controversial.

Hepatotoxicity of Aloe-Emodin

Multidrug resistance-associated protein 2 (MRP2) is an important efflux transporter involved in intracellular detoxification, inflammation, excretion of drug metabolites, and regulation of oxidative stress.[105] In mice treated with aloe-emodin (0.8 and 1.6 g/kg) for 11 weeks, aloe-emodin inhibited the transport activity of MRP2, triggered oxidative stress and apoptosis, and led to a decrease in intracellular GSH, mitochondrial dysfunction, and activation of apoptosis in HepG2 cells.[85] In addition, aloe-emodin (0.01505 mg/mL for 72 hours) caused liver lesions in zebrafish, leading to obvious hepatitis cell infiltration and necrosis. A significant increase was observed in the expression levels of P65, JNK, and P53

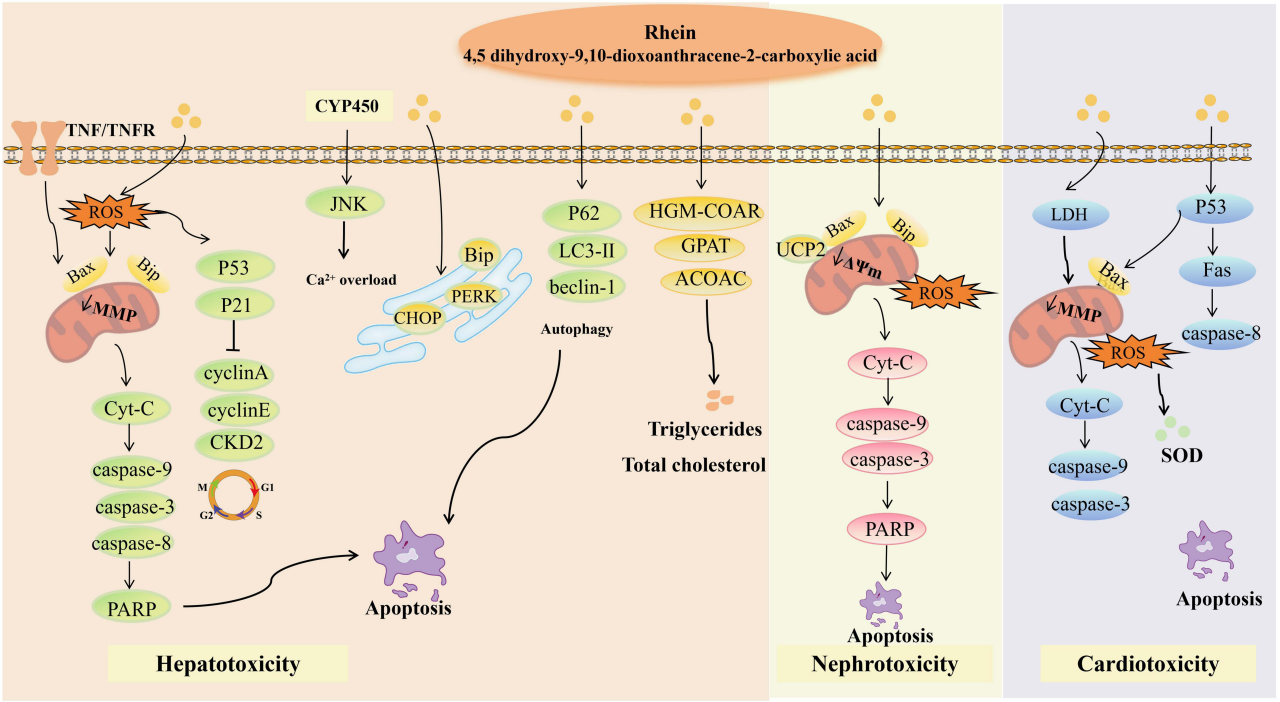

Figure 3 The toxic mechanism of rhein.

proteins in zebrafish hepatocytes. This increase was found to be associated with the activation of the NF-κB inflammatory pathway and the P53 apoptosis pathway.[86] Similarly, aloe-emodin (20, 40 μM for 48 h) reduced the viability of HL-7702 cells in a dose-dependent and time-dependent manner, induced cell cycle arrest at the S and G2/M phases, and dose-dependently up-regulated the level of Fas, P53, P21, Bax/Bcl-2, Caspase-3, −8, −9, as well as PARP cleavage. The mechanisms of hepatotoxicity involved the Fas death pathway and the mitochondrial apoptosis pathway.[87] In addition, aloe-emodin (40 μmol/L) led to increased expression of heme oxygenase-1 mRNA and quinone oxidoreductase-1 mRNA in L02 and HepaRG cells, thereby inducing mitochondrial damage and imbalance of hepatocyte oxidative stress, leading to hepatocyte apoptosis.[88]

Nephrotoxicity of Aloe-Emodin

Aloe-emodin (100 μM for 48 hours) was demonstrated to significantly reduce the viability of HK-2 cells and induce G2/M arrest in the HK-2 cells cycle. The mechanism underlying the apoptosis induced by aloe-emodin was found to be associated with Caspase-3 and ERS. This association involved eukaryotic initiation factor-2α phosphorylation, X-box binding protein 1 mRNA splicing, JNK phosphorylation, as well as an increased accumulation of GRP78 and CHOP.[89] Dosage is a factor affecting the nephrotoxicity of aloe-emodin. The aloe-emodin group (1.6 g/kg) showed elevated blood urea nitrogen and serum creatinine levels, and severe renal tubular and glomerular damage. The low-dose group (0.8 g/kg) showed milder renal tubular damage. In both groups, SOD levels were significantly decreased; TGF-β1, PPPPα and IL-6 were significantly increased. The mechanism of nephrotoxicity might be related to oxidative stress, apoptosis, and transforming growth factor-β (TGF-β) signaling pathway.[90]

In addition to the two toxicities mentioned above, aloe-emodin also has certain genetic toxicity. Genotoxicity corresponds to the ability of substances to intercalate into DNA. Topoisomerase II inhibitors are compounds with DNA-intercalating properties,[106] and it was demonstrated that both emodin and aloe-emodin were capable of inhibiting the activity of topoisomerase II, but the inhibitory effect exerted by emodin was found to be stronger than that of aloe-emodin,[107] which suggests that emodin may also be genetically toxic. Primary DNA damage was observed in the liver and kidneys of mice treated with aloe-emodin (2000, 1000, and 500 mg/kg/day (×2)), demonstrating that aloe-emodin might cause organ-specific genetic toxicity.[108] Studies have found that aloe-emodin at a dose of 2000 mg/kg body

weight/day does not induce DNA chain breaks in male mouse kidney and colon single cell preparations, but does cause damage to kidney and colon cells, indicating that aloe-emodin is not genotoxic.[109] In addition, Heidemann et al performed in vivo cytogenetic analysis on bone marrow cells of wistar rats and found that doses up to 2000 mg/kg did not cause toxic effects or chromosomal structural aberrations.[110] Therefore, the kidney and colon may be the toxic target organs of aloe-emodin, but whether there is genetic toxicity still deserves further study.

In summary, The hepatotoxicity and nephrotoxicity mechanisms of physcion both involve oxidative stress, inflammatory responses, and apoptosis. In addition, the nephrotoxicity mechanism is related to ERS. However, inconsistent conclusions exist regarding aloe-emodin genotoxicity, with a lack of in-depth research. The toxic mechanism of aloe-emodin is shown in Figure 4.

Toxicity and Mechanism of Physcion

The toxicity of physcion mainly includes hepatotoxicity, nephrotoxicity, erythrotoxicity, and neurotoxicity. The mechanism involved in hepatotoxicity is the regulation of choline efflux transporters and drug-metabolizing enzymes. The nephrotoxicity of physcion has not been reported. Additionally, the erythrotoxicity and neurotoxicity of physcion also deserve further attention.

Hepatotoxicity of Physcion

Hepatotoxicity is the common toxic effect of physcion, but its nephrotoxicity has not been systematically reported. In addition, physcion may also exert erythrotoxicity and neurotoxicity.

MRP2 and Bsep are key transporters involved in bilirubin metabolism and choline efflux, and their inhibition can exacerbate cholestatic injury and induce hepatotoxicity.[111,112] Kang L et al have demonstrated that physcion alters the distribution of total choline or individual choline molecules in the bile, liver, and plasma of mice by regulating choline efflux transporters, thereby inducing liver damage. Administration of physcion (100 mg/kg) to mice for 14 days resulted in mild inflammatory infiltration in the liver cell and induction of bile acid accumulation. The mechanism of toxicity

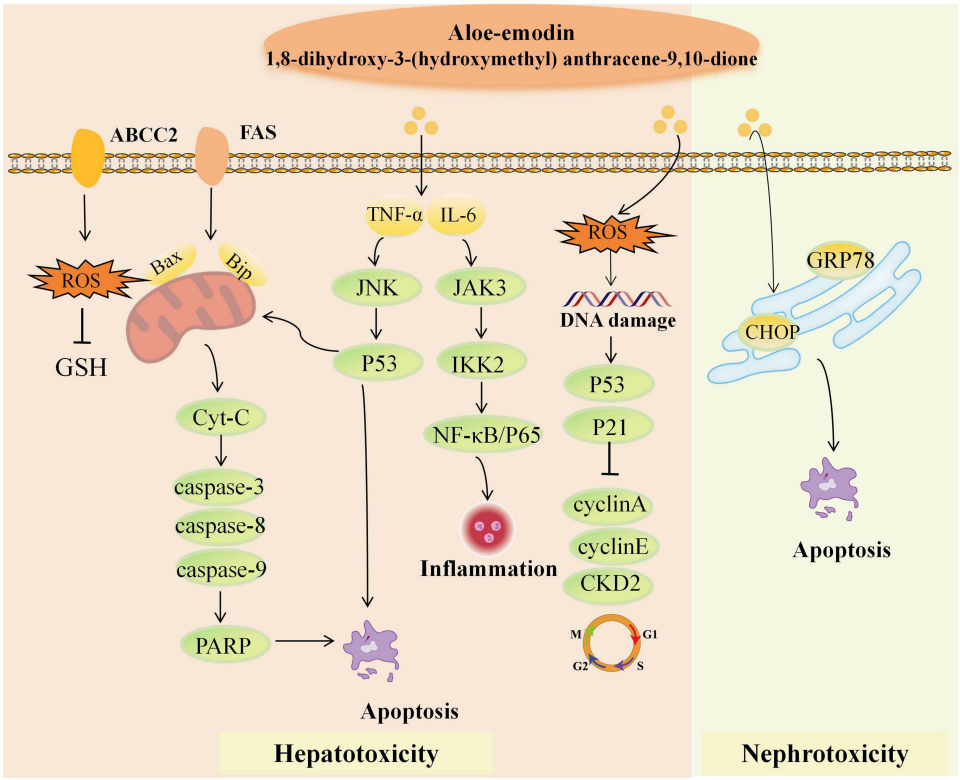

Figure 4 The toxic mechanism of aloe-emodin.

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involved down-regulation of Bsep and MRP2 expression.[92] Physcion metabolites may be the main components of its hepatotoxicity. Studies have found that the metabolites of physcion had a strong inhibitory effect on Uridine diphosphate glucuronosyltransferase (UGT) 1A1 enzyme.[113] Exogenous substances that inhibit the UGT1A1 enzyme will lead to an inability to convert bilirubin into conjugated bilirubin or a decrease in the amount of bilirubin converted. This results in bilirubin metabolism disorders, induces bilirubin accumulation in liver cells and blood, and ultimately causes liver toxicity.[114] Zheng et al discovered that physcion exhibits high toxicity towards normal L02 cells. The mechanism underlying hepatotoxicity is associated with the inhibitory effect of physcion on CYP1A2, CYP2C9, CYP2D6, and CYP3A4, which competitively blocks the rate-limiting enzyme UGT1A1 in the bilirubin metabolism process.[93]

The potential toxicity of physcion to red blood cells and nerves still needs to be explored. Studies have demonstrated that physcion (50 and 100 μM) induces significant hemolysis in red blood cells, which is associated with programmed cell death.[94] In terms of neurotoxicity, the survival rate of human SH-SY5Y neuroblastoma cells incubated with physcion (10 μM) for 24 hours dropped to approximately 55%, while under the same conditions, aloe-emodin-induced mortality was less than 15%, indicating that physcion was more neurotoxic than aloe-emodin. However, physcion still exhibits certain limitations in studies on erythrocyte toxicity and neurotoxicity. For instance, its toxicity mechanism has not been fully elucidated to date.[115]

In summary, the hepatotoxic mechanism of physcion involves the regulation of choline efflux transporters and drug-metabolizing enzymes. Although studies on erythrotoxicity and neurotoxicity are relatively limited, they also deserve attention.

Toxicity and Mechanism of Chrysophanol

Toxicity reports on chrysophanol are rare. At present, several studies suggest that it may possess hepatotoxicity, reproductive toxicity, and genotoxicity.

Chrysophanol seems to be the least toxic of the five AQs. Currently, there are very few studies on the toxicity of chrysophanol. Regarding hepatotoxicity, toxicity test results based on zebrafish indicate that chrysophanol is a potential hepatotoxicity marker of rhubarb.[116] However, chrysophanol exhibits relatively low toxicity. The study revealed that chrysophanol (50 μM for 15 minutes) merely induced an elevation in Ca^{2+} concentration in rat hepatocytes, and it did not trigger apoptosis. However, following 30 minutes of exposure, cell viability was markedly diminished, indicating that the hepatotoxicity of chrysophanol is closely associated with the duration of exposure. Interestingly, exposure to physcion (50 μM) for 15–60 minutes exhibited no significant impact on hepatocyte viability, suggesting that chrysophanol demonstrates greater hepatotoxicity than physcion. Furthermore, following 10 minutes of treatment with rhein (50 μM), intracellular reduced GSH was entirely depleted. In contrast, chrysophanol (50 μM) reduced GSH levels to 60% of their initial value after 60 minutes of exposure. Therefore, the hepatotoxicity of chrysophanol is less severe than that of rhein.[78] However, studies on the hepatotoxicity of chrysophanol are lacking or insufficient. With regard to reproductive toxicity, a study investigating the potential of chrysophanol to induce chromosome breakage in hamster ovary cells revealed that chrysophanol (30 μg/mL) exhibited almost no ability to cause chromosome breakage.[117] A study using resonance light scattering spectroscopy to confirm the interaction between chrysophanol and DNA found that chrysophanol could insert into the base pairs of double-helix DNA in a manner similar to a DNA intercalator, thereby producing toxic effects. This finding indicated that chrysophanol exhibited certain genetic toxicity, and its toxicity was associated with environmental factors, including amino acids, sodium chloride, glucose, and pH. The genetic toxicity of chrysophanol is comparable to that of ethidium bromide, mitoxantrone, and doxorubicin.[118]

In summary, the above toxicity studies have obvious limitations: the hepatotoxic mechanism has not been thoroughly elucidated, the concentration settings for reproductive toxicity are restricted, and genotoxicity has not been verified in vivo or in vitro. These indicate that the toxicity of chrysophanol has not yet been comprehensively and systematically revealed, and many unknown safety risks require further exploration.

Relationship Between Pharmacokinetics and Toxicity of AQS

The quinone structure of AQs is fat-soluble, and the main absorption site is the intestine rather than the stomach.[53] A study investigated the pharmacokinetics in rats after oral administration of rhubarb extract (300 mg/kg). The results

showed that these five AQs were rapidly and widely distributed 10 minutes after oral administration, mainly in the stomach, liver, intestine, and kidneys. They were excreted unchanged in urine in small amounts and in feces in larger amounts. Thus, fecal excretion is the primary route of elimination for five AQs. However, the urinary excretion rate of rhein was much higher than that of other AQs. This may be related to the fact that other AQs are readily converted into rhein in the body.[119,120] About 84 hours after administration, the cumulative urinary excretion of the five AQs reached more than 90% of the total excretion.[119] In addition, the metabolic pathways of AQs in the body are complex, and the metabolism of the five AQs may affect each other's Tmax, Cmax, $T_{1/2}$, etc.[121] Therefore, it is necessary to explore the relationship between the metabolic transformation and toxicity of AQs, although research in this area is currently insufficient. Drug metabolites produced in the body often increase polarity, enhance water solubility, and decrease or completely inactivate pharmacological activity.[122] Therefore, metabolic reactions are generally considered to be biological detoxification processes. However, in some cases, therapeutic drugs can be converted into active metabolites under the action of various metabolic enzymes and produce toxicity.[123] Drug biotransformation is a key factor in the early stages of new drug development and is divided into two types of reactions: Phase I (oxidation, reduction, and hydrolysis) and Phase II (conjugation). Phase I metabolic reactions are typically catalyzed by the CYP450 enzyme system in the liver. Phase II metabolic reactions are typically catalyzed by transferases in the liver, such as glucuronyltransferases, sulfotransferases, and methyltransferases.[123]

Metabolism and Toxicity of Emodin

Emodin rarely exists in its original form after entering the body. Following oral administration of *polygonum cuspidatum* to rats, free emodin was predominantly detected in the liver, whereas glucuronic acid/sulfate-conjugated emodin was found in the blood, kidneys, and lungs.[124] A comprehensive study investigating the pharmacokinetic metabolism of emodin in rats has revealed that approximately 56% of emodin remained unabsorbed and was predominantly excreted in the feces in its original form. The original form and metabolites of emodin that entered the systemic circulation were mainly distributed in the kidneys. The hydroxylated metabolites were primarily excreted via urine and feces, whereas the glucuronidated metabolites were mainly excreted via urine and bile.[125] The metabolism of emodin in the body is very complex. Bai et al employed UHPLC-Q-Exactive MS to detect the metabolites of emodin following oral administration to rats. The results showed that emodin underwent multiple metabolic pathways in addition to glucuronidation and hydroxylation. Moreover, these metabolites were extensively distributed in plasma, bile as well as heart, liver, spleen, lung, kidney, and brain tissues.[126] Furthermore, upon intravenous injection of emodin (5.0 mg/kg) in rats, emodin was rapidly transformed into its hydroxylated derivatives or conjugates with glucuronic acid/sulfate. By contrast, following oral administration of emodin (20.0 mg/kg and 40.0 mg/kg), only emodin glucuronide was identifiable in the serum.[127] In addition, gender is also an influential factor in the metabolism of emodin.[128] The study demonstrated that regardless of whether rats were subjected to intraperitoneal injection (20 mg/kg or 40 mg/kg) or oral administration (20 mg/kg, 40 mg/kg and 80 mg/kg, 12 weeks) of emodin, the peak concentration of glucuronidated emodin was attained at 1 hour and was eliminated within 12 hours. It was noteworthy that female mice appeared to metabolize emodin more rapidly than male mice.[129] These factors determine that the toxicity study of emodin metabolites is a difficult task.

The metabolic enzymes CYP1A1, CYP2C19, CYP3A4, UGT1A9, and UGT2B7 play a key role in the hepatotoxicity induced by emodin.[64] 5-hydroxy-emodin is a hydroxylated metabolite of emodin, which is primarily produced by CYP1A2, and is more toxic than emodin. The toxic mechanism is associated with the activation of the aromatic hydrocarbon receptor (AHR). The Ahr-Cyp1a1 pathway potentiates the ROS/ERS effect and CYP1A2 enzyme activity.[63] In addition, promoting the oxidative metabolism of emodin by CYP2C19 and CYP3A4 enzymes and promoting the formation of hydroxyl compounds might be a mechanism for reducing the toxicity of emodin.[130] Although this view requires further confirmation. Current research generally believes that the toxicity of emodin is stronger than its glucuronide derivative. Chen et al reported that UGT2B7 exhibited the most pronounced glucuronidation effect on emodin, presenting percentage distributions of 54.9% in human liver microsomes and 53.1% in human kidney microsomes. Long-term or high-dose use of emodin inhibited the activity of UGT2B7 by suppressing the expression of hepatocyte nuclear factor 4-α (HNF4α), which led to the accumulation and toxicity of emodin in hepatocytes.[131] Therefore, inducing the simultaneous expression of HNF4A and UGT2B7 is showing promise as

a novel detoxification means for emodin. Emodin-3-O-β-D-glucuronide is the main glucuronidation metabolite of emodin and has certain toxicity. In comparison, emodin had a larger area under the curve (AUC) and more pronounced hepatotoxicity, significantly increasing ALT and AST levels, indicating that emodin is more toxic. It was speculated that the higher hepatic accumulation of emodin might be the cause of its greater hepatotoxicity. In addition, GSH depletion has been associated with emodin-induced hepatotoxicity. Liu et al identified an emodin–cysteine adduct in cultures of normal human liver L-02 cells exposed to emodin, the level of which increased in an emodin-concentration-dependent manner. Decreased levels of GSH and oxidized glutathione (GSSG), together with elevated levels of glutamate, a GSH metabolite, were also observed in the cultures. Marked alterations in acylcarnitines suggested that GSH depletion-induced disorder of fatty acid oxidation contributes to hepatotoxicity.[132] Acylcarnitines play an important role in mitochondrial function, and mitochondrial dysfunction has been reported to be associated with hepatotoxicity.[133] Moreover, the differences in emodin metabolism between male and female rats account for the sex-related differences in the toxicokinetics of emodin.[134] It is worth noting that the increased expression of MRP2 induced by emodin might accelerate the elimination of emodin-3-O-β-D-glucuronide, which was also one of the detoxification mechanisms.[134]

Therefore, the author speculates that to reduce the toxicity of emodin, in addition to adhering to conventional usage and dosage guidelines, it is of great importance to prevent emodin from accumulating in the body. For instance, a study has found that TSG significantly inhibits the phase II metabolism of emodin, thereby increasing the bioavailability of emodin and elevating the risk of cumulative poisoning.[135] Moreover, it is also essential to avoid using emodin in combination with CYP3A enzyme inducers or UGT2B7 enzyme inhibitors.[125] Additionally, AhR, HNF4A, and MRP2 are detoxification targets that warrant further investigation.

Metabolism and Toxicity of Rhein

Following oral administration to rats, mice, rabbits, and beagle dogs, rhein was predominantly distributed to the liver, followed by the stomach, intestines, lungs, spleen, kidneys, heart, and brain. The absorbed rhein was metabolized into glucuronides and sulfates, which were subsequently excreted in the bile and entered the enterohepatic circulation. In the intestine, glucuronides and sulfates might undergo conversion to other AQs.[136] Rhein contains two hydroxyl groups and one carboxyl group, which endow it with strong polarity and electrochemical redox properties.[103] Three metabolites were detected in the feces of rats administered rhubarb AQs using HPLC, and rhein was identified as the metabolite that accumulated to the greatest extent over time, which may be related to the in vivo conversion of aloe-emodin and sennoside A into rhein.[133] Studies have demonstrated that the metabolites of rhein primarily consist of three mono-glucuronides, corresponding to two hydroxy glucuronides and one acyl glucuronide (connected through the carboxyl group).[137] Therapeutic drugs can be metabolized into active metabolites by various metabolic enzymes in the body and subsequently covalently bind to biological macromolecules (such as proteins or DNA), thereby leading to increased toxicity.[123] A study also revealed that a monohydroxylated metabolite was detected in the urine and bile of rats administered with rhein. This metabolite was likewise detected in rat and human liver microsome cultures exposed to rhein. Meanwhile, a total of three GSH conjugates were identified in rat bile. These GSH conjugates might be formed through the combination of rhein or its metabolites.[138] Furthermore, it is likely that the majority of rhein was converted into acyl glucuronides in the liver. Rhein acyl glucuronides covalently bound to GSH to form rhein-GSH adducts, which might eventually induce toxicity. UGT1A1, UGT1A9, and UGT2B7 are the enzymes that catalyze the conversion of rhein acyl glucuronides.[137] CYP2C19, serving as the main metabolic enzyme of rhein, activated rhein and converted it into active metabolites. This enzyme formed electrophilic centers in rhein, which then combined with GSH to generate GSH-rhein adducts and monohydroxyrhein, ultimately leading to hepatotoxicity.[123,139] Studies have demonstrated that rhein is primarily metabolized into chemically reactive acyl glucuronides in primate (human and monkey) liver microsomes. In contrast, the predominant metabolite of rhein in rats, mice, and dogs liver microsomes is a monoglucuronide formed on the hydroxyl group, which is unreactive. The interspecies differences in rhein glucuronidation are likely attributable to variations in the content of UGTs in the livers of different species.[140] In addition, in vitro data from rat and human hepatic systems indicate that rhein metabolism involves a combination of weak Phase I and intensive Phase II processes. Almost all rhein elimination is mediated by hepatic metabolism, and total hepatic clearance comprises metabolic clearance via CYP450, UGT, and SULT enzymes.[141] Following oral administration of

rhein in beagle dogs, rhein glucuronides and sulfates were rapidly formed in plasma, pharmacokinetic parameters revealed that the $AUC_{0\text{-}10h}$ of rhein glucuronides/sulfates was significantly elevated, suggesting that these conjugates play a more prominent role in the systemic circulation of rhein in vivo.[142] However, the potential toxic effects and mechanisms associated with rhein biotransformation via the above Phase I and Phase II metabolic pathways remain unclear and inadequately investigated.

Metabolism and Toxicity of Aloe-Emodin

After oral administration of rhubarb extract in rats, aloe-emodin was mainly distributed in the stomach, and was also found in tissues including the liver, kidney, lung, heart and brain. A large amount of aloe-emodin was converted into rhein. Aloe-emodin was mainly excreted in its prototype form, with a urinary excretion rate of 1.14% and a fecal excretion rate of 46.83%.[119,120] After oral administration of aloe-emodin to rats, the main components in the blood were aloe-emodin glucuronide and rhein glucuronide, while the parent aloe-emodin was barely detectable in blood. However, when aloe-emodin was intravenously administered to rats, the main components in the blood were aloe-emodin glucuronide and rhein sulfate.[143] Hence, the metabolites of aloe-emodin are likely to emerge as the key factors exerting a more pronounced impact on the bioactivity. The metabolites of aloe-emodin show considerable complexity. CYP1A2, 2B6, 2C19, 3A1 and 3A4 are the main enzymes involved in the metabolism of aloe-emodin.[144] Studies have shown that the metabolites detected in rat and human liver microsomes exposed to aloe-emodin include two hydroxylated metabolites and four GSH conjugates. The GSH conjugates were derived from aloe-emodin or its hydroxylated metabolites, while the hydroxylation of aloe-emodin was mainly catalyzed by CYP 1A2, 3A4 and 3A5.[145] In addition, CYP3A1 is the main metabolic enzyme in rat liver microsomes. Studies have shown that aloe-emodin is metabolized into hydroxy metabolites and rhein in rat liver microsomes.[88] CYP3A4, as the main metabolic enzyme in human liver microsomes, is closely related to the hepatotoxicity of aloe-emodin. Inhibition of CYP3A4 would aggravate the damage of aloe-emodin to L02 and HepaRG cells.[88] Quinones also induce toxicity through various other mechanisms, including protein binding, DNA binding, and interaction with GSH.[146] Panigrahi et al found that the cytotoxicity of aloe-emodin might be attributed to its strong GSH depletion ability and DNA binding affinity.[147] This was consistent with the observation that a large amount of GSH compounds were detected in liver microsomes incubated with aloe-emodin. Phase II metabolism of aloe-emodin contributes to its GSH depletion capacity. Sulfotransferases are responsible for mediating the Phase II metabolic activation of aloe-emodin. The benzyl hydroxyl group of aloe-emodin is likely to undergo sulfation reaction to form aloe-emodin sulfate, which was chemically reactive and could react with endogenous GSH to form aloe-emodin-GSH conjugates (SN1 mechanism) or be directly attacked by GSH (SN2 mechanism). Both pathways lead to intracellular GSH depletion.[148] However, the toxicity research of aloe-emodin metabolites is relatively limited and needs further research. For example, the toxicity research of aloe-emodin metabolites is still insufficient, and the mechanism of hepatocellular damage caused by CYP3A4 inhibition still deserves further exploration. In addition, intestinal commensal microbes encode a variety of enzymes that perform structural modifications on drugs, mediating drug activation, inactivation, toxicity induction, altered stability, reduced bioavailability, and rapid excretion, thus playing an important role in drug metabolism.[149] The gut microbiota is essential for the biotransformation of aloe-emodin. Aloe-emodin undergoes rapid oxidation and conjugation in the gastrointestinal tract before entering the systemic circulation, and the resulting metabolites may exhibit altered biological activity and bioavailability.[150] However, studies on the gut microbiota–metabolism–toxicity axis of aloe-emodin remain scarce to date.

Metabolism and Toxicity of Physcion

There are few systematic studies on the pharmacokinetic properties of physcion. In studies of AQs co-cultured with human intestinal flora in vitro, physcion is likely to be metabolized by intestinal flora into aloe-emodin, emodin, chrysophanol, or other metabolites before entering the blood.[151] Therefore, physcion was often difficult to detect after oral administration of different rhubarb preparations.[152] Four monohydroxylated metabolites and one O-demethylated metabolite, as well as emodin, were detected in incubations of physcion with rat liver microsomes. Emodin was speculated to be the primary metabolite of physcion contributing to hepatotoxicity. CYP2C19, CYP1A2, CYP2B6, CYP3A4 predominantly participated in the hydroxylation reaction of physcion. Furthermore, three N-acetylcysteine

(NAC) conjugates were observed in rat liver microsomes supplemented with NAC as a trapping agent.[153] The metabolites of physcion displayed a strong inhibitory effect on the UGT1A1, while physcion showed minimal inhibitory activity towards the UGT1A1. It was hypothesized that the inhibition of UGT1A1-mediated bilirubin metabolism by metabolites might pose a potential risk of hepatotoxicity.[113] In fact, physcion is converted into multiple metabolites in vivo. Nevertheless, the study of toxic mechanisms associated with metabolites is also insufficient. Future studies should focus on the effects of physcion metabolites on UGT1A1-mediated bilirubin metabolism and the hepatotoxicity caused by NAC depletion.

Metabolism and Toxicity of Chrysophanol

After oral administration of chrysophanol to rats, chrysophanol was widely distributed in the body, mainly in tissues with rich blood supply such as the heart, spleen and liver. The concentration of chrysophanol in the kidney was higher than that in the liver, which indicated that it was eliminated through excretion rather than metabolism.[154] There are few reports on the toxicity of chrysophanol. Sun Y et al showed that hydroxylation of chrysophanol on alkyl carbon and aromatic carbon was the main metabolic pathway. Three hydroxylated metabolites, aloe-emodin, 7-hydroxy-emodin, and 2-hydroxy-emodin, were detected in rat and human liver microsomes exposed to chrysophanol. Chrysophanol and its oxidative metabolites reacted with GSH in both in vitro and in vivo conditions. CYP1A2 was the main enzyme for chrysophanol bioactivation, while CYP2B6 and CYP3A4 also contributed to the generation of its oxidative metabolites.[155] Aloe-emodin is one of the main metabolites of chrysophanol.[155] Studies have shown that aloe-emodin is more toxic to the liver and kidneys than chrysophanol.[156] Although CYP450 is involved in the metabolism of chrysophanol, its toxicity mechanism has not yet been confirmed, and whether aloe-emodin is the main toxic metabolite of chrysophanol still needs further confirmation.

In summary, the metabolic pathways and toxicity mechanisms of AQs are highly complex. Both Phase I and Phase II metabolites of AQs are associated with toxicity. AQs have numerous metabolites, which are interconvertible and undergo multiple chemical reactions in vivo. This makes toxicity studies of AQs extremely challenging. Furthermore, the metabolism of AQs is dependent on the route of administration, gender, and species, making appropriate animal experimental design crucial. The following strategies are proposed for studies on AQs-induced toxicity: (1) Use advanced methods and technologies to analyze metabolites; (2) Use artificial intelligence (AI) technology to screen and predict metabolites; (3) Further explore inhibitors or activators of metabolic enzymes. The metabolic toxicity of AQs is shown in Figure 5.

Application of Cutting-Edge Technologies in Toxicological Research on AQs

With the development of modern science and technology, multi-omics technologies, single-cell sequencing, AI and computer simulation technologies, as well as microfluidic technologies and mass spectrometry imaging (MPI), will serve as effective tools for the toxicological research of rhubarb AQs in the future. Progress in the application of cutting-edge technologies in toxicological research on AQs is presented in Figure 6.

Multi-Omics Technologies and Single-Cell Sequencing

Omics is an interdisciplinary discipline integrating technologies from multiple fields including biology, statistics, and computer science, and such multi-omics integration technology plays an indispensable role in various scientific fields.[157] Multi-omics is a comprehensive multi-level analytical method that encompasses genomics, transcriptomics, proteomics, and metabolomics.[158] Transcriptomics enables the in-depth revelation of intrinsic correlations between the toxicological effects and detoxification of traditional Chinese medicines (TCMs) in terms of gene regulation and life activity processes, thereby acquiring more specific information regarding functional genes and related signaling pathways.[159,160] Proteomics allows rapid and comprehensive analysis of protein expression differences in cells, tissues and organs under toxic conditions, which facilitates screening differentially expressed proteins, identifying functional biomarkers and clarifying their underlying molecular mechanisms.[161] Metabolomics can dynamically monitor the progression of toxicity via

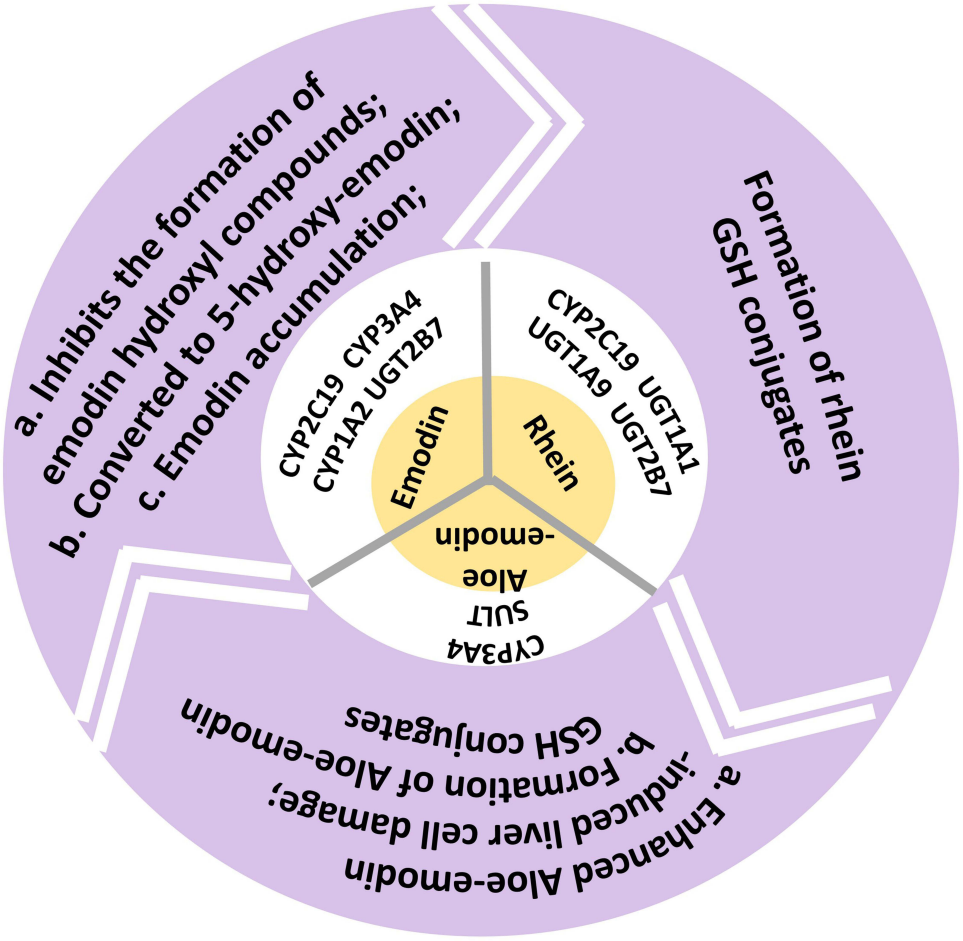

Figure 5 Metabolic toxicity of AQs.

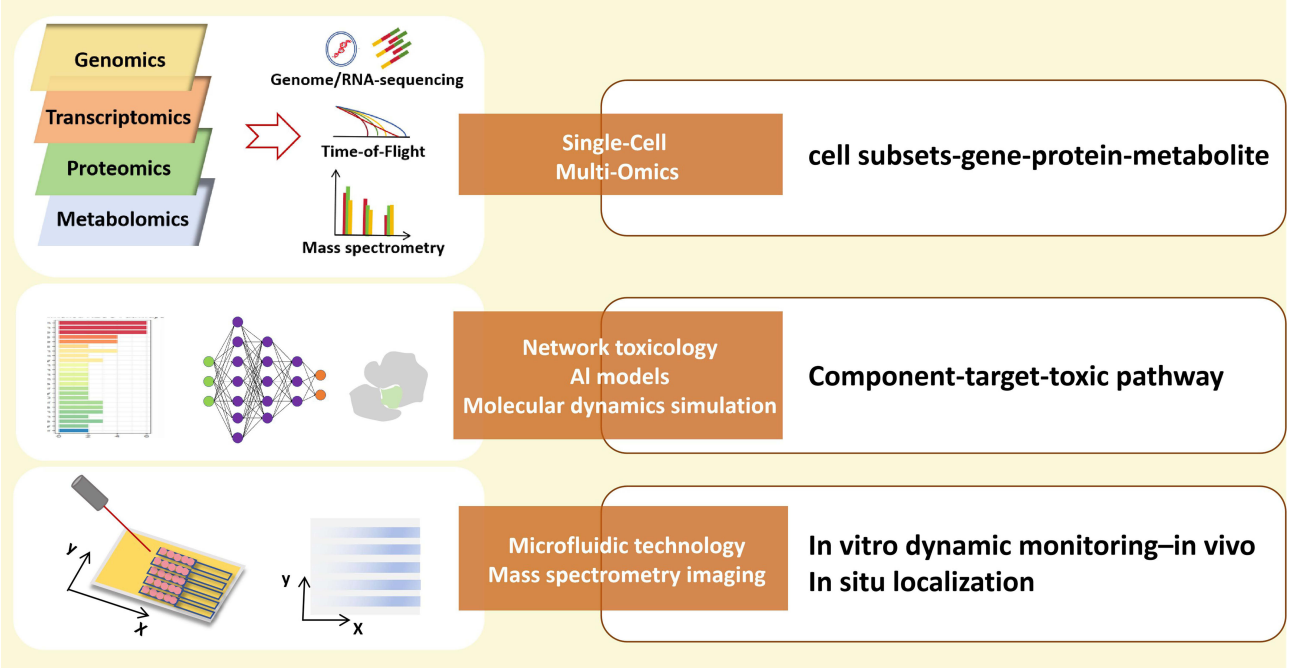

Figure 6 Application of cutting-edge technologies in toxicological research on **five AQs**.

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continuous sampling, and more intuitively identify the target organs of TCM-induced toxicity as well as their associated mechanisms of action.[162] Therefore, multi-omics enables targeted high-throughput screening for toxicological research of TCMs characterized by the "multi-component and multi-target" property. For instance, Xin Li et al integrated transcriptomics and metabolomics to define the biological pathways underlying liver toxicity induced by Cortex Dictamni, linking its toxic mechanisms to oxidative stress and mitochondrial dysfunction.[163] Q Ran et al employed transcriptomics and metabolomics to identify key targets and metabolites associated with the liver toxicity of Rhododendron, laying a foundation for the safety evaluation of this herb in clinical application.[164] Through combined hepatic transcriptomic, proteomic and metabolomic analyses, X Liu et al demonstrated that chronic hepatotoxicity caused by Emilia sonchifolia correlates with cholestasis, oxidative stress and ferroptosis, which are mediated by Cyp2c29, Cyp3a41a, Ugt2b1 and Hsd3b3.[165] However, multi-omics-based toxicological research on rhubarb AQs remains scarce currently. We recommend that multi-omics be employed in future studies to rapidly, comprehensively and precisely characterize the toxic targets and mechanisms of rhubarb AQs. Unlike multi-omics, single-cell sequencing enables analysis of individual omics layers (transcriptome, epigenome, proteome) in single cells, facilitating in-depth insight into complex molecular alterations in compound-exposed single cells and offering novel perspectives on toxicant response mechanisms at cell-type resolution.[166] For example, R Sun et al employed single-cell sequencing to identify key pathways underlying benzene-induced carcinogenesis and confirmed the hematopoietic cell lineage as a critical toxic target subpopulation.[167] Yu et al investigated the toxicity of PFOA in nine cell populations of zebrafish, identifying cardiac cells as the most severely affected subpopulation, as well as the key targets and mechanisms underlying its cardiotoxicity.[168] Furthermore, Chen et al demonstrated that DQ induces an oxidative stress microenvironment in endothelial and parenchymal cells, leading to multi-organ injury, based on a single-cell atlas covering over 270,000 cells from mouse lung, liver and kidney.[169] Single-cell sequencing overcomes the averaging limitation of bulk sequencing, enabling accurate discrimination of heterogeneous cell subpopulations, identification of compound-targeted subpopulations, and precise dissection of cell-specific molecular responses and regulatory networks. Similarly, rhubarb AQs induce multi-organ injury with differential toxicity across target organs. Thus, we propose constructing a multi-organ single-cell atlas and applying single-cell sequencing to identify the core cell populations and injury mechanisms of drug toxicity, thereby further elucidating the pathophysiological and molecular mechanisms underlying rhubarb AQ-induced multi-organ injury.

Notably, the integration of scRNA-seq with other multi-omics has emerged as a research frontier. This integration not only accurately and comprehensively characterizes cellular states but also maps their intrinsic correlations, which are expected to become the standard toolkit for molecular and cellular biology research.[170] Kiran Makhani et al employed single-cell multi-omics to analyze arsenic-induced transcriptomic and epigenetic alterations in distinct immune cells.[171] Wu Zhi et al utilized scRNA-seq and multi-omics analysis to identify the mechanism of bladder tumorigenesis induced by tobacco carcinogens, facilitating integrated multi-omics insights into the risk assessment and toxic mechanisms of tobacco carcinogens.[172] Thus, toxicological research based on single-cell multi-omics is likely to become one of the future research trends for rhubarb AQs.

AI and Computer Simulation Technology

With the continuous advancement of toxicology, omics technologies and AI prediction techniques, multiple specialized toxicology databases have been established. AI is exerting a profound impact on drug-induced toxicity assessment, with broad coverage ranging from the basic prediction of toxicity potential at different biological levels to the identification of toxic biomarkers and biological network analysis.[173] A study summarized the datasets and tools applied in drug toxicity prediction, including 55 databases (toxicity, chemical, omics, and benchmark databases) and 12 tools, which can be used to evaluate drug-induced hepatotoxicity, nephrotoxicity, cardiotoxicity and neurotoxicity.[174] These offer promising directions for machine learning-driven drug toxicity research, where the selection and development of high-quality models are critical. For example, the random forest model achieves 83% accuracy in cardiac datasets and 85% accuracy in hepatitis prediction.[175] Furthermore, ensemble models may be more favorable. An ensemble model was generated via probability averaging of eight effective and widely used machine learning algorithms (Naïve Bayes, K-nearest neighbor, Kstar, AdaBoostM1, Bagging, J48, Random Forest, and Deeplearning4j). Compared with previous models, this model exhibits significant superiority and is applicable for DILI risk assessment in early drug development stages.[176] In

nephrotoxicity prediction, among the deep learning model based on 8 fingerprints and Rdkit descriptors and 27 machine learning models, the deep neural network model performed better in five-fold cross-validation, while the extra tree model exhibited superior performance on test data.[177] In addition, the deephERG model constructed by the support vector machine algorithm and multitask deep neural network (DNN) algorithm is also recognized as a high-quality model for nephrotoxicity prediction.[178] In cardiotoxicity prediction, the most commonly used models are support vector machine (SVM) and random forest (RF) classifiers.[179] However, the deephERG model constructed by the multitask deep neural network (DNN) algorithm and the XGBoost model built via the ensemble method are proven to be suitable models for cardiotoxicity.[180] Meanwhile, the extra tree regressor submodel and the predictive model (NB-03) based on eight molecular descriptors and ECFP_10 fingerprints exhibit favorable predictive performance for neurotoxicity.[181,182] Specialized AI models are required for target organ specificity, compound physicochemical properties, and toxicity patterns.[183,184] Therefore, with the support of big data, it is imperative to explore suitable AI models to integrating into the toxicity prediction of rhubarb AQs on various target organs.

Microfluidic Technology

Microfluidic technology features high throughput, low sample consumption and precise cell manipulation, enabling real-time in-situ monitoring of cellular process biomarkers.[185] Microfluidic platforms for drug toxicity analysis include drug dilution generators, 3D cell co-cultures and organ chip.[186] Organ chips developed based on microfluidic chip technology have attracted extensive attention; they can simulate the microenvironments of various living cells, tissues and organs in vitro, enable real-time dynamic monitoring of intracellular drug toxicity-related indicators, and reflect the key structural and functional characteristics of human tissues and organs.[187] Currently, organ chips used for simulation include the lung, liver, heart, intestine, brain, kidney and others.[188] For example, Xu et al established a analyzable high-throughput microparticle model integrating endocardial and myocardial functions via microfluidic technology based on 3D cell co-culture, which can accurately analyze the effects of metabolites on cardiotoxicity.[189] YC Toh et al developed a microfluidic 3D hepatocyte chip (3D HepaTox Chip) designed to maintain the synthetic and metabolic functions of hepatocytes, which was applied for in vitro drug toxicity testing to predict in vivo drug-induced hepatotoxicity.[190] Yang et al established an integrated microfluidic array system; using Danio rerio embryos as biological models, it enables accurate evaluation of time- and dose-dependent damage in chemically exposed embryos.[191] In recent years, multi-organ chips have been regarded as capable of bridging the gap between the dynamic characteristics of human biology and achieved remarkable development. In a constructed liver-kidney chip model, hepatocytes can not only abundantly and stably express metabolism-related biomarkers but also enable the investigation of compound biotransformation and toxicity.[192] Theobald et al established a tri-organ gut-vascular-neural axis chip, coupled with an integrated solid-phase extraction mass spectrometer, and found that metabolites of PFAS in intestinal epithelial cells could induce neurological dysfunction, promoting axial changes in oxidative stress responses and inflammatory signaling.[193] Aleksander Skardal et al described a tri-tissue organ chip system consisting of liver, heart and lung for evaluating bleomycin-induced cardiotoxicity. Interestingly, this multi-organ chip revealed that bleomycin exhibits both cardiotoxicity and pulmonary toxicity, whereas the isolated cardiac system failed to recapitulate such cardiotoxicity, indicating that bleomycin may induce secondary mediators derived from other tissues rather than directly affecting cardiac organoids.[194] Furthermore, Xin Wang developed a system-on-a-chip incorporating seven interacting microphysiological systems, encompassing the brain, pancreas, liver, lung, heart, intestine and endometrium, to identify changes in metabolites of compounds and biomarkers. This model reveals that oxidation, reduction and conjugation reactions are the most critical metabolic pathways for compounds.[195] Given the characteristics of rhubarb AQs involving multi-organ toxicity and complex metabolism, multi-organ chips can not only track their in vivo metabolic pathways, identify toxic metabolites and toxicity mechanisms, but also investigate the toxicity mechanisms under systemic crosstalk, thus being well-suited for toxicity studies of rhubarb AQs.

Mass Spectrometry Imaging

Compared with conventional immunofluorescence microscopy, positron emission tomography and magnetic resonance imaging, mass spectrometry imaging (MSI) serves as a mature label-free technique for the simultaneous mapping of

multiple molecular distributions in tissues.[196] At the molecular level, MSI delivers key information regarding the spatial specificity of ADMET (absorption, distribution, metabolism, excretion, and toxicity) of xenobiotics.[197] Cao M Z et al used DESI-MSI to reveal that the major metabolites of VX were distributed in metabolically active tissues (heart, liver, kidney, etc), with the heart showing the highest accumulation of VX metabolites during exposure. It was successfully applied to assessing the dynamic distribution of VX and its metabolites in zebrafish.[198] Md Monirul Islam et al employed AP-MALDI-MSI to observe the pharmacokinetics of imipramine and chloroquine, as well as the spatially differential distribution of their metabolites, in the kidneys of male and female mice.[199] Furthermore, the quantitative MALDI imaging method developed by Weiwei Tang et al not only meets the requirements of specificity, sensitivity and linearity but also reveals the tissue-specific spatiotemporal distribution patterns of tetraamines in rat lungs, livers, kidneys, spleens and hearts, which are of great significance for the study of drug-induced organ toxicity.[200] Owing to the complex metabolism and abundant components of rhubarb AQs, MSI is proposed to track their real-time metabolic pathways and clarify their in vivo spatial distribution, improves the understanding of rhubarb AQ pharmacokinetics and plays a critical role in investigating metabolite toxicity.

In conclusion, cutting-edge technologies including multi-omics, single-cell sequencing, AI and computer simulation, microfluidics, and MSI possess unique technical advantages and application values in rhubarb AQ toxicity research, addressing intractable complex issues in traditional toxicology from multiple dimensions: molecular mechanisms, toxicity prediction, real-time monitoring, and in situ localization.

Discussion

Diacerein (for osteoarthritis) and mitoxantrone (for antitumor treatment), two clinically approved drugs, are both AQs. Clinical studies on rhubarb-based therapy have shown that rhubarb exhibits certain therapeutic efficacy in stage 3–4 chronic kidney disease, atherosclerosis, and acute pancreatitis.[201,203] Furthermore, in a single-center, parallel, double-blind, randomized controlled trial, nano-emodin (n-Emo)-mediated photodynamic therapy (PDT) was shown to promote wound healing in patients undergoing free gingival graft (FGG) treatment. The clinical application of rhubarb AQs has gradually attracted increasing attention. However, related clinical toxicity has also been documented.[204] A 72-year-old patient developed hepatocellular injury after taking an AQ-containing formula continuously for 14 days. Using the updated Roussel Uclaf Causality Assessment Method, this adverse event was presumed to be associated with AQs in the formula.[205] In Japan, an analysis of herb-related drug-induced liver injury (DILI) was performed among 287 patients diagnosed with DILI, and rhubarb was identified as a common herb involved.[206] Therefore, it is important to study and understand their toxicity and mechanism.

Among the five AQs, the toxic mechanisms of emodin, rhein, and aloe-emodin have been extensively investigated. Mitochondrial dysfunction, oxidative stress, and downstream apoptotic signaling constitute the most fundamental toxic mechanisms. These core pathways mediate organ damage, dominated by hepatotoxicity and nephrotoxicity, which are also the most commonly reported adverse effects of AQs. Emodin has been well studied with substantial toxicological evidence, including hepatotoxicity, nephrotoxicity, and reproductive toxicity. ERS and ferroptosis mediate its hepatotoxicity and nephrotoxicity, respectively, while IGF receptor-related pathways are involved in its reproductive toxicity. In contrast, rhein is the only AQ that has been definitively shown to induce cardiotoxicity, warranting caution in clinical applications. Its hepatotoxic mechanism is more complex, additionally involving autophagy and lipid imbalance. The hepatotoxicity and nephrotoxicity of aloe-modin have been reasonably confirmed, yet its genotoxicity remains inconclusive. Research on physcion has mainly focused on hepatotoxicity mediated by the inhibition of transporters and metabolic enzymes. Although evidence suggests potential erythrocyte toxicity and neurotoxicity, the underlying mechanisms remain to be elucidated. Chrysophanol is considered the least toxic among the five AQs. However, recent evidence indicates that it may exert potential genotoxicity via DNA intercalation, and relevant studies are still scarce.[51,61,73,82] Aloe-emodin, rhein, and emodin are confirmed hepatotoxic components.[207,208] while there are few studies on the toxicity of chrysophanol and physcion. A study used a combination of cytotoxicity tests and computer-simulated reverse dose methods to evaluate the potential human hepatotoxicity of 16 AQs and their derivatives. The results showed that rhein was identified as a potential hepatotoxic compound among the 16 AQs due to comprehensive factors such as cytotoxicity, plasma concentration and daily intake.[208] In addition, Gati Krushna Panigrahi et al reported that the higher cytotoxicity

observed for rhein, emodin, and aloe-emodin among AQs might be attributed to their strong binding affinity for DNA and GSH.[147] Furthermore, different AQs exhibit varying degrees of hepatotoxicity. A study examined the effects of AQs on the proliferation of primary rat hepatocyte microtissues and found that their hepatotoxicity was dose- and time-dependent, with the order of hepatotoxicity being rhein > emodin > physcion.[147] Similarly, an evaluation of the cytotoxicity of five AQs (rhein, emodin, aloe-emodin, physcion, and chrysophanol) in rat primary hepatocytes and HepG2 cells demonstrated that rhein exhibited the highest toxicity, followed by emodin, aloe-emodin, physcion, and chrysophanol in descending order of potency.[209] A study compared the toxicity of rhein and chrysophanol in rat liver cells and revealed that rhein exhibited significantly higher efficacy in generating ROS and inducing apoptosis. In contrast, chrysophanol induced only a transient elevation in intracellular Ca^{2+} concentration without causing detectable oxidative damage, thereby indirectly supporting its lower toxicity relative to rhein.[78]

Numerous factors influence the toxicity of AQs, with dosage, treatment duration, and drug metabolism being the most extensively investigated. Chronic toxicity was observed in rats following continuous oral administration of rhubarb extract at doses ranging from 381 to 608 mg/kg over a 52-week period.[210] In rats, emodin administered at doses of 80–144 mg/kg/day induced significant maternal toxicity, characterized by alterations in body weight. Conversely, no statistically significant maternal toxicity was observed at a dose of 57 mg/kg/day. In contrast, persistent maternal toxicity was evident in mice following chronic exposure to emodin at 1005 mg/kg/day.[211] Furthermore, acute exposure to rhein at a single dose of 4000 mg/kg resulted in a 40% mortality rate in mice. In a subchronic toxicity study, oral administration of rhein at 375 mg/kg/day for 75 days induced a 55.5% mortality rate.[74] Aloe-emodin administered at doses of 500, 1000, and 2000 mg/kg induced primary DNA damage in both hepatic and renal tissues.[108] However, the dose levels of aloe-emodin found in food supplements or herbal remedies are unlikely to cause toxicity, ROS generation, or Nrf2 activation in the liver and kidneys.[108] Therefore, the dosage and duration of administration must be carefully considered to ensure the safe and effective use of AQs in clinical or therapeutic applications. However, the safe dosage range and duration of administration required to achieve a satisfactory benefit/risk balance for AQs remain unknown and require further research.[212] The use of CYP450 inhibitors to inhibit the toxicity of AQs may significantly elevate the plasma concentrations of other drugs. Furthermore, interindividual variability in CYP450 enzyme expression levels contributes to differential susceptibility to drug-drug interactions. Excessive CYP450 inhibition may cause serious side effects or even death.[213,214] Therefore, when considering using CYP450 enzyme inhibitors to reduce the toxicity of AQs, attention should be paid to monitoring the plasma concentrations of other drugs. In addition, glucuronidation is the major metabolic pathway of AQs, and all glucuronides of aloe-emodin, emodin, chrysophanol, and physcion are formed by multiple human UGT isozymes, particularly UGT1A9. In contrast, the UGT2B subfamily exhibits high activity only towards β-OH.[215] Therefore, it is recommended to conduct further research on the metabolic pathways related to UGT1A9 and UGT2B, with particular attention to toxicity mechanisms. It is important to note that factors including gender, species, disease status, and route of administration significantly influence the metabolism of AQs. The expression level of UGT2B1 in the liver of male mice is higher than that in female mice, resulting in the intestine of male mice being able to metabolize emodin more efficiently than that of female mice. In addition, notable differences exist in the glucuronidation metabolism of emodin across different species (mice, guinea pigs, dogs and humans).[128] Interestingly, when rats were fed a Chinese herbal extract containing AQs such as emodin and chrysophanol as active ingredients, it was observed that long-term administration of AQs caused numerous adverse changes, and male rats appeared to be more vulnerable than females, with irreversible changes in the weight of the testicles, epididymis, and spleen relative to body weight.[216] In addition, it has been reported that gender-related disparities in gastric emptying and gastric pH may contribute to variations in drug solubility and dissolution rate.[217] Moreover, the impact of diseases on the pharmacokinetics of AQs should not be ignored. Plasma concentrations of rhein, emodin, aloe-emodin, and chrysophanol were significantly elevated in rats with acute liver injury when compared to those in normal rats.[218] Normal and NAFLD rats were orally given different doses of AQs, and the concentrations of emodin, rhein, aloe-emodin, physcion and chrysophanol in plasma were determined individually. It was found that the AQs in the NAFLD rat group had a longer retention time, a slower absorption rate, but higher bioavailability and peak concentration.[219] Furthermore, following intravenous administration of emodin, the identified metabolites encompassed emodin glucuronide, ω-hydroxyemodin (ω-OHE), and ω-OHE sulfate/glucuronide. In contrast, when emodin was administered orally, only emodin glucuronide was detected as a metabolite, whereas emodin, ω-OHE, and ω-OHE sulfate/glucuronide were not observed.[127] Therefore, the toxicity study of AQs should consider multiple factors. It is advisable to select animal models similar to humans for research, design experimental groups with diverse genders, and choose appropriate administration methods.

In cases of toxicity induced by GSH depletion, it is advisable to supplement with specific antioxidants capable of preserving and elevating GSH levels in both serum and tissues, including NAC α-lipoic acid, silymarin flavonoids (derived from milk thistle) and L-glutamine. Consequently, concurrent administration of GSH-associated antioxidants alongside AQs is recommended. For example, concurrent administration of dihydromyricetin (a flavanone that increases GSH levels) at the same time as taking AQs showed significantly less liver damage than taking AQ monotherapy.[220,221] Furthermore, Co-treatment with the natural compound baicalin markedly attenuates rhein-mediated oxidative damage and exerts cytoprotective effects.[222] This provides a practical method for effectively preventing AQs-induced hepatotoxicity and nephrotoxicity.

Gut microbiota-mediated drug metabolism and toxicity deserve considerable attention. A large number of microorganisms colonize the human gastrointestinal tract. After oral administration, intestinal bacteria can enhance drug activity, attenuate toxicity, or induce toxicity via metabolism or biotransformation of the drug active ingredients.[223] For example, gut microbiota dysbiosis affects the metabolism of indomethacin, thereby influencing intestinal injury induced by the drug.[224] Irinotecan is converted to its active form in the liver; however, when it enters the intestine, the normally harmless gut microbiota can transform it into a toxic form.[225] An imbalance of the gut microbiota caused by broad-spectrum antibiotics results in metabolic dysfunction of olanzapine in rats.[226] Therefore, the gut microbiota play an equally important role as the liver in the metabolic transformation of drugs. A pharmacokinetic study on the excretion of RPM extract after oral administration in rats found that the excretion rate of emodin in bile and feces was higher than that in urine, indicating that emodin may be mainly metabolized in the liver. Rhein was excreted primarily through bile and was not detected in urine or feces, suggesting that rhein may undergo enterohepatic circulation and be further metabolized after intestinal reabsorption. In addition, the mean cumulative excretion-time curves of chrysophanol and aloe-emodin rose slowly after administration, which may be related to the elimination of individual components, gastrointestinal tissue hydrolysis of other components, or metabolic transformation by hepatic drug-metabolizing enzymes.[227] Other studies have confirmed that metabolites of aloe-emodin produced under the action of gut microbiota in the gastrointestinal tract may exhibit certain biological activities,[150] suggesting that the changes in toxicity of rhein and aloe-emodin mediated by gut microbiota warrant further investigation. However, studies on the toxicity of rhubarb AQs after metabolism by gut microbiota are still lacking at present.

In addition, MRP2 and HNF4α may be considered as important key regulatory factors for the detoxification of AQs. MRP2 plays an important role in the trans-plasma membrane excretion of various organic anions, GSH conjugates, conjugated sulfates and unconjugated metabolites, and phase II drug metabolites, and is believed to contribute to the excretion of environmental toxins.[228] HNF4α predominantly regulates the expression of drug-metabolizing enzymes and transporters in human hepatocytes, coordinating the regulation of bile acid stasis and xenobiotic metabolism for drug metabolism and detoxification.[229,230]

AQs are characterized by complex metabolic profiles and intricate structure-activity relationships, and there is currently a lack of animal and human toxicology data. A rapid and accurate toxicological evaluation of AQs is needed.[215,231] Cutting-edge technologies, including multi-omics, single-cell sequencing, AI and computer simulation, microfluidics, and MSI, can serve as promising future research directions for rhubarb AQs toxicity studies. Single-cell sequencing facilitates elucidation of cellular heterogeneity mechanisms, while multi-omics enables comprehensive dissection of molecular regulatory mechanisms. The combined application of multi-omics and single-cell sequencing represents the most cutting-edge strategy currently, realizing multi-dimensional correlation analysis of "cell subsets-genes-proteins-metabolites"; for instance, Hayley M. Bennett et al employed mass spectrometry-based single-cell proteomics to detect approximately 1,500/2,500 proteins.[232] Building on single-cell sequencing and multi-omics technologies, we may attempt to establish a linked molecular regulatory network underlying rhubarb AQs toxicity, thereby comprehensively revealing its toxicity mechanisms spanning molecular, cellular and organ levels. Currently, AI-based network toxicology also serves as an important research direction in drug toxicity. This technology can successfully construct a complex network of "rhubarb AQs-targets-toxicity pathways". By incorporating machine learning algorithms such as RF and SVM, it can quantify the interaction strength between components and targets, eliminate false-positive targets, and predict potential toxic targets of unknown AQ metabolites.[233] Furthermore, deep learning-based synergistic toxicity prediction models can be used to establish concentration-toxicity response models and determine the

concentration thresholds of AQs toxicity. Hu S et al constructed an integrated model consisting of four base models: RF, extreme gradient boosting (XGBoost), least absolute shrinkage and selection operator (Lasso), and multilayer perceptron (MLP). Their model predicted that liver toxicity would occur when the dose of emodin exceeded 45.74 mg/kg per day or the administration duration exceeded 30.41 days.[234] This provides a reference for rational dosing regimens. In addition, molecular dynamics (MD) simulation, which mimics atomic-level experimental conditions, can well simulate the molecular-target docking mechanism, endowing studies with high efficiency, cost-effectiveness and predictability.[235] Furthermore, MD simulation integrated with machine learning/deep learning can dynamically generate adaptive force fields.[236] For example, Zhenping Bao et al integrated MD simulation, transcriptomics, metabolomics and machine learning to predict the toxic effects of Triton X.[237] Furthermore, Hu S et al employed meta-analysis and machine learning prediction to reveal the balance between the hepatoprotective and hepatotoxic effects of emodin in rodent models. By identifying dose-and duration-dependent responses, they found that a dose exceeding 45.74 mg/kg/day or a treatment duration longer than 30.41 days may represent critical thresholds at which emodin shifts from exerting hepatoprotective effects to inducing hepatotoxicity. This finding may facilitate the development of more rational dosing and therapeutic regimens.[234] Therefore, computer simulation technology incorporating network toxicology, machine learning and MD simulation can be used to simulate the interaction patterns between rhubarb AQs, their metabolites and toxic target proteins, thereby further revealing the molecular mechanisms of their toxic effects. At the same time, AI models can also predict the synergistic toxicity of rhubarb AQs with multiple components, resolving the long-standing challenge in traditional research of quantifying how multi-component interactions affect toxicity. It is worth noting that microfluidic technology coupled with mass spectrometry is increasingly prevalent in the field of biomarker discovery.[238] By simulating the in vivo physiological microenvironment and constructing 3D organoid models, microfluidic chip technology can recapitulate the entire process of absorption, metabolism, and toxic effects of AQs. Through the real-time detection module of microfluidic chips, toxicological indicators such as cell viability, lactate dehydrogenase (LDH) release, and ROS production can be dynamically monitored,[185,239] providing more accurate data for in vitro toxicity assessment. MSI technology enables the in situ localization and quantitative analysis of rhubarb AQs and their metabolites in vivo tissues,[240] addressing the issue that traditional tissue homogenization methods fail to locate toxic target tissues and reveal tissue-specific metabolic differences. In the future, integrating microfluidics with MSI can achieve integrated analysis of "in vitro dynamic monitoring-in vivo in situ localization" for rhubarb AQs. For example, microfluidic chips can be used to acquire real-time response data of cells exposed to rhubarb AQs, combined with MSI to clarify their distribution and metabolic characteristics in vivo tissues, further correlating molecular mechanisms with tissue damage phenotypes and providing multi-dimensional technical support for comprehensive dissection of rhubarb AQs toxicity. However, several aspects of cutting-edge technological research still warrant improvement in the future. First, the interpretation of species differences remains insufficient. Most existing studies are limited to rodent models; it is necessary to clarify the species-specific metabolism and toxicity (such as differences in CYP450 enzyme activity) using humanized organoid models combined with human single-cell sequencing data.[241] Second, research on indirect toxicity is inadequate. Single-cell multi-omics and MSI techniques should be employed to explore the regulatory effects of rhubarb AQs on the gut microbiota-toxicity site axis.[242,243] Third, the technical system lacks standardization, and there are no unified protocols for multi-technology integrated research (eg., single-cell data processing, optimization standards for force-field parameters in MD simulations). Therefore, relevant standardized guidelines for toxicity evaluation need to be established to improve research quality.

Furthermore, exploring the application of innovative dosage forms emerges as a crucial avenue for alleviating the toxicity of AQs. Rhubarb total free AQs oral colon-specific drug delivery granules, which are formulated through a microbial-triggering system in conjunction with a pH-dependent system, exhibit a markedly lower level of nephrotoxicity.[244,245] A novel detoxification method is injecting exosome-loaded miRNAs into toxic sites. Exosomes serve as natural kacarriers for drug delivery, possessing high affinity and inherent targeting capability, which enable the long-distance transport of miRNAs into cells and can thus be used as a new delivery vector for miRNA therapy.[246] Ma Chunhua's study found that B-exo-miRNA-499a-5p and C-B-exo-miRNA-499a-5p effectively ameliorated doxorubicin-induced cardiotoxicity by inhibiting the CD38/MAPK/NF-κB signaling pathway,[247] providing new ideas and approaches for rhein-induced cardiotoxicity. In addition, it is recommended to use rhubarb that has been processed by traditional

methods rather than raw rhubarb to prevent toxicity. Studies have found that the AQ content of rhubarb is significantly reduced after multiple cooking and drying processes.[248] However, the clinical use of rhubarb lacks standardized guidelines for its processing and detoxification.[249] Moreover, although the establishment of pharmacovigilance (PV) procedures for herbal medicines is gaining increasing attention,[250] the development of standard guidelines and PV systems is challenging due to the inherent characteristics of natural products. Notably, the combined use of certain natural products considered to have "detoxification" effects with rhubarb can alleviate the toxic side effects of rhubarb while enhancing its therapeutic efficacy. For instance, glycyrrhizic acid (GL), glycyrrhetinic acid (GA), and liquiritigenin (LI), the main active components of licorice, interact with Pgp, BCRP, MRP2, and Claudin1, promoting the excretion of rhein and emodin and exerting a certain detoxification effect.[251] Through synergistic interventions via multiple approaches including dosage form modification, standardized processing, and rational compatibility, it is expected to effectively reduce the toxicity of AQs while preserving the clinical efficacy of rhubarb, thereby providing a scientific basis for the safe and rational application of rhubarb and other similar AQs-containing herbs.

In conclusion, AQs exhibit significant medicinal research potential owing to their diverse pharmacological effects. However, AQs also possess inherent toxicity. The toxic target organs vary among different AQs, and the research on toxicity mechanism is still lacking. Current studies have clearly demonstrated that there is a significant association between the metabolites and the toxicity of AQs. Nevertheless, the in vivo metabolism of AQs presents a substantial challenge due to its complexity, which involves both phase I and phase II metabolisms. Furthermore, AQs can be converted into various chemical structures and produce a large number of metabolites in vivo. However, empirical studies and theoretical frameworks regarding the complex relationship between AQs metabolism and its toxicity are still lacking. Consequently, there is an urgent need for comprehensive research to clarify this relationship. These factors make the exploration of the toxicity of AQs a significant challenge. In the future, alongside the iteration and integration of diverse technologies, it is anticipated to construct an integrated technical framework for rhubarb AQs toxicity research, featuring multi-omics screening, single-cell precise localization, AI-based prediction and validation, microfluidic real-time monitoring, and MSI-mediated in situ visualization. This will achieve full-process coverage covering toxic target screening, mechanistic elucidation, risk prediction and detoxification strategy optimization.

Abbreviations

AQs, anthraquinones; UGT, Uridine diphosphate glucuronosyltransferase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; GSH, glutathione; ERS, endoplasmic reticulum stress; ATP, adenosine triphosphate; mTOR, mammalian target of rapamycin; MAPK, Mitogen-activated protein kinase; Bcl-2, B-cell lymphoma/Leukemia-2; Nrf2, Nuclearfactor erythroidderived 2-like 2; ROS, reactive oxygen species; ERK, extracellular signal-regulated protein kinases; PI3K/AKT, phosphatidylinositol 3-kinase/protein kinase B; LPS, lipopolysaccharide; SOD, superoxide dismutase; CHOP, CCAAT/enhancer binding protein homologous protein; AI, artificial intelligence; MMP, mitochondrial membrane potential; GPX4, glutathione peroxidase 4; JNK, c-Jun N-terminal kinase; TLR4, Toll-like receptor 4.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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