



## LJMU Research Online

Zhou, Y-X, Xia, W, Yue, W, Peng, C, Rahman, K and Zhang, H

**Rhein: A Review of Pharmacological Activities**

<http://researchonline.ljmu.ac.uk/id/eprint/2171/>

### Article

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

**Zhou, Y-X, Xia, W, Yue, W, Peng, C, Rahman, K and Zhang, H (2015) Rhein: A Review of Pharmacological Activities. EVIDENCE-BASED COMPLEMENTARY AND ALTERNATIVE MEDICINE, 2015. pp. 1-10. ISSN 1741-427X**

LJMU has developed **LJMU Research Online** for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact [researchonline@ljmu.ac.uk](mailto:researchonline@ljmu.ac.uk)

<http://researchonline.ljmu.ac.uk/>

## Review Article

# Rhein: A Review of Pharmacological Activities

Yan-Xi Zhou,<sup>1,2</sup> Wei Xia,<sup>3</sup> Wei Yue,<sup>1</sup> Cheng Peng,<sup>2</sup> Khalid Rahman,<sup>4</sup> and Hong Zhang<sup>1,5</sup>

<sup>1</sup>Central Laboratory, Shanghai Seventh People's Hospital, Shanghai 200137, China

<sup>2</sup>Key Laboratory of Standardization of Chinese Herbal Medicines of Ministry of Education, Pharmacy College, Chengdu University of Traditional Chinese Medicine, Chengdu 610075, China

<sup>3</sup>Department of Nuclear Medicine, Shanghai Seventh People's Hospital, Shanghai 200137, China

<sup>4</sup>School of Pharmacy and Biomolecular Sciences, Faculty of Science, Liverpool John Moores University, Liverpool L3 3AF, UK

<sup>5</sup>Department of Pharmaceutical Botany, School of Pharmacy, Second Military Medical University, Shanghai 200433, China

Correspondence should be addressed to Cheng Peng; pccxycd@126.com and Hong Zhang; zhanghong@smmu.edu.cn

Received 7 March 2015; Revised 13 May 2015; Accepted 25 May 2015

Academic Editor: Antonella Fioravanti

Copyright © 2015 Yan-Xi Zhou et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Rhein (4, 5-dihydroxyanthraquinone-2-carboxylic acid) is a lipophilic anthraquinone extensively found in medicinal herbs, such as *Rheum palmatum* L., *Cassia tora* L., *Polygonum multiflorum* Thunb., and *Aloe barbadensis* Miller, which have been used medicinally in China for more than 1,000 years. Its biological activities related to human health are being explored actively. Emerging evidence suggests that rhein has many pharmacological effects, including hepatoprotective, nephroprotective, anti-inflammatory, antioxidant, anticancer, and antimicrobial activities. The present review provides a comprehensive summary and analysis of the pharmacological properties of rhein, supporting the potential uses of rhein as a medicinal agent.

## 1. Introduction

Rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid, Figure 1) is a lipophilic anthraquinone extensively found in medicinal herbs *Rheum palmatum* L., *Cassia tora* L., *Polygonum multiflorum* Thunb. and *Aloe barbadensis* Miller, and so on, which have been used medicinally in China for more than 1,000 years. Diarrhea, the most common side effect, is well tolerated in humans. Rhein exhibits linear pharmacokinetics between 50 and 200 mg [1] and has many pharmacological effects, including hepatoprotective, nephroprotective, anti-inflammatory, antioxidant, anticancer, and antimicrobial activities (summarized in Table 1). These pharmacological effects lay the foundation for the treatment of hepatic disease [2], osteoarthritis [3], diabetes [4], atherosclerosis [5], and various cancers, such as nasopharyngeal carcinoma [6], tongue cancer [7], hepatocellular carcinoma [8], and lung cancer [9]. The aim of the present review was to give a comprehensive summary and analysis of the pharmacological properties of rhein, supporting the potential uses of rhein as a medicinal agent.

## 2. Pharmacology

**2.1. Hepatoprotective Activity.** Rhein has been shown to modulate cytochrome P450 (CYP) enzymes in rat liver microsomes. For example, rhein significantly inhibited CYP2E1; inhibition constant ( $K_i$ ) = 10  $\mu$ m (mixed); CYP2C9 and CYP3A were also inhibited evidently;  $K_i$  = 38  $\mu$ m (mixed) and  $K_i$  = 30  $\mu$ m (mixed), respectively; but rhein revealed only mild inhibitory effects on CYP1A2 ( $K_i$  = 62  $\mu$ m, uncompetitive) and CYP2D6 ( $K_i$  = 74  $\mu$ m, mixed) [10].

In hepatitis B virus-transgenic mice with nonalcoholic steatohepatitis induced by a high-fat (HF) diet, rhein was found to attenuate the serum levels of total cholesterol, triglyceride, and fasting plasma glucose, ameliorating glucose and lipid metabolism [11]. Oral administration of rhein significantly accelerated energy expenditure and decreased the levels of cholesterol and liver triglyceride. It lowered body weight, the expression of the lipogenic enzyme sterol regulatory element-binding protein-1c (SREBP-1c) and its target genes in liver, and the transcriptional activity of SREBP-1c through its upstream regulator, liver X receptor (LXR). Rhein

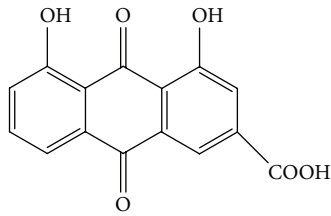


FIGURE 1: Chemical structure of rhein.

also improved insulin resistance and hepatic steatosis and normalized alanine aminotransferase (ALT) levels in HF diet-induced obese mice. Moreover, rhein regulated the T helpers Th1/Th2 responses by inhibition of T-box expressed in T-cells (T-bet) expression and enhancement of GATA-binding protein-3 expression through increased signal transducer and activator of transcription 6 phosphorylation [12].

Fibrosis, characterized by extracellular matrix accumulation and disruption of normal tissue structure, is a common cause of chronic failure of many organs [13]. The recent evidence supports rhein as an antifibrotic agent in hepatic disorders. In carbon tetrachloride/ethanol-induced liver fibrosis rats, rhein downregulated the levels of serum ALT, hyalauronic acid, procollagen type III, and liver malondialdehyde (MDA), upregulated the liver superoxide dismutase (SOD) level, and inhibited the expression of transforming growth factor beta 1 (TGF- $\beta$ 1) and alpha-smooth muscle actin ( $\alpha$ -SMA), the collagen staining positive area and the grade of fibrosis in the liver [2]. Furthermore, rhein markedly improved histological changes of fibrosis and attenuated the expression of  $\alpha$ -SMA and TGF- $\beta$ 1 in the liver, suggesting its protective effect from hepatocyte injury and hepatic fibrosis [14].

**2.2. Nephroprotective Activity.** Several researches have demonstrated the nephroprotective property of rhein both *in vivo* and *in vitro*. In Sprague-Dawley rats with immune globulin anephropathy (IgAN), rhein enhanced the expression of intestinal epithelial tight junction proteins zona occludens protein-1 and occludin, repaired damaged tight junctions, and protected the intestinal barrier [15]. Oral administration of rhein (150 mg/kg/d) evidently ameliorated renal interstitial fibrotic lesions and attenuated the expression of  $\alpha$ -SMA and deposition of fibronectin (FN) in mice with renal interstitial fibrosis induced by unilateral ureteral obstruction. Rhein also suppressed TGF- $\beta$ 1 and its type I receptor expression in obstructed kidneys. *In vitro*, rhein abolished the  $\alpha$ -SMA and FN expression in rat kidney interstitial fibroblasts cells (NRK-49F) induced by TGF- $\beta$ 1, suggesting that rhein is a potent inhibitor of renal interstitial fibrosis [16].

Rhein markedly ameliorated the glomerular hypertrophy, mesangial expansion, excessive extracellular matrix, and renal capsule dilation in IgAN rats. Additionally, rhein administration evidently decreased IgA deposition in glomerulus, the volume of urinary red blood cells, 24-h urinary protein excretion, and the expression of upregulated FN and  $\alpha$ -SMA in renal tissue [17]. In chronic allograft

nephropathy rat models, rhein improved renal function through reductions of renal fibrosis and interstitial inflammation and increases of bone morphogenetic protein 7 and hepatic growth factor levels. Furthermore, both FN and collagen IV were reduced in the extracellular matrix [18].

Rhein was capable of protecting against renal injury progression and ameliorating pathological changes by regulation of the activities of nuclear factor-kappa B (NF- $\kappa$ B) and caspase-3 in the early phase of glomerulosclerosis induced by both unilateral nephrectomy and injection with adriamycin into caudal vein in rats. One of the possible molecular mechanisms by which rhein alleviated renal tissue cell apoptosis in glomerulosclerosis is that caspase-3 expression in kidney is downregulated [19]. Furthermore, rhein inhibited the hypertrophy of renal proximal tubular epithelial cells induced by high glucose (30 mM) and angiotensin II ( $10^{-7}$  M) in rats through significantly decreasing increased cell size,  $^3$ H-leucine incorporation, and cellular protein content [20].

**2.3. Chondroprotective Activity.** There has been a large amount of research on the effects of rhein on osteoarthritis (OA) chondrocytes and tissue separated from human or other animals. Interleukin-1 $\beta$  (IL-1 $\beta$ ) plays a fundamental role in OA pathophysiology and cartilage destruction [3]. Several cells in articular joint tissue produce IL-1 $\beta$ , such as macrophages, synovial cells, and chondrocytes. This cytokine contributes to degeneration of articular cartilage by stimulating the cells to produce proteolytic enzymes and by decreasing the anabolism of the chondrocytes [3]. Rhein ( $10^{-5}$  M) enhanced by 46.5% of aggrecan and 50% of prostaglandin E $_2$ , while it reduced by 17–30% of interleukin-6 (IL-6), matrix metalloproteinase (MMP)-3, nitric oxide (NO), and macrophage inflammatory protein-1 $\beta$  in human osteoarthritic chondrocytes incubated with  $10^{-10}$  M IL-1 $\beta$  [21]. Rhein markedly decreased IL-1 converting enzyme protein production [3] and partially increased tissue inhibitor of metalloproteinase-1 (TIMP-1) synthesis and NO production of IL-1 $\beta$  [22, 23]. Moreover, rhein slightly decreased monocyte chemotactic protein-1 (MCP-1) production, while it increased the levels of IL-1 receptor antagonist (IL-1RA), cytokine receptors IL-6R, soluble tumor necrosis factor (sTNF) R I and R II, and some chemokines or intercellular adhesion molecule (ICAM)-1 in IL-1 (1 ng/mL)-stimulated chondrocytes from osteoarthritic patients [24]. Rhein (5–20 mg/mL) inhibited 1,25(OH) $_2$ D $_3$ -induced osteocalcin release, urokinase plasminogen activator (u-PA) production, and plasminogen activator inhibitor (PAI)-1 levels but increased the levels of insulin-like growth factor-1, prostaglandin E $_2$ , and cyclooxygenase-2 in human OA primary subchondral osteoblasts [25].

IL-1 plays an important role in the OA pathogenesis. Rhein ( $10^{-7}$ – $10^{-5}$  M) notably blocked IL-1 $\beta$  production and NO release stimulated by lipopolysaccharide (1  $\mu$ g/mL) in human OA cartilage and synovial tissue cultures. Rhein also reversed the inhibitory effect of lipopolysaccharide (LPS) on cartilage  $^{35}$ S uptake and increased IL-1RA content in cartilage culture media [26]. Rhein also effectively inhibited the synthesis of IL-1 $\beta$  in human OA synovium, as well as

TABLE 1: Summary of the *in vitro* and *in vivo* evidence for the biological activities of rhein.

Pharmacological effect	<i>In vitro</i> evidence	<i>In vivo</i> evidence
Hepatoprotective activity [2, 7, 10–12]		It modulates cytochrome P450 enzymes, protects hepatocyte from injury and prevents the progress of hepatic fibrosis in rats, alleviates glucose and lipid metabolism, increases energy expenditure, and restrains proinflammatory cytokine expression in mice.
Nephroprotective activity [15–20]	It abolishes the $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) and fibronectin expression of NRK-49F cells.	It alleviates renal fibrosis in mice. It reduces intestinal permeability and protects the intestinal mucosa in immune globulin A nephropathy (IgAN), halts the progression of IgAN, prevents the development of glomerulosclerosis, improves renal function, reduces renal fibrosis and interstitial inflammation, and inhibits the hypertrophy of renal proximal tubular epithelial cells in rats.
Chondroprotective activity [3, 21, 25, 26, 28–35]	It inhibits cytokines (IL-1 $\beta$ , LPS, TNF- $\alpha$ , and rhIL-1 $\alpha$ )-induced effects in human osteoarthritic (OA) chondrocytes, human chondrosarcoma cell line HEM-C55, human OA cartilage and synovial tissue cultures, human umbilical vein endothelial cells (HUVECs), and bovine and rabbit articular chondrocytes. In particular, it stimulates aggrecan production, promotes matrix formation, decreases the production of certain proinflammatory mediators (IL-1 $\beta$ , IL-6, IL-8, and prostaglandin E <sub>2</sub> ), corrects the matrix metalloproteinases/metalloproteinases imbalance, decreases IL-1 converting enzyme protein production, inhibits proliferation of synoviocytes and chondrocytes, and suppresses cathepsin B activity and proteoglycan release.	
Anti-inflammatory activity [36, 37]	It reduces the transcription and expression of endothelial cell adhesion molecules.	It inhibits nicotinamide adenine dinucleotide phosphate oxidase (p22phox gp91phox) and cytokines (matrix metalloproteinase-2, activating transcript factor 6, and p66Shc).
Antioxidant activity [38–41]	Antioxidant properties in human peripheral neutrophils, HUVECs, and beef heart submitochondrial particles.	It prevents the drug-induced oxidative damage in rats.
Anticancer activity [6–9, 42–52]	Anticarcinogenic effects in mouse epidermal cell JB6 line, human colon adenocarcinoma cells (Caco-2), human nasopharyngeal carcinoma (NPC) cells, HUVECs, and tongue cancer SCC-4 cells induce apoptosis in human hepatocellular carcinoma BEL-7402 cells, human cervical cancer Ca Ski cells, human promyelocytic leukemia cells (HL-60), human NPC cells, human tongue cancer cell line (SCC-4), human hepatoblastomaG2 (HepG2) cells, KB cells, and A-549 human lung cancer cells.	
Antidiabetic activity [4, 53–62]	It inhibits transforming growth factor $\beta$ <sub>1</sub> and/or glucose transporter 1 overexpression in human and rat mesangial cells, inhibits glucose uptake in Ehrlich ascites tumor cells and human glomerular mesangial cells, and enhances glucose tolerance in 3T3-L1 adipocytes.	It decreases glucose concentrations, increases insulin secretion, and/or improves glucose tolerance in db/db mice.
Antimicrobial activity [63, 64]	Antimicrobial effects against bacterium <i>Helicobacter pylori</i> and staphylococcus aureus ( <i>S. aureus</i> ).	
Purgative activity [49, 65, 66]	It induces ion secretion in human CaCo-2 monolayer cells and stimulates electrogenic chloride secretion in guinea pig colon.	It increases Na <sup>+</sup> and H <sub>2</sub> O flow in rat colon <i>in-situ</i> .
Lipid-lowering activity [67, 68]	It regulates cholesterol homeostasis and lipid and energy metabolism in 3T3-L1 and HepG2 cells.	It protects against obesity in mice.

the action of this cytokine on the cartilage, by reducing the content of chondrocyte IL-1 receptors [27].

Rhein ( $10^{-5}$  M) had a weak action on  $\alpha 4/\beta 1$  or  $\alpha 5/\beta 1$  receptors in TNF- $\alpha$  or recombinant human IL-1 $\alpha$ - (rhIL-1 $\alpha$ -) stimulated chondrocytes (human chondrosarcoma cell line HEM-C55) [28]. Rhein was found to downregulate the proliferation rate of both synoviocytes and chondrocytes, decrease caspase-3/7 activities, and increase the expression of p21 and/or p27, but not cyclin D1 [29].

After bovine articular chondrocytes were cultured in low oxygen tension with rhein ( $10^{-5}$  M) for 24 h, IL-1 $\beta$  (10 ng/mL)-activated mitogen activated protein kinase (MAPK) pathway, DNA binding of NF- $\kappa$ B, and activator protein-1 (AP-1) were inhibited significantly. NF- $\kappa$ B and AP-1 are two key factors related to the expression of several proinflammatory genes in chondrocytes. Furthermore, rhein could prevent the procatabolic action of the cytokine by inhibition of the collagenase synthesis and increase the synthesis of matrix components, such as type II collagen and aggrecan, which might be the mechanism of its disease-modifying effect in OA [30]. Rhein ( $10^{-4}$  M) evidently prevented increases of MMPs and aggrecanase-1, NF- $\kappa$ B, and AP-1 DNA binding, phosphorylation of extracellular signal-regulated protein kinase (ERK), and c-Jun NH2-terminal kinase in bovine chondrocytes stimulated by IL-1 $\beta$  (10 ng/mL) *in vitro* [31]. Rhein dose-dependently inhibited IL-1 $\beta$ -induced degradation of the inhibitor  $\kappa$ B- $\alpha$  protein, translocation of the protein p65 (a member of the NF- $\kappa$ B family) to the nucleus, and NF- $\kappa$ B binding to a specific (gamma-(32)P)-labelled oligonucleotide probe. Rhein also inhibited inducible NO synthase mRNA and protein synthesis and NO production in a dose-dependent manner [32]. In cultured rabbit articular chondrocytes, rhein (0.1–30  $\mu$ M) dose-dependently suppressed the rhIL-1 $\alpha$ -induced proteoglycan degradation, MMPs activity, and the expression of proMMPs-1, -3, -9, and -13, while it increased the production of TIMP-1 [33, 34]. Rhein at 20  $\mu$ M inhibited the activity of cathepsin B from human liver. In cultured rabbit cartilage challenged with IL-1 $\beta$ , rhein at 100  $\mu$ M suppressed cathepsin B activity and proteoglycan release. After treatment with oral diacerein, the prodrug of rhein, at the dose of 25 mg/day for 3 months, the progression of OA lesions and osteophyte formation were restrained in the experimental OA rabbit model [35].

**2.4. Anti-Inflammatory Activity.** Reducing the expression of endothelial cell adhesion molecules (ECAMs) is known to decrease inflammation-induced vascular complications. The transcription and expression of ECAMs, including ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and E-SELECTIN, could be reduced by the rhein treatment (10 and 20  $\mu$ M) in human umbilical vein endothelial cells (HUVECs). In the presence of LPS stimulation, the transcription and expression of VCAM-1 were also inhibited by treatment with rhein (10 and 20  $\mu$ M) [36].

Adjuvant injection elicited the inflammatory edema in rat paw, accompanied by activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (p22phox gp91phox), transcript factor 6 (ATF6) and p66Shc, elevation

of cytokines including MMP-2, and an increase of the p-Akt/Akt ration, which were notably reversed by rhein [37]. Rhein (20  $\mu$ M) almost completely inhibited intersegmental blood vessels formation at both 48 and 72 h after fertilization (hpf) and completely prevented subintestinal vessel plexus formation at 72 hpf in wild type zebra fish embryos. Rhein affected multiple molecular targets related to angiogenesis, particularly *angpt2* and *tie2*, and also blocked endothelial cell migration [69].

**2.5. Antioxidant Activity.** The superabundant production of reactive oxygen species (ROS) is involved in many pathophysiological processes such as aging, atherosclerosis, cancer, neurodegenerative disorders, chronic inflammation, and degenerative rheumatic disease. Rhein was observed to inhibit the ROS production in human peripheral neutrophils activated by N-formyl-methionyl-leucyl-phenylalanine or phorbol-12-myristate-13-acetate *in vitro* [38]. Besides, many other mechanisms of the antioxidant effect of rhein have been revealed. Pretreatment with different rhein concentrations (2, 4, 8, and 16  $\mu$ M) significantly downregulated the mRNA expression of Bid, caspase-3, -8, and -9 and the content of MDA and lactate dehydrogenase, while it increased NO content and activities of NO synthase, SOD, and glutathione peroxidase (GSH-PX) in hydrogen peroxide- ( $H_2O_2$ -) insulted HUVECs, reversing  $H_2O_2$ -induced cell apoptosis [39]. Rhein dramatically decreased acetaminophen-induced serum glutamate-pyruvate transaminase, glutamate-oxaloacetic transaminase, creatinine and urea nitrogen levels in the liver, ROS production, NO and MDA levels, and GSH concentration in the liver and kidney of rats. Rhein also significantly ameliorated the histopathological damage of the liver and kidney [40]. Rhein eliminated the biphasicity of ubiquinone oxidoreductase- (NADH-) induced reaction and caused a substantial stimulation of NADH-induced lipid peroxidation in beef heart submitochondrial particles. Furthermore, rhein facilitated both NADH- and NADPH-induced lipid peroxidation [41].

**2.6. Anticancer Activity.** Anticarcinogenic effects of rhein on proliferation and metastasis in cells have been investigated *in vitro*. Rhein inhibited hypertrophic scar fibroblasts proliferation in a dose-dependent manner [70]. Rhein also dose-dependently inhibited 12-O-tetradecanoylphorbol-13-acetate- (TPA-) induced cell transformation and AP-1 activation, prevented the phosphorylation of c-Jun protein and c-Jun NH2-terminal kinase (JNK), did not restrain the phosphorylation of ERK and p38 kinase in mouse epidermal cell JB6 line [42]. Rhein (0.1 and 1 mg/mL) evidently suppressed cell proliferation and mitogen-activated protein (MAP) kinase activation in human colon adenocarcinoma cells (Caco-2) but significantly lessened  $H_2O_2$ -induced DNA damage and the elevated MDA and ROS levels induced by  $H_2O_2/Fe^{2+}$  at the concentrations of 0.1–10 mg/mL [43].

Cancer invasion is believed to be dependent on extracellular matrix remodeling elicited by tumor cells. Rhein inhibited invasion and migration in human nasopharyngeal carcinoma (NPC) cells through downregulation of the expression of MMP-9, vascular endothelial growth

factor (VEGF), growth factor receptor bound protein 2, son of sevenless-1 and Ras, inhibition of the phosphorylation of ERK, p38 MAPK, and activation of transcription factor NF- $\kappa$ B [6]. Rhein prevented HUVEC tube formation, proliferation, and migration stimulated by vascular endothelial growth factor (VEGF<sub>165</sub>) under normoxic and hypoxic conditions. Moreover, rhein inhibited the activation of phosphatidylinositol 3-kinase (PI3K), phosphorylated-AKT (p-AKT), and phosphorylated ERK, suppressing *in vitro* angiogenesis. Rhein restrained cell cycle and viability of hormone-dependent breast cancer cells (MCF-7) and hormone-independent breast cancer cells (MDA-MB-435s) under normoxic or hypoxic conditions. In addition, rhein decreased the expression of hypoxia-inducible factor (HIF)-1 $\alpha$ , VEGF<sub>165</sub>, epidermal growth factor (EGF), the phosphorylation of NF- $\kappa$ B inhibitor, and the activity of heat shock protein 90 $\alpha$  (Hsp90 $\alpha$ ) under normoxic or hypoxic conditions [44].

Rhein prevented the mRNA expression of MMP-9, which plays an important role and is the most associated with tumor invasion and metastasis in various human cancers, decreased the levels of MMP-2 and urokinase u-PA, and inhibited the migration and invasion in human tongue cancer SCC-4 cells [7]. A further study demonstrated that rhein dose-dependently induced DNA damage in SCC-4 cells, followed by the inhibition of the mRNA expression of DNA repair-associated O (6)-methylguanine-DNA methyltransferase (MGMT) [45]. The mitosis was inhibited in *Allium cepa* root tips incubated with rhein in a dose-dependent manner [71].

Apoptosis, a physiological process for eliminating malignant cells including cancer cells, does not result in the damage to normal cells or surrounding tissues. Rhein-induced apoptosis has been reported in various human cancer cells. Incubation of human hepatocellular carcinoma BEL-7402 cells with rhein at 50–200  $\mu$ M for 48 hours caused an increasing apoptosis, the features of which included cellular morphological change and chromatin condensation. Additionally, rhein induced cell cycle S-phase arrest, decreased c-Myc gene expression, and increased caspase-3 gene expression [8]. Rhein induced the abrogation of mitochondrial membrane potential and cleavage of Bid protein in human cervical cancer Ca Ski cells. Rhein decreased the level of Bcl-2 while increased the levels of Fas, p53, p21, Bax, and cytoplasmic Ca<sup>2+</sup> and the activities of both caspase-8 and -9, promoted caspase-3 activation, and resulted in DNA fragmentation [46].

Rhein induced apoptosis in human promyelocytic leukemia cells (HL-60) through facilitating the loss of mitochondrial membrane potential, cytochrome c release from mitochondrion to cytosol, and cleavage of Bid protein. Rhein also increased the generation of ROS and the phosphorylation of c-Jun N-terminal kinase and p38 kinase [47]. Rhein elevated nuclear condensation and DNA fragmentation, resulting in apoptosis of human NPC cells. Furthermore, rhein increased the activation of caspase-3, -8, -9, and -12 as well as the levels of glucose-regulated protein 78 (GRP 78), PKR-like ER kinase, ATF6, and CCAAT, induced the rapid accumulation

of calcium (Ca<sup>2+</sup>), and lessened the mitochondrial membrane potential. Then Cytochrome c and apoptosis-inducing factor were released [48].

Incubation of human CaCo-2 monolayer cells with 50  $\mu$ M rhein induced nitrate production and a time-dependent polymorphonuclear leukocytes chemotaxis. Overnight rhein incubation produced an increasing number of apoptotic cells in the culture supernatant [49]. The treatment with 30  $\mu$ M rhein for 24 h showed the most efficient apoptosis induction in SCC-4 cells. Rhein inhibited p53, cyclin A and E, resulting in S-phase arrest of the cells. The ratio of Bax/Bcl-2 was changed by rhein through inhibition of Bcl-2 level. Rhein increased ROS production and Ca<sup>2+</sup> release, decreased the mitochondrial membrane potential level, and activated caspase-3, -8, and -9 [50]. Rhein significantly increased the protein expression of p53 and p21/WAF1 and the levels of CD95 and its two forms of ligands, membrane-bound CD95 ligand and soluble CD95 ligand, in human hepatoblastoma G2 (HepG2) cells, which not only inhibited HepG2 cell growth but also induced cell apoptosis [51]. The IC<sub>50</sub> values of rhein for KB, hepatoma BEL-7402, and mammary carcinoma MCF-7 cells were 11.5, 14.0, and 18.4 mg/mL, respectively. In KB cells treated with rhein for 96 h, the increase of 71% was observed in apoptotic cells [52]. The apoptosis was observed when A-549 human lung cancer cells were incubated with rhein at 50  $\mu$ M for 12 h and up to 72 h. Rhein induced G<sub>0</sub>/G<sub>1</sub> arrest through inhibition of cyclin D3, Cdk4, and Cdk6. Rhein promoted ROS and Ca<sup>2+</sup> production, caspase-3 activation, and cytochrome c release from mitochondria, increased the levels of GADD153 and GRP78, both hallmarks of endoplasmic reticulum stress, induced the loss of mitochondrial membrane potential ( $\Delta\Psi$ m), and led to apoptosis in A-549 human lung cancer cells. Rhein also increased the levels of p53, p21, and Bax, while it reduced the level of Bcl-2 [9].

**2.7. Antidiabetic Activity.** *In vivo* (in db/db mice), a significant decrease in area under curve (AUC) of glucose concentrations, simultaneously, and increases in AUC of insulin level and first-phase insulin secretion were observed after administration of rhein (120 mg/kg) for 8 weeks. Furthermore, rhein treatment greatly protected  $\beta$  cell mass and inhibited  $\beta$  cell apoptosis [53, 54]. Oral administration of rhein (120 mg/kg) for 8 or 16 weeks notably reduced fasting blood glucose level and improved glucose tolerance. After localized at  $\beta$ -cell mitochondria, rhein could protect mitochondrial ultrastructure from hyperglycemia-induced mitochondrial fission protein dynamin-related protein 1 expression, resulting in inhibition of  $\beta$ -cell apoptosis [55]. Intragastric treatment with rhein (120 mg/kg) significantly downregulated blood glucose concentrations at 0, 30, 60, and 120 min after glucose load, suppressed pancreatic  $\beta$ -cell apoptosis, and elevated the early-phase insulin secretion in mice, suggesting the potential of rhein as a novel therapeutic agent for type 2 diabetes [4].

Rhein decreased urinary albumin excretion, extracellular matrix level, and TGF- $\beta$ <sub>1</sub> and FN expression in renal tissue and also reduced the plasma levels of cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C), and ApoE in db/db mice with diabetic nephropathy (DN) [72]. In rat

mesangial cells transfected with human glucose transporter 1 (GLUT 1) gene, rhein dose-dependently decreased 2-deoxyglucose uptake, reversed cell hypertrophy, and lowered the enhanced glutamine: fructose-6-phosphate aminotransferase activity of the human GLUT 1 gene, suggesting an inhibitory effect on the GLUT 1 overexpression in diabetic nephropathy [56].

It was observed that rhein significantly lowered the secretion of FN and inhibited the proliferation of human mesangial cells in mimic hyperglycemic environment of diabetic nephropathy, the possible mechanism of which might be related to suppression of the bioactivities of TGF- $\beta$ 1 and p38MAPK [57]. TGF- $\beta$ 1 stimulates the glucose uptake by enhancing the GLUT 1 mRNA expression in both human and rat glomerular mesangial cells, which could be antagonized by rhein [58, 59].

Rhein strongly inhibited the uptake of both 2-deoxyglucose and 3-O-methylglucose in Ehrlich ascites tumor cells by alteration of membrane-associated functions [60]. In addition, rhein greatly decreased the induction of ROS in both the NIT-1 cells and isolated islets. Rhein enhanced insulin-stimulated glucose uptake in 3T3-L1 adipocytes, while it decreased triglyceride accumulation in streptozotocin-induced diabetic mice [61]. Rhein markedly attenuated the increased glucose uptake and GLUT1 mRNA expression stimulated by TGF- $\beta$ 1 in a dose-dependent manner in human glomerular mesangial cells [62]. Rhein reversed the abnormal changes of MMP-9/TIMP-1 ratio and impeded overexpression of integrin-linked kinase in high glucose-induced epithelial-mesenchymal transition of HK-2 cells [73].

**2.8. Antimicrobial Activity.** Like many herbal monomer, rhein has a potential antibacterial property. For example, rhein inhibited Arylamine N-acetyltransferase activity and growth in the bacterium *Helicobacter pylori* from peptic ulcer patients [63]. In the other *in vitro* study, rhein showed a good antibacterial activity against all 21 tested *staphylococcus aureus* (*S. aureus*) strains. 28 transporter genes of *S. aureus* ATCC25923 were differentially regulated by rhein. In particular, rhein increased the transcription of genes (srtB and isdABCDEFGHI) encoding iron-regulated surface determinants system and genes (nrdIEF and nrdDG) involved in ribonucleotide reductase systems, while it prevented the transcription of genes (pflAB, nirBDR, narGH, Idh1, COL-SA0660, COL-SA2363, and COL-SA2386) responsible for anaerobic respiration and fermentation [64].

Bacterial DNA/CpG DNA is recognized as a key molecule during the pathogenesis of sepsis. Therefore, preventing CpG DNA from binding to its receptor is considered as the most promising strategy. Rhein was demonstrated to have high affinity for CpG DNA. It could significantly reduce CpG DNA- and LPS-induced TNF- $\alpha$  release in RAW264.7 cells [74].

**2.9. Purgative Activity.** Rhein ( $4 \times 10^{-3}$  M) reduced net H<sub>2</sub>O and Na<sup>+</sup> absorption in rat colon *in-situ*. However, its secretory

effect was associated with neither inhibition of Na<sup>+</sup> and K<sup>+</sup>-ATPase nor damage of the colon epithelium [65]. Mucosal or serosal application of rhein (10 nM–0.5 mM) activated chloride secretion by excitation of submucosal neurons and release of acetylcholine and endogenous prostaglandins [66]. In addition, rhein induced ion secretion in human CaCo-2 monolayer cells [49].

**2.10. Lipid-Lowering Activity.** LXRs play important roles in regulating cholesterol homeostasis and lipid and energy metabolism. After bounding directly to LXRs in C57BL/6J mice fed a HF diet, rhein suppressed the expression levels of LXR target genes in both 3T3-L1 and HepG2 cells *in vitro*. In white adipose tissue, muscle, and liver, rhein reprogrammed the expression of LXR target genes related to adipogenesis and cholesterol metabolism. Rhein activated uncoupling protein 1 (UCP1) expression in brown adipose tissue (BAT) in wild-type mice, suggesting that rhein may protect against obesity and related metabolic disorders through LXR antagonism and regulation of UCP1 expression in BAT [67]. Rhein downregulated the mRNA levels of adipogenesis-specific transcription factors PPAR $\gamma$  and C/EBP $\alpha$  and their downstream target genes involved in adipocyte differentiation, such as CD36, AP-2, and acyl CoA oxidase in both 3T3-L1 preadipocytes and C57BL/6 mice. Furthermore, the expression of C/EBP $\beta$  was reduced by rhein in 3T3-L1 preadipocytes. HF diet-induced weight gain and adiposity were reversed by rhein in C57BL/6 mice [68].

**2.11. Other Activities.** Rhein was found to have estrogenic activity and the EC<sub>50</sub> value was 18.96  $\mu$ g/mL in the yeast-based estrogenicity assay system [75]. LIGHT is known to act as a novel mediator for atherogenesis. Rhein inhibited LIGHT-induced human monocyte migration, ROS generation, the expression of chemokine receptor (CCR)1, CCR2, and ICAM-1, the production of IL-8, MCP-1, TNF- $\alpha$ , and IL-6, and the activation of the p38 MAPK and NF- $\kappa$ B [76]. Vascular smooth-muscle cell proliferation plays an important role in atherosclerosis and restenosis. Treatment with rhein resulted in the induction of apoptosis, release of cytochrome c into the cytosol, loss of mitochondrial membrane potential, decrease in Bcl-2 and Bcl-xL, and the increase in Bax and Bak expression in TNF- $\alpha$ -induced human aortic smooth-muscle cells (HASMCs), thus inhibiting the proliferation of these cells [5].

Rhein had a potent (>76%) inhibitory effect on mast-cell degranulation in rats at a 5 mg/kg dose and suppressed lipoxygenase (LOX) enzyme activity with the IC<sub>50</sub> value of 3.9  $\mu$ g/mL, proposing that rhein has antiallergic activity [77]. Rhein dose-dependently blocked the increase of PAI-1 mRNA expression and protein production and inhibited the activity of phosphor-p44/p42 MAP kinase induced by TGF- $\beta$ 1 in human endothelial cells, suggesting the protective effect on the endothelial dysfunction. Rhein might be a potential agent for the treatment of vascular diseases [78]. Furthermore, rhein inhibited IL-1-induced secretion of MMPs and aggrecanases and apoptosis in intervertebral disc cells, which prevented the progression of intervertebral disc degeneration [79].

### 3. Conclusion

As reviewed here, different pharmacological experiments in a number of *in vitro* and *in vivo* models have convincingly demonstrated the abilities of rhein to exhibit hepatoprotective, nephroprotective, anti-inflammatory, antioxidant, anticancer, and antimicrobial activities, lending support to the rationale behind several of its potential medicinal uses.

However, further studies need to be carried out in order to explore the concealed areas. Although various bioactivities of rhein are substantiated by using laboratory animal or cell models, the molecular mechanisms and targets involved are still unknown, which will count against further clinical applications of this agent.

### Conflict of Interests

The authors have declared that there is no conflict of interests.

### Authors' Contribution

Yan-Xi Zhou and Wei Xia contributed equally to this work.

### Acknowledgments

This work was supported by funds from Shanghai Municipal Health and Family Planning Commission (ZY3-JSFC-2-2010, 20134090, and ZYXK2012010), Pudong New Area Science and Technology Commission (PKJ2013-Y22), Pudong New Area Health and Family Planning Commission (PWZz2013-02 and PWRd2013-03), and the State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicine Resources.

### References

- [1] B. Layek, T. S. Kumar, R. K. Trivedi, R. Mullangi, and N. R. Srinivas, "Development and validation of a sensitive LC-MS/MS method with electrospray ionization for quantitation of rhein in human plasma: application to a pharmacokinetic study," *Biomedical Chromatography*, vol. 22, no. 6, pp. 616–624, 2008.
- [2] M.-Z. Guo, X.-S. Li, D.-M. Shen, X.-Q. Guan, H.-R. Xu, and J. Gao, "Effect of Rhein on the development of hepatic fibrosis in rats," *Zhonghua Gan Zang Bing Za Zhi*, vol. 11, no. 1, pp. 26–29, 2003.
- [3] F. Moldovan, J. P. Pelletier, F.-C. Jolicoeur, J.-M. Cloutier, and J. Martel-Pelletier, "Diacerhein and rhein reduce the ICE-induced IL-1 $\beta$  and IL-18 activation in human osteoarthritic cartilage," *Osteoarthritis and Cartilage*, vol. 8, no. 3, pp. 186–196, 2000.
- [4] H. Du, J.-Q. Shao, P. Gu, J. Wang, and Z.-H. Liu, "Effect of early intervention with rhein on islet function in db/db mice," *Nan Fang Yi Ke Da Xue Xue Bao*, vol. 31, no. 9, pp. 1526–1529, 2011.
- [5] S.-K. Heo, H.-J. Yun, W.-H. Park, and S.-D. Park, "Rhein inhibits TNF- $\alpha$ -induced human aortic smooth muscle cell proliferation via mitochondrial-dependent apoptosis," *Journal of Vascular Research*, vol. 46, no. 4, pp. 375–386, 2009.
- [6] M. L. Lin, J. G. Chung, Y. C. Lu, C. Y. Yang, and S. S. Chen, "Rhein inhibits invasion and migration of human nasopharyngeal carcinoma cells *in vitro* by down-regulation of matrix metalloproteinases-9 and vascular endothelial growth factor," *Oral Oncology*, vol. 45, no. 6, pp. 531–537, 2009.
- [7] Y.-Y. Chen, S.-Y. Chiang, J.-G. Lin et al., "Emodin, aloe-emodin and rhein inhibit migration and invasion in human tongue cancer SCC-4 cells through the inhibition of gene expression of matrix metalloproteinase-9," *International Journal of Oncology*, vol. 36, no. 5, pp. 1113–1120, 2010.
- [8] P. Shi, Z. Huang, and G. Chen, "Rhein induces apoptosis and cell cycle arrest in human hepatocellular carcinoma BEL-7402 cells," *The American Journal of Chinese Medicine*, vol. 36, no. 4, pp. 805–813, 2008.
- [9] T. C. Hsia, J. S. Yang, G. W. Chen et al., "The roles of endoplasmic reticulum stress and Ca<sup>2+</sup> on rhein-induced apoptosis in A-549 human lung cancer cells," *Anticancer Research*, vol. 29, no. 1, pp. 309–318, 2009.
- [10] J.-C. Tang, H. Yang, X.-Y. Song et al., "Inhibition of cytochrome P450 enzymes by rhein in rat liver microsomes," *Phytotherapy Research*, vol. 23, no. 2, pp. 159–164, 2009.
- [11] D.-X. Bian, J. Liu, L. Lu et al., "Protective effects of rhein on hepatic progression in HBV-transgenic mice with nonalcoholic steatohepatitis induced by a high-fat diet," *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi*, vol. 27, no. 5, pp. 328–331, 2013.
- [12] X. Sheng, M. Wang, M. Lu, B. Xi, H. Sheng, and Y. Q. Zang, "Rhein ameliorates fatty liver disease through negative energy balance, hepatic lipogenic regulation, and immunomodulation in diet-induced obese mice," *The American Journal of Physiology—Endocrinology and Metabolism*, vol. 300, no. 5, pp. E886–E893, 2011.
- [13] T. A. Wynn, "Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases," *The Journal of Clinical Investigation*, vol. 117, no. 3, pp. 524–529, 2007.
- [14] M.-Z. Guo, X.-S. Li, H.-R. Xu, Z.-C. Mei, W. Shen, and X.-F. Ye, "Rhein inhibits liver fibrosis induced by carbon tetrachloride in rats," *Acta Pharmacologica Sinica*, vol. 23, no. 8, pp. 739–744, 2002.
- [15] D. He, L. Lee, J. Yang, and X. Wang, "Preventive effects and mechanisms of rhein on renal interstitial fibrosis in obstructive nephropathy," *Biological and Pharmaceutical Bulletin*, vol. 34, no. 8, pp. 1219–1226, 2011.
- [16] S.-N. Peng, H.-H. Zeng, A.-X. Fu, X.-W. Chen, and Q.-X. Zhu, "Effects of rhein on intestinal epithelial tight junction in IgA nephropathy," *World Journal of Gastroenterology*, vol. 19, no. 26, pp. 4137–4145, 2013.
- [17] S.-N. Peng, H.-H. Zeng, A.-X. Fu, X.-W. Chen, and Q.-X. Zhu, "Protection of rhein on IgA nephropathy mediated by inhibition of fibronectin expression in rats," *Indian Journal of Pharmacology*, vol. 45, no. 2, pp. 174–179, 2013.
- [18] J. Su, L. P. Yin, X. Zhang, B. B. Li, L. Liu, and H. Li, "Chronic allograft nephropathy in rats is improved by the intervention of rhein," *Transplantation Proceedings*, vol. 45, no. 6, pp. 2546–2552, 2013.
- [19] Z. Q. Ji, C. W. Huang, C. J. Liang, W. W. Sun, B. Chen, and P. R. Tang, "Effects of rhein on activity of caspase-3 in kidney and cell apoptosis on the progression of renal injury in glomerulosclerosis," *Zhonghua Yi Xue Za Zhi*, vol. 85, no. 26, pp. 1836–1841, 2005.
- [20] D.-Q. Yu, Y. Gao, and X.-H. Liu, "Effects of Rhein on the hypertrophy of renal proximal tubular epithelial cells induced by high glucose and angiotensin II in rats," *Zhong Yao Cai*, vol. 33, no. 4, pp. 570–574, 2010.
- [21] C. Sanchez, M. Mathy-Hartert, M. A. Deberg, H. Flicheux, J.-Y. L. Reginster, and Y. E. Henrotin, "Effects of rhein on human articular chondrocytes in alginate beads," *Biochemical Pharmacology*, vol. 65, no. 3, pp. 377–388, 2003.



- [22] D. Borderie, A. Hernvann, H. Lemarechal, C.-J. Menkes, and O. Ekindjian, "Inhibition of the nitrosothiol production of cultured osteoarthritic chondrocytes by rhein, cortisol and diclofenac," *Osteoarthritis and Cartilage*, vol. 9, no. 1, pp. 1–6, 2001.
- [23] J.-P. Pelletier, F. Mineau, J. C. Fernandes, N. Duval, and J. Martel-Pelletier, "Diacerhein and rhein reduce the interleukin 1 $\beta$  stimulated inducible nitric oxide synthase level and activity while stimulating cyclooxygenase-2 synthesis in human osteoarthritic chondrocytes," *Journal of Rheumatology*, vol. 25, no. 12, pp. 2417–2424, 1998.
- [24] J. Deffaud, M. Kirchmeyer, F. Domagala et al., "Modulatory effect of rhein on IL-1 $\alpha$ -induced responses in human chondrocytes: a comparative study between antibody microarrays and specific ELISAs," *Biorheology*, vol. 45, no. 3-4, pp. 439–455, 2008.
- [25] J.-P. Pelletier, D. Lajeunesse, P. Reboul et al., "Diacerhein reduces the excess synthesis of bone remodeling factors by human osteoblast cells from osteoarthritic subchondral bone," *Journal of Rheumatology*, vol. 28, no. 4, pp. 814–824, 2001.
- [26] M. Yaron, I. Shirazi, and I. Yaron, "Anti-interleukin-1 effects of diacerhein and rhein in human osteoarthritic synovial tissue and cartilage cultures," *Osteoarthritis and Cartilage*, vol. 7, no. 3, pp. 272–280, 1999.
- [27] J. Martel-Pelletier, F. Mineau, F.-C. Jolicoeur, J.-M. Cloutier, and J.-P. Pelletier, "In vitro effects of diacerhein and rhein on interleukin 1 and tumor necrosis factor- $\alpha$  systems in human osteoarthritic synovium and chondrocytes," *The Journal of Rheumatology*, vol. 25, no. 4, pp. 753–762, 1998.
- [28] C. Gigant-Huselstein, D. Dumas, E. Payan et al., "In vitro study of intracellular IL-1 $\beta$  production and  $\beta$ 1 integrins expression in stimulated chondrocytes—effect of rhein," *Biorheology*, vol. 39, no. 1-2, pp. 277–285, 2002.
- [29] F. Legendre, A. Heuze, K. Boukerrouche et al., "Rhein, the metabolite of diacerhein, reduces the proliferation of osteoarthritic chondrocytes and synoviocytes without inducing apoptosis," *Scandinavian Journal of Rheumatology*, vol. 38, no. 2, pp. 104–111, 2009.
- [30] G. Martin, P. Bogdanowicz, F. Domagala, H. Ficheux, and J.-P. Pujol, "Rhein inhibits interleukin-1 $\beta$ -induced activation of MEK/ERK pathway and DNA binding of NF- $\kappa$ B and AP-1 in chondrocytes cultured in hypoxia: a potential mechanism for its disease-modifying effect in osteoarthritis," *Inflammation*, vol. 27, no. 4, pp. 233–246, 2003.
- [31] F. Legendre, P. Bogdanowicz, G. Martin et al., "Rhein, a diacerhein-derived metabolite, modulates the expression of matrix degrading enzymes and the cell proliferation of articular chondrocytes by inhibiting ERK and JNK-AP-1 dependent pathways," *Clinical and Experimental Rheumatology*, vol. 25, no. 4, pp. 546–555, 2007.
- [32] A. F. Mendes, M. M. Caramona, A. P. de Carvalho, and M. C. Lopes, "Diacerhein and rhein prevent interleukin-1 $\beta$ -induced nuclear factor-kappaB activation by inhibiting the degradation of inhibitor kappaB-alpha," *Pharmacology and Toxicology*, vol. 91, no. 1, pp. 22–28, 2002.
- [33] T. Tamura, N. Kosaka, J. Ishiwa, T. Sato, H. Nagase, and A. Ito, "Rhein, an active metabolite of diacerhein, down-regulates the production of pro-matrix metalloproteinases-1, -3, -9 and -13 and up-regulates the production of tissue inhibitor of metalloproteinase-1 in cultured rabbit articular chondrocytes," *Osteoarthritis and Cartilage*, vol. 9, no. 3, pp. 257–263, 2001.
- [34] T. Tamura and K. Ohmori, "Rhein, an active metabolite of diacerhein, suppresses the interleukin-1 $\alpha$ -induced proteoglycan degradation in cultured rabbit articular chondrocytes," *Japanese Journal of Pharmacology*, vol. 85, no. 1, pp. 101–104, 2001.
- [35] L. Savarino, A. Fioravanti, G. Leo, R. Aloisi, and M. Mian, "Anthraquinone-2,6-disulfonic acid as a disease-modifying osteoarthritis drug: an *in vitro* and *in vivo* study," *Clinical Orthopaedics and Related Research*, no. 461, pp. 231–237, 2007.
- [36] G. Hu, J. Liu, Y.-Z. Zhen et al., "Rhein inhibits the expression of vascular cell adhesion molecule 1 in human umbilical vein endothelial cells with or without lipopolysaccharide stimulation," *The American Journal of Chinese Medicine*, vol. 41, no. 3, pp. 473–485, 2013.
- [37] X.-D. Cong, M.-J. Ding, D.-Z. Dai, Y. Wu, Y. Zhang, and Y. Dai, "ER stress, P66shc, and P-Akt/Akt mediate adjuvant-induced inflammation, which is blunted by argirein, a supermolecule and rhein in rats," *Inflammation*, vol. 35, no. 3, pp. 1031–1040, 2012.
- [38] T. Tamura, T. Yokoyama, and K. Ohmori, "Effects of diacerhein on indomethacin-induced gastric ulceration," *Pharmacology*, vol. 63, no. 4, pp. 228–233, 2001.
- [39] X.-F. Zhong, G.-D. Huang, T. Luo, Z.-Y. Deng, and J.-N. Hu, "Protective effect of rhein against oxidative stress-related endothelial cell injury," *Molecular Medicine Reports*, vol. 5, no. 5, pp. 1261–1266, 2012.
- [40] Y.-L. Zhao, G.-D. Zhou, H.-B. Yang et al., "Rhein protects against acetaminophen-induced hepatic and renal toxicity," *Food and Chemical Toxicology*, vol. 49, no. 8, pp. 1705–1710, 2011.
- [41] M. A. Glinn, C. P. Lee, and L. Ernster, "Pro- and anti-oxidant activities of the mitochondrial respiratory chain: factors influencing NAD(P)H-induced lipid peroxidation," *Biochimica et Biophysica Acta—Bioenergetics*, vol. 1318, no. 1-2, pp. 246–254, 1997.
- [42] S. Lin, J.-J. Li, M. Fujii, and D.-X. Hou, "Rhein inhibits TPA-induced activator protein-1 activation and cell transformation by blocking the JNK-dependent pathway," *International Journal of Oncology*, vol. 22, no. 4, pp. 829–833, 2003.
- [43] G. Aviello, I. Rowland, C. I. Gill et al., "Anti-proliferative effect of rhein, an anthraquinone isolated from *Cassia* species, on Caco-2 human adenocarcinoma cells," *Journal of Cellular and Molecular Medicine*, vol. 14, no. 7, pp. 2006–2014, 2010.
- [44] V. E. Fernand, J. N. Losso, R. E. Truax et al., "Rhein inhibits angiogenesis and the viability of hormone-dependent and -independent cancer cells under normoxic or hypoxic conditions *in vitro*," *Chemico-Biological Interactions*, vol. 192, no. 3, pp. 220–232, 2011.
- [45] Y. Y. Chen, S. Y. Chiang, J. G. Lin et al., "Emodin, aloe-emodin and rhein induced DNA damage and inhibited DNA repair gene expression in SCC-4 human tongue cancer cells," *Anticancer Research*, vol. 30, no. 3, pp. 945–952, 2010.
- [46] S.-W. Ip, Y.-S. Weng, S.-Y. Lin, N.-Y. Tang, C.-C. Su, and J.-G. Chung, "The role of Ca<sup>2+</sup> on rhein-induced apoptosis in human cervical cancer Ca Ski cells," *Anticancer Research*, vol. 27, no. 1A, pp. 379–390, 2007.
- [47] S. Lin, M. Fujii, and D.-X. Hou, "Rhein induces apoptosis in HL-60 cells via reactive oxygen species-independent mitochondrial death pathway," *Archives of Biochemistry and Biophysics*, vol. 418, no. 2, pp. 99–107, 2003.

- [48] M.-L. Lin, S.-S. Chen, Y.-C. Lu et al., "Rhein induces apoptosis through induction of endoplasmic reticulum stress and Ca<sup>2+</sup>-dependent mitochondrial death pathway in human nasopharyngeal carcinoma cells," *Anticancer Research*, vol. 27, no. 5, pp. 3313–3322, 2007.
- [49] F. Raimondi, P. Santoro, L. Maiuri et al., "Reactive nitrogen species modulate the effects of rhein, an active component of senna laxatives, on human epithelium in vitro," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 34, no. 5, pp. 529–534, 2002.
- [50] W. W. Lai, J. S. Yang, K. C. Lai et al., "Rhein induced apoptosis through the endoplasmic reticulum stress, caspase- and mitochondria-dependent pathways in SCC-4 human tongue squamous cancer cells," *In Vivo*, vol. 23, no. 2, pp. 309–316, 2009.
- [51] P. L. Kuo, Y. L. Hsu, L. T. Ng, and C. C. Lin, "Rhein inhibits the growth and induces the apoptosis of Hep G2," *Planta Medica*, vol. 70, no. 1, pp. 12–16, 2004.
- [52] Y.-H. Huang and Y.-S. Zhen, "Rhein induces apoptosis in cancer cells and shows synergy with mitomycin," *Yao Xue Xue Bao*, vol. 36, no. 5, pp. 334–338, 2001.
- [53] H. Du, J. Shao, P. Gu, B. Lu, X. Ye, and Z. Liu, "Improvement of glucose tolerance by rhein with restored early-phase insulin secretion in db/db mice," *Journal of Endocrinological Investigation*, vol. 35, no. 6, pp. 607–612, 2012.
- [54] H. Du, J. Shao, P. Gu, B. Lu, J. Wang, and Z. Liu, "Effect of rhein treatment on first-phase insulin secretory function in db/db mice," *Zhongguo Zhongyao Zazhi*, vol. 35, no. 20, pp. 2764–2767, 2010.
- [55] J. Liu, Z. Chen, Y. Zhang et al., "Rhein protects pancreatic  $\beta$ -cells from dynamin-related protein-1-mediated mitochondrial fission and cell apoptosis under hyperglycemia," *Diabetes*, vol. 62, no. 11, pp. 3927–3935, 2013.
- [56] J. Zhu, Z. Liu, and Y. Li, "Inhibition of glucose transporter 1 overexpression in mesangial cells by rhein," *Zhonghua Nei Ke Za Zhi*, vol. 40, no. 8, pp. 537–542, 2001.
- [57] Z. H. Tan, Y. J. Shen, J. N. Zhao, H. Y. Li, and J. Zhang, "Effects of rhein on the function of human mesangial cells in high glucose environment," *Yaoyue Xuebao*, vol. 39, no. 11, Article ID 0513-4870(2004)11-0881-06, pp. 881–886, 2004.
- [58] Z. Liu, Y. Li, and J. Zhang, "Modulatory effect of transforming growth factor-beta and Rhein on glucose transporter-1 in human glomerular mesangial cells," *Zhonghua Yi Xue Za Zhi*, vol. 79, no. 10, pp. 780–783, 1999.
- [59] J. Zhang, Z. Liu, Z. Chen, Y. Li, and L. Li, "Effect of rhein on glucose transporter-1 expression and its function in glomerular mesangial cells," *Chinese Medical Journal*, vol. 112, no. 12, pp. 1077–1079, 1999.
- [60] S. Castiglione, M. Fanciulli, T. Bruno et al., "Rhein inhibits glucose uptake in Ehrlich ascites tumor cells by alteration of membrane-associated functions," *Anti-Cancer Drugs*, vol. 4, no. 3, pp. 407–414, 1993.
- [61] S. B. Choi, B. S. Ko, S. K. Park, and J. S. Jang, "Insulin sensitizing and  $\alpha$ -glucoamylase inhibitory action of sennosides, rheins and rhaponticin in Rhei Rhizoma," *Life Sciences*, vol. 78, no. 9, pp. 934–942, 2006.
- [62] Z.-H. Liu, Y.-J. Li, Z.-H. Chen, D. Liu, and L.-S. Li, "Glucose transporter in human glomerular mesangial cells modulated by transforming growth factor-beta and rhein," *Acta Pharmacologica Sinica*, vol. 22, no. 2, pp. 169–175, 2001.
- [63] J. G. Chung, M. F. Tsou, H. H. Wang et al., "Rhein affects arylamine *N*-acetyltransferase activity in *Helicobacter pylori* from peptic ulcer patients," *Journal of Applied Toxicology*, vol. 18, no. 2, pp. 117–123, 1998.
- [64] L. Yu, H. Xiang, J. Fan et al., "Global transcriptional response of *Staphylococcus aureus* to Rhein, a Natural Plant Product," *Journal of Biotechnology*, vol. 135, no. 3, pp. 304–308, 2008.
- [65] E. Leng-Peschlow, "Sennoside-induced secretion is not caused by changes in mucosal permeability or Na<sup>+</sup>, K<sup>+</sup>-ATPase activity," *Journal of Pharmacy and Pharmacology*, vol. 45, no. 11, pp. 951–954, 1993.
- [66] T. Frieling, C. Rupprecht, and M. Schemann, "Rhein stimulates electrogenic chloride secretion by activation of submucosal neurons in guinea pig colon," *Pharmacology*, vol. 47, no. 1, pp. 70–76, 1993.
- [67] X. Sheng, X. Zhu, Y. Zhang et al., "Rhein protects against obesity and related metabolic disorders through liver X receptor-mediated uncoupling protein 1 upregulation in brown adipose tissue," *International Journal of Biological Sciences*, vol. 8, no. 10, pp. 1375–1384, 2012.
- [68] Q. Liu, X.-L. Zhang, R.-Y. Tao et al., "Rhein, an inhibitor of adipocyte differentiation and adipogenesis," *Journal of Asian Natural Products Research*, vol. 13, no. 8, pp. 714–723, 2011.
- [69] Z.-H. He, R. Zhou, M.-F. He et al., "Anti-angiogenic effect and mechanism of rhein from *Rhizoma Rhei*," *Phytomedicine*, vol. 18, no. 6, pp. 470–478, 2011.
- [70] Q. Wang, N.-N. Zhang, H.-Y. Li, M. Jiang, J. Gao, and G. Bai, "Active ingredients in rhubarb with anti-proliferative effects on scar fibroblasts," *Yaoyue Xuebao*, vol. 47, no. 12, pp. 1618–1622, 2012.
- [71] F. A. Badria and A. S. Ibrahim, "Evaluation of natural anthracene-derived compounds as antimetabolic agents," *Drug Discoveries & Therapeutics*, vol. 7, no. 2, pp. 84–89, 2013.
- [72] Q. Gao, W.-S. Qin, Z.-H. Jia et al., "Rhein improves renal lesion and ameliorates dyslipidemia in db/db mice with diabetic nephropathy," *Planta Medica*, vol. 76, no. 1, pp. 27–33, 2010.
- [73] L. Peng, J. Yang, C. Ning et al., "Rhein inhibits integrin-linked kinase expression and regulates matrix metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio in high glucose-induced epithelial-mesenchymal transition of renal tubular cell," *Biological and Pharmaceutical Bulletin*, vol. 35, no. 10, pp. 1676–1685, 2012.
- [74] X. Liu, J. Cheng, X. Zheng et al., "Targeting CpG DNA to screen and isolate anti-sepsis fraction and monomers from traditional Chinese herbs using affinity biosensor technology," *International Immunopharmacology*, vol. 9, no. 9, pp. 1021–1031, 2009.
- [75] S. C. Kang, C. M. Lee, E. S. Choung et al., "Anti-proliferative effects of estrogen receptor-modulating compounds isolated from *Rheum palmatum*," *Archives of Pharmacol Research*, vol. 31, no. 6, pp. 722–726, 2008.
- [76] S.-K. Heo, H.-J. Yun, E.-K. Noh, and S.-D. Park, "Emodin and rhein inhibit LIGHT-induced monocytes migration by blocking of ROS production," *Vascular Pharmacology*, vol. 53, no. 1-2, pp. 28–37, 2010.
- [77] B. Singh, J. R. Nadkarni, R. A. Vishwakarma, S. B. Bharate, M. Nivsarkar, and S. Anandjiwala, "The hydroalcoholic extract of *Cassia alata* (Linn.) leaves and its major compound rhein exhibits anti-allergic activity via mast cell stabilization and lipoxygenase inhibition," *Journal of Ethnopharmacology*, vol. 141, no. 1, pp. 469–473, 2012.

- [78] J. Zhu, Z. Liu, H. Huang, Z. Chen, and L. Li, "Rhein inhibits transforming growth factor  $\beta$ 1 induced plasminogen activator inhibitor-1 in endothelial cells," *Chinese Medical Journal*, vol. 116, no. 3, pp. 354–359, 2003.
- [79] H. Li, C. Liang, Q. Chen, and Z. Yang, "Rhein: a potential biological therapeutic drug for intervertebral disc degeneration," *Medical Hypotheses*, vol. 77, no. 6, pp. 1105–1107, 2011.