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PHARMACOLOGICAL POTENTIAL ACTION OF RHEIN AND ITS DIVERSE SIGNAL TRANSDUCTION: A SYSTEMATIC REVIEW

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ABSTRACT

Herbal medicines are widely used in clinical research due to their three "low-effect": Low toxicity, low number of side effects, and low cost. Many components of common plants, fruits, vegetables, molds, and lichens have been well-documented for their various bioactivities. Rhein (1,8-dihydroxy-3-carboxyanthraquinone) is a natural occurring anthraquinone derivative isolated from roots and barks of Polygonaceae family plants, e.g., *Rheum palmatum* and *Polygonum multiflorum*. Rhein has been known to possess purgative, vasorelaxant, anti-bacterial, antifungal, anti-diabetic, anti-cancerous, anti-tumorigenic, anti-inflammatory, anti-viral, neuroprotection, hepatoprotection, and nephroprotection activities. Rhein also exerts antifeedant and tumorigenic activity. These bioactivities of rhein have been exhibited in various *in vitro* as well *in vivo* experimental models. With its ability of pleiotropic effect, rhein is a bioactive molecule capable to interact with several molecular targets pathways including IL-1, p53, p21, TNF-α, IFNγ, LRX, SREBP-1c, MCP-1, CCR1, CCR2, ICAM, HVEM, p38 MAPK, NF-κB, IKB-α, LOX, COX, MAPK/AP-1, PPAR-γ, TGF-β, androgen receptors, and HER2/neu signaling, The present mini-review summarizes and presents the information concerning rhein and its various bioactivities and signaling pathways Involved in various diseases.

Keywords: Rhein, Hydroxyanthraquinones derivatives, Bioactivity, signaling pathways.

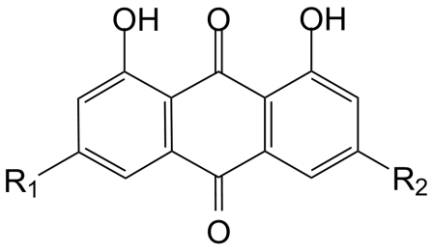
INTRODUCTION

Most diseases are the results of dysregulation of multiple cell signaling pathways. The need of drugs that target multiple cell signaling pathways has been increased over the years [1]. Medicinal herbs are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals [2], and over recent decades, a considerable mounting of evidence has demonstrated a wide range of pharmacological activities for a number of medicinal plants. The investigation of crude plant extracts or isolated compound through ethno-pharmacological evaluation of plants with medicinal value, have shown numerous plants exert medicinal properties which may include anti-oxidant, anti-inflammatory, anti-aging, anti-diabetic, antimicrobial and anti-tumorigenic activities [3]. Ongoing research has been aimed to discover alternative non-toxic drugs and combinational therapies that can replace the existing conventional methods of treatment. Herbal medicines have brought great awareness not only for their multi-potential activities but also for their low costs and most importantly for improving the quality of patient's life and survival [4]. Numerous scientific published papers have reported the use of phytochemical compounds such as anthraquinone rhein and its derivatives extracts from herbal medicines, including *Allium cepa L.* [5], *Cassia alata* [6], *Cassia fistula* [7,8], *Cassia nodosa* [9], *Cassia tora* [10], *Polygonum multiflorum* [11], *Rheum emodi* [12], *Rheum palmatum* [13,14], *Rheum rhaponticum* [15], *Rheum undulatum* [16] and *Senna tora* [17].

To further understand more about the therapeutic functions of Traditional Chinese Medicine (TCM), experiments are needed to identify the functional ingredients and ascertain the molecular mechanisms of these compounds. *Polygonum multiflorum* (*Polygonaceae*) also known as "*He shou wu*" or "*Fo-Ti*" is one of the traditional Chinese herbal medicines commonly used in northeast Asia. Its rhizomes and roots have been widely used as anti-allergic, anti-tumor, antibacterial, hemostatic, spasmolytic, analgesics, anti-alopecia, vasorelaxant and anti-aging agent for many centuries in Asian traditional medicine [18-21]. Mounting data of pharmacological effects of this herb and its components, including anti-inflammation, anti-oxidative, and neuroprotective, as well as improved learning and memory, have been recently published [22-25].

Major compounds of therapeutic importance in rhizomes or roots of this herbal medicine are derivatives of anthraquinone, including emodin, rhein, aloe-emodin, danthron, chrysophanol and physcion [26]. Among these compounds, rhein has been studied for its promising

therapeutic potential due to its multi-signaling target pathways in various diseases. Various published reports suggested that rhein significantly suppresses multiple cell signaling pathways in cancer diseases [27-29]. Besides, rhein has been also exerting other bioactivities including anti-osteoarthritic [30], anti-atherosclerotic [31], anti-diabetic [32], antimicrobial and antiviral [8-10, 14, 33]. This mini-review will emphasize on various molecular targets signaling pathways modulated by rhein (1,8-dihydrohyanthraquinone-3-carboxylic acid, Fig. 1) and its potential actions in various diseases.



Compound	R_1	R_2
Aloe-emodin	H	CH_2OH
Chrysophanol	H	CH_3
Danthron	H	H
Emodin	OH	CH_3
Physcion	OCH_3	CH_3
Rhein	H	COOH

Fig.1: Chemical structures of Aloe-emodin, Chrysophanol, Danthron, Emodin, Physcion and Rhein

1. IDENTIFICATION AND QUANTITATIVE ANALYSIS METHODS

A variety of constituents have been isolated and identified from rhubarb, *Polygonum multiflorum* and other herbal medicines species. They are classed as anthraquinones, dianthrones, stilbenes, anthocynins, flavonoids, anthraglycosides, polyphenols, essential oil, organic acids, chromenes, chromanone, chromone glycosides and vitamins [34], in which anthraquinone rhein is accepted to be one of the important active component. Their structures are characterized by ^{1}H-, ^{13}C-, ^{31}P-NMR, IR, mass and elemental analysis [15, 35]. Several analytical techniques have been used to identify and to analyze the anthraquinone rhein and other anthraquinone derivatives. The capillary zone electrophoresis (CZE)[36], High performance liquid chromatography (HPLC)[26,36,37], Micellar electrokinetic chromatography (MEKC)[38], and High-speed counter-current chromatography (HSCCC)[39] have been

identified and used as methods for separation and analysis of anthraquinones compounds. Multi-constituents analysis by liquid chromatography coupled with diode array detector and electrospray ionization tandem mass spectrometry (LC/DAD/ESI/MS/MS) is a simple and powerful analytical tool for the analysis of the known compounds in complex matrix [40]. Zuo and colleagues have developed a highly selective and sensitive capillary gas chromatography coupled with flame ionization and mass spectrometric detection (GC-MS) method for the separation and quantification of anthraquinones, aloe-emodin, emodin, rhein and physcion, in roots of *Polygoni multiflori* [11]. In contrast to GC methods, HPLC does not require derivatisation prior to the quantitative determination. As such, it has become the most commonly used technique for the isolation, identification and quantification of phenolics and anthraquinones in plant and pharmaceutical materials [41]. However, some of these HPLC methods require a complicated mobile phase, and most of them must use gradient elution methods. For extraction efficiency, several solvents including water, methanol and ethanol have been mainly used and compared. In roots from *Polygonum multiflorum* samples, high amount of hydroxyanthraquinone were extracts with 70% ethanol, following 70% methanol, 100% methanol, 100% ethanol and hot water [26].

However, to be able to quantify efficiency bioactive compounds isolated from herbal medicines, high purity chemical standards or chemical reference substances are needed. Usually these chemical standards are often expensive and insufficient, especially when the component is of low content level and hard to be purified from plant. Moreover, some constituents of herbal medicines become unstable when they are purified from a complicated matrix [42]. An analytical method was successfully developed to quantify other co-existing components using a single standard substance. The method (UV relative correction factors, UV-RCF) was assessed to be simple, low-cost and beneficial especially for determination of unstable constituents in herbal medicines. Thus, novel determination and analytical methods are constantly in need to further efficiency to analyze these herbal bioactive compounds.

2. PHARMACOKINETICS AND PHARMACODYNAMICS PARAMETERS ANALYSIS OF RHEIN

Drugs can resemble a double-edged sword in that they can both help and harm the patient. Rational utilization of drugs requires a thorough understanding of their pharmacokinetics and pharmacodynamics profile. While pharmacokinetics (PK) principles deal with the absorption, distribution, binding, biotransformation and excretion of drugs and their metabolites in the

body [43], pharmacodynamics (PD) is the term used to reflect the relationship between measurements of drug exposure in serum, tissues, and body fluids and the pharmacological and toxicological effects of drugs [44]. By fully appreciating the nature of pharmacokinetics, pharmacodynamics principles, and drug–drug interactions, healthcare professionals can drastically reduce unwanted side effects and at the same time enhance the therapeutic efficacy and usefulness of drugs. Furthermore, PK-PD relationships are playing an increasingly important role in decisions making on the rational utilization of drugs [45].

To assess the toxicity and the therapeutic activity of anthraquinone rhein and others free anthraquinones, a comparative study was conducted on the tissues distribution of rhubarb anthraquinones (AQs) in normal and CCl_4-injured rats orally administered rhubarb extract [13]. As most of the studies today have focused on hepatotoxicity and nephrotoxicity [46], this study had also assessed the toxicity of rhein in spleen. Moreover, the concentration of rhein in spleen was revealed significantly higher than in the liver and kidney (Table 1) [13]. The distribution of rhein was not only significantly different between the two different groups of rats, but was also significantly different among the tissues [13]. The Table 1 showed that the concentration of rhein in the spleen and kidney of normal rats was significantly higher than in the same tissues of the pathological model rats ($p < 0.01$), but the concentration of rhein in the liver of normal rats was also higher than in model rats, with no statistically significance. These data indicate that the pharmacokinetics of AQs in normal animals and pathological animals may be different, which would cause the tissue concentrations of AQs in normal rats to be higher than those in the pathological model rats [13]. However it is important to emphasize that the tissue distributions of rhein ($p< 0.001$), aloe-emodin ($p < 0.001$) and emodin ($p < 0.05$) in normal rats were higher than those in model rats with rhein > aloe-emodin > emodin in kidney and spleen tissues and aloe-emodin > rhein > emodin in liver tissues [13].

Table 1: Amount of anthraquinone rhein (μg/g) in rat tissue after oral administration for 12 weeks [13]

Tissues	Control group (mean ± SD, n=18)	Model group (mean ± SD, n=18)	*P*
Liver	0.43±0.11	0.30±0.76	0.7421
Kidney	1.59±0.74	0.68±0.23	0.0015
Spleen	2.68±1.49	0.87±0.13	<0.0001

The table 2 summarized some PK-PD parameters of rhein after oral administration from few pharmacological animal studies [47-50]. In these studies, regardless the source of rhein, and

comparatively to the control groups, the PK-PD parameters of rhein after oral administration were found significantly important and interesting in rat model groups. For instance, rhein was present at quite high levels in serum (C_{max}=0.48±0.13 μg.mL^{-1}), suggesting good bioavailability [49]. In fact, the rhein serum level peaked at only 0.5h [47], 1.75h [49], 0.13h [50] and its AUC values were several orders of magnitude higher than those of the other components from the same animal groups [47,49].

Table 2: Pharmacokinetics-Pharmacodynamics parameters of rhein after oral administration from pharmacological animal studies

References	47	48	49	50
Experimental conditions				
Source of Rhein	Dahuang Fuzi Tang Decoction	Xiexin Decoction	Da-Cheng-Qi Decoction	Onpi-to
Dose	1.44g/100g body weight, 24h	3.34 g/kg body weight	4.5mg/kg body weight	125mg/kg, 48h
Route	Oral (In Water)	Oral	Oral (In water)	Oral (In water)
Animal used	6 Male SD Rats	7 Male SD Rats	8 Male SD Rats	6 Male SD Rats
Parameters				
$AUC_{(0-t)}$ (μg.mL^{-1}.h^{-1})	3.28±0.91	3.56±1.58 (t=8h)	N/A	N/A
$AUC_{(0-\infty)}$ (μg.mL^{-1}.h^{-1})	3.71±1.13	N/A	2.87±0.56	0.75±0.32
C_{max} (μg.mL^{-1})	1.41±0.68**	1.326±1.833	0.48±0.13	1.30±0.93
$MRT_{(0-t)}$ (h)	6.4±0.9	N/A	7.82±3.37	N/A
CL/F (mL.kg^{-1}.h^{-1})	4.1±1.5**	N/A	N/A	N/A
T_{max} (h)	0.5±0.3*	N/A	1.75±1.25	0.13±0.09
$T_{1/2}$ (h)	N/A	N/A	4.367±2.33	N/A

Dahuang Fuzi Tang (DFT) is composed of three herbs (Radix et Rhizoma Rhei, Radix Aconiti Lateralis Praeparata and Radix et Rhizoma Asari)
Xiexin Decoction (XXD) is composed of Radix et Rhizoma Rhei (Rheum palmatum L.), Rhizoma Coptidis (Coptis chinesis Franch) and Radix Scutellaria (Scutellaria baicalensis Georgi)
Da-Cheng-Qi decoction (DCQD) contains *Radix et Rhizoma Rhei*, *Magnolia officinalis* REHD., *Fructus Aurantii Immaturus*, and *Natrii Sulfas*
Onpi-to is composed of five crude drugs (Rhein rhizome, Glycyrrhizae Radix, Ginseng Radix, Zingiberis Rhizoma and Aconiti Tuber)
N/A*:* Non available, $T_{1/2}$*:* Elimination half-life, $AUC_{(0-\infty)}$: Area under concentration-time curve from t=0 to last time; $MRT_{(0-\infty)}$: Mean residence time, T_{max}: Time to read peak concentration; C_{max}: Maximum plasma concentration; CL: Clearance; F: Bioavailability

While administrating a drug, it is quite important to know the advantages and disadvantages of the various therapeutic routes. The PK-PD information regarding herbal medicines are still not fully elucidated. To further assess the PK-PD parameters free anthraquinones, especially rhein, few studies have been done on healthy human volunteers (Table 3) [16, 51, 52].

Table 3: Pharmacokinetics-Pharmacodynamics parameters of rhein from clinical studies

References	16	52		
Study conditions				
Source of Rhein	*Rheum undalatum L*	*Rheum officinalis* Bails		
Dose	Single dose of 100mg/kg of Rhubarb extract	Single dose of 50mg/kg rhubarb extract		
Route	Oral (In 100ml Water)	Oral (In 150ml water)		Retention Enema (In 150ml water)
Population	12 healthy volunteers (9men/3women)	8 healthy male volunteers		8 healthy male volunteers
Parameters			***P***	
$AUC_{(0-\infty)}$ ($\mu g.mL^{-1}.h^{-1}$)	9.75 ±/1.01	18.64 ± 5.67	0.001	8.58 ± 4.43
C_{max} ($\mu g.mL^{-1}$)	1.84±0.16	2.79 ± 1.02	0.041	1.60 ± 0.56
$MRT_{(0-t)}$ (h)	5.57±/0.49	5.67 ± 2.93	0.596	4.81 ± 2.75
CL_0 ($mL.kg^{-1}.h^{-1}$)	12.02±/1.72	3.10 ± 1.25	0.063	8.34 ± 0.56
T_{max} (h)	1.34±0.12	0.99 ± 0.48	0.465	0.89 ± 0.55
K (1/h)	0.23±/0.02	N/A		N/A
$T_{1/2}$ (h)	3.375±/0.335	3.33 ± 1.06	0.116	2.57 ± 0.87
$Vd_{SS,0}$ ($mL.kg^{-1}$)	63.31±/8.00	14.89 ± 6.44	0.013	30.92 ± 11.27
AUC/bw ($\mu g.mL^{-1}.h^{-1}.kg^{-1}$)	0.15 ± 0.16	N/A		N/A

N/A*:* Non available, $T_{1/2}$*:* Elimination half-life, $AUC_{(0-\infty)}$: Area under concentration-time curve from t=0 to last time; $MRT_{(0-\infty)}$: Mean residence time, T_{max}: Time to read peak concentration; C_{max}: Maximum plasma concentration, CL: Clearance, F: Bioavailability, $Vd_{SS,0}$: Volume of distribution, K: Elimination rate constant, bw: body weight

Among anthraquinones, and regardless the route of administration, few publications reported that rhein was the only anthraquinone absorbed by the body after oral administration [16]. The

PK results of the rhein after oral and retention enema administration showed that plasma concentration-time curve fits two-compartment model and one-compartment model, respectively. Meanwhile, It was noted that in terms of the bioavailability, while the levels in aloe-emodin, emodin and chrysophanol (Fig. 1) in rhubarb extracts were much higher than rhein level, only rhein was selectively absorbed by the body even if rhein is structurally similar to other anthraquinones [16].

From the several published data and regardless the mode of administration, it showed that compared to other anthraquinones, rhein has a rapid absorption. Moreover we can notice, by retention enema administration, the bioavailability of rhein was reduced compared to oral administration [52]. Compared to others compounds, the high bioavailability of rhein can be explained by the fact that rhein can be easily bio-transformed from aloe-emodin [51,53]. By analysis of the route administration, the absorption of weak acids such as rhein may be optimal in the acidic environment of the stomach, whereas their absorption might be unfavorable in the relatively alkaline situation of the small intestine. Retention enema therapy requires multiple, higher daily doses due to poor bioavailability if the same plasma rhein concentration as oral therapy is to be achieved [52].

4. BIOACTIVITY AND PHARMACOLOGICAL MECHANISM OF RHEIN

4.1. Anti-inflammatory and anti-allergic activity

Rhein is a bioactive molecule capable of to interact with several molecular targets pathway by inhibiting several inflammatory biomarkers that play a crucial role in the development of various diseases. In recent years, in similarities with other anthraquinones derivatives, researchers have drawn important attention to the anti-inflammatory effects in order to explore the molecular mechanisms and therapeutic potential of rhein in the prevention and treatment of various inflammatory disorders [4]. Inflammatory diseases including liver fibrosis, pancreatitis, atherosclerosis, osteoarthritis, asthma and glomerulonephritis have been used to investigate the anti-inflammatory mechanism of rhein in *in vitro* or *in vivo* study models.

4.1.1. Liver fibrosis

As others anthraquinones, rhein has long been used as a therapeutic bioactive agent for treating liver fibrosis. Liver fibrosis results from chronic damage to the liver in conjunction with accumulation of extracellular matrix (ECM) proteins, which is a characteristic of most types of chronic liver diseases [54]. In others words, fibrosis occurs as a result of initial liver injury, including hepatocyte damage, kuffer cell activation, hepatic stellate cell (HSC)

activation and proliferation [55]. Decreased activity of ECM-removing, matrix metalloproteinases (MMPs) is mainly due to overexpression of their specific inhibitors [56]. Yinchenhao Tang (YCHT) is one of the famous TCM formulas efficient in treating hepatic injury (HI) and jaundice syndrome [57]. Rhein has been well identified as one of main bioactive component of YCHT [58]. In a bile duct ligated (BDL) rats with hepatic fibrosis experimental model, the potential of rhein from YCHT, has been assessed [57]. YCHT treatment caused a statistically significant down-regulation in the secretion of monocyte chemoattractant protein-1 (MCP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) in BDL rats with fibrosis [57]. In the same study, Haptoglobin (a α2-glycoprotein synthesized by liver and down-regulated after liver injuries) and plectin (a major intermediate filament (IF)-based cytolinker protein that stabilizes cells and tissues mechanically) were decreased and increased, respectively following treatment with YCHT. α-smooth muscle actin (α-SMA) and transforming growth factor β1 (TGF-β1) are two other keys proteins involved in hepatic fibrosis. In another *in vivo* study, the down-regulation of the expressions of these two proteins were suggested to be part of the main mechanism of anti-fibrosis of rhein, associated with suppressing the activation of HSC and the synthesis of ECM [55]. Furthermore, in Caco-2 cells, rhein inhibited P-glycoprotein (P-gp) function by decreasing expression of cyclooxygenase-2 (COX-2) through the MAPK/AP-1 pathway [59].

4.1.2. Arthritis

Osteoarthritis (OA) is a slowly progressive disease of unknown cause and obscure pathogenesis. One of its characteristic features is synovial angiogenesis which is supposed to be mediated by various pro-inflammatory cytokines [60]. It is clinically characterized by pain, deformity, enlargement of the joints, loss of joint stability and limitation of motion. Pathologically, the disease is characterized by focal destructive cartilage lesions, subchondral sclerosis, cyst formation and large osteophytes at the margins of the joint [61].

IL-1 is one of the important factors involved in cartilage destruction in osteoarthritis. IL-ra is a protein produced by several cell types, including monocytes, synoviocytes and chondrocytes, and is able to block several of the effects of IL-1 [62]. In presence of pro-inflammatory cytokines such IL-1, TNF-α, IL-17 or IL-18, chondrocytes over produce Nitric Oxide (NO) [30]. Published studies have reported that Nitric Oxide (NO) contributes to inflammatory and arthritic tissue destruction by inhibition of cartilage macromolecule synthesis, such as collagen type II and proteoglycan [63], and by enhancing matrix

metalloproteases (MMPs) [64]. In OA articular sites, chondrocytes appear to be a significant source of NO, and in contrast to normal cartilage, OA-affected cartilage can spontaneously release NO, enabling cartilage damage in ex-vivo conditions [65]. Several reports have proven that after Lipopolysaccharide (LPS) stimulation of synovial tissue and cartilage cultures, rhein significantly inhibited the production of NO, IL-1β and IL-1ra [30, 60], and therefore can be suitable to treat osteoarthritis.

4.1.3. Atherosclerosis

Atherosclerosis is a dynamic process that involves inflammation at all stages [66], and the abnormal growth of Vascular Smooth Muscle Cells (VSMCs) and inflammation of VSMC, endothelial cells and macrophages/monocytes play an important role in vascular diseases, including atherosclerosis and restenosis after angioplasty [67]. Several inflammation cytokines and/or chemokines including MCP-1, IL-8, TNF-α which play an important role in mediating inflammatory/proliferative responses and in the pathogenesis of atherosclerosis are secreted by VSMC in atherosclerotic lesions [31,68]. LIGHT is a member of the tumor necrosis factor (TNF) family that binds to three distinct TNF receptors, Herpes virus entry mediator (HVEM), lymphotoxin β receptor, and soluble receptor 3 [69]. Heo et al. showed that rhein inhibit LIGHT-induced inflammatory response such as migration expression of ICAM-1, CCR1, CCR2 and HVEM, and production of IL-8, MCP-1, TNF-α, and IL-6, as well as activation of p38 mitogen-activated protein kinase (MAPK), IKB-α and NF-κB in THP-1, and these effects occurs via suppression of reactive oxygen species (ROS) production [31]. Therefore, these results indicated that rhein has the potential use as anti-atherosclerosis drug.

4.1.4. Asthma

Allergic diseases are second most frequent complaint in the population which significantly affects the quality of life and among these, asthma being one of the most common allergic diseases accounting for significant morbidity and mortality in both children and adults [70]. Mast cells are important mediators of allergy anaphylaxis reactions. Degranulated mast cells release a number of constituents and *de novo* synthesized mediators which include histamine, leukotrienes and prostaglandins [71]. Thus, mast cell stabilization is a key factor in controlling the occurrence of asthma [6]. In a mast-cell stabilization model, Singh et al. demonstrated that rhein, one of the two main chemical constituents of *Cassia alata* showed significant anti-allergic/antiasthmatic activity in a dose-dependent manner by exhibiting lipoxygenase (LOX) inhibitory activity, further justifying the anti-allergic activity of *Cassia alata* [6]. Thus,

through mast cell stabilization and LOX inhibition, rhein has been proven to be potential alternative treatment for allergic diseases.

4.1.5. Glomerulonephritis

Glomerulonephritis (GN) is inflammation of the kidney's glomeruli characterized by accumulation of extracellular matrix (ECM). IgA nephropathy (IgAN) also known as Berger's disease or Berger's syndrome is a form of glomerulonephritis which is the most common primary glomerular disease worldwide [72]. Intestinal mucosal damage has been reported to be one key feature in the incidence and aggravation of IgAN [73]. The integrity of the tight junction protein (ZO) plays a key role in the function of the intestinal mucosal barrier; therefore a decrease in tight junction proteins increases intestinal permeability and leads to dysfunction of the intestinal mucosal barrier [74]. Moreover, occludin, a novel integral membrane protein localizing at tight junctions has been reported to play an important role in the stabilization of these tight junctions [75]. According to the study conducted by Peng et al. in IgAN induced rats, rhein reduced intestinal permeability by protecting intestinal epithelial tight junction proteins ZO-1 and occludin, which alleviates the damage to the intestinal mucosa in IgAN [76].

4.2. Hepatoprotection, nephroprotection and anti-diabetic activity

4.2.1. Hepatoprotection activity

Scientific studies have demonstrated that anthraquinones derivatives including aloe-emodin, rhein and emodin have hepatoprotective biological activity [13]. In a cholangiolitic hepatitis induced by α-naphthylisothiocyanate in rats, the intragastrical administration (40mg/kg/day bodey weight) of rhein, aloe-emodin and physcion significantly reduced the serum level of both glutamate-pyruvate transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and the serum total bilirubin (TBIL), direct bilirubin (DBIL), alkaline phosphatase (ALP), γ-glutamyltransferase (γ-GT) and total bile acid (TBA) [77]. Moreover, in the same study, compared to others anthraquinones, the concurrent intragastrical administration of rhein was found the most significant to reduce the severity of all morphological alternation, especially the neutrophil infiltration and sinusoid congestion.

Furthermore, in another study conducted on acetaminophen (APAP)-induced hepatic and renal toxicity in rats, results showed that GPT, GOT, urea nitrogen (UREA), creatinine (CREA) levels and ROS production were reduced dramatically, nitric oxide (NO), malondiadehyde (MDA), glutathione (GSH) contents were restored remarkably by rhein

administration, as compared to the APAP alone treated rats [78]. Tsang et al. studied the effect of rhein on pancreatic stellate cells in mice, in experimental chronic pancreatitis model. In their *in-vivo* and *in-vitro* experiments, the prolonged administration of rhein at 50 mg/kg/day significantly decreased immunoreactivities of α-SMA and TGF-β on pancreatic sections implicating the activation of PSCs [79]. Rhein also significantly reduced the deposition of extracellular matrix proteins fibronectin 1 (FN1) and type I collagen (COL I-α1) in exocrine parenchyma [79]. Additionally, the sonic hedgehog (SHH) and its immediate effector GLI1 in pancreatic tissues were reduced. The SHH/GLI1 signaling pathway was consequently suppressed.

4.2.1 Anti-diabetic and nephroprotection activity

Diabetes is a metabolic disorder associated with either improper functioning of the beta-cells or wherein cells fail to use insulin properly. Insulin, the principal hormone regulates uptake of glucose from the blood into most of the cells except central nervous system [80]. Obesity has been historically known as a risk factor for type-e diabetes. Nonalcoholic fatty liver disease (NAFLD) is associated with obesity, insulin resistance, and inflammatory disorders. In a high-fat diet-induced obese (DIO) mice study model, it was found that oral administration of rhein for 40 days significantly increased energy expenditure, reduced body weight, particularly body fat content, improved insulin resistance, and lowered circulating cholesterol levels in DIO mice without affecting food Intake [81]. Rhein also down-regulated the expression of TNF-α, interferon-γ (INFγ) and IL-12, inhibited the lipogenic enzyme sterol regulatory element-binding protein-1c (SREBP-1c), by bounding directly to its upstream regulator liver receptor (LXR) and activating uncoupling protein I (UCPI) expression [82]. Consequently it further increased IL-4, IL-10 and IL-12P70 [81]. Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated nuclear transcription factor that plays key roles in regulating glucose homeostasis, lipogenesis and adipocytes differentiation [83]. PPARγ inhibitors have been proven to be potent insulin sensitizing agent for treating type II diabetes [84]. The gastric gavage administration of rhein (100mg/kg for 2 weeks) to diet-induced obese (DIO) C57BL/6 mice, blocked high-fat diet induced obesity, decreased fat mass and size of white and brown adipocytes, and lowered serum cholesterol, LDL cholesterol and fasting blood glucose level [85]. In addition, rhein also inhibited PPARγ signaling, proving its PPARγ antagonism activity and being a potential candidate for obesity therapy [85].

Diabetic nephropathy (DN), a major complication of diabetes, has been a major cause of end-

stage renal disease [86]. Hyperglycaemia, a major risk factor for the development of DN, causes abnormalities in blood flow and increased vascular permeability, overproduction and deposition of EMC [87]. The hexosamine biosynthetic pathway has been reported to be involved in the development of insulin resistance and diabetic vascular complications [88]. Published data reported that rhein inhibited the overactivity of the hexosamine pathway and decreased TGF-β1 and p21 expression, which contributed to the decreased cellular hypertrophy and ECM synthesis in MCGT1 and LLC-PK1 cells [32,87], thus exerting its therapeutic role in diabetic nephropathy.

4.3. Antimicrobial, antibacterial, antifungal and larvicidal activity.

4.3.1 Antimicrobial and antibacterial activity

The antimicrobial activity of rhein has been investigated against some probiotic or pathogenic microbial strains. In a microcalorimetric assay, the antimicrobial potential of rhein against *Bifidobacterium adolescentis* (*B.adolescentis*) was investigated by Wang et al. [14]. *B.adolescentis* is among the common species of probiotics that play a key role in the human intestinal balance microflora [89]. Lack of Bifidobacteria could lead to increase the possibility of morbidity, such as inflammation bowel disease [90]. The results of that study showed that, among the five hydroxyanthraquinones (HAQs) tested, rhein was found more potent than the four others [14]. The sequence of antimicrobial activity was rhein > emodin > aloe-emodin > chrysophanol > physcion. Hatano et al. studied the phenolic constituents of Cassia Seeds and antibacterial effects of some naphthalenes and anthraquinones on Methicillin-Resistant *Staphylococcus aureus* (MRSA). Along with others HAQs, rhein showed significant antibacterial activity against four strains of MRSA and also a strain of methicillin-sensitive *Staphylococcus aureus* (MSSA) [10].

Furthermore, by microdilution method, the antimicrobial activity of rhein was carried out against fours bacterial microorganisms. With a minimum inhibitory concentration (MIC) of 2×10^3 mg/mL, rhein exerted a potent antibacterial activity against *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) [9].

4.3.2 Antifungal activity

Dermatophytosis is a clinical condition caused by fungal infection of the skin in humans, pets such as cats, and domesticated animals such as sheep and cattle. Dermatophytes, the most frequent pathogens, are a common label for a group of three types of fungus (*Microsporum, Epidermophyton and Trichophyton*) that commonly causes skin disease in animals and

humans [91]. The antidermatophytic activity of rhein, isolated from *Cassia fistula* Pod Pulp, was assessed in a study done by Chewchinda et al. [91]. Rhein exerted significant potent antifungal bioactivity against *Microsporum gypseum (M. gypseum), Trichophyton rubrum (T. rubrum) and Trichophyton mentagrophytes (T. mentagrophytes)* at the MIC of 500µg/mL, 250µg/mL and 500µg/mL, *respectively.* Furthermore, in others studies, rhein was also proven to be an active antifungal agent by acting on *T. mentagrophytes* and *Epidermophyton floccosum (E.floccosum)* at MIC of 25 µg/mL and 31.25 µg/mL [7,12]. Studies suggested that the antimicrobial or antifungal potency of hydroxyanthraquinones depends on the functional groups carboxyl, hydroxyl and hydroxyl methyl on phenyl ring, at C1 and C8. The polarity and sequence of this bioactivity was carboxyl > hydroxyl > hydroxylmethyl > methyl and methoxyl [12,14].

4.3.3 Larvicidal activity

The notion of pesticides and their excessive use have concerned various researchers in terms of environment safety. Researching alternatives sources of synthetic pesticides ad fungicides based on natural plants extracts and essential oils has been an interesting and promising area [92]. The Extracts of *Cassia fistula L.* (*also known as Golden Shower*) have been recommended for pest and disease control [93]. Rhein, isolated from the flowers of *Cassia fistula L.* was found to exert larvicidal pharmacological action against lepidopteron pests *Spodoptera litura* (*S. litura*) and *Helicoverpa armigera* (*H.armigera*) [8]. The results reported by Duraipandiyan et al. showed that rhein exhibited larvicidal activity of 67.5% against *H. armigera* with LC_{50} value of 506.5 ppm and 36.25% against *S. litura* with the LC_{50} value of 1192.55 ppm. Moreover, treatment with rhein had showed malformation and mortality in larval, pupal and adult stages [8]. Furthermore, in terms of pesticides based on natural plants, similar findings were recorded in hexane fractions of *Atalantia monophylla* against *H. armigera* [94].

4.4. Antiviral activity

Human cytomegalovirus (HCMV) is a ubiquitous herpes virus that leads to a life-long persistence. The frequency of infection ranges from 50% to 100% in the general adult population. HCMV causes severe and often fatal disease in immunocompromised individuals including recipients of organ or tissue transplants, cancer and AIDS patients [95]. Moreover, intrauterine HCMV infections are second only to Down's syndrome as known as major cause of mental retardation [96]. Prolonged therapy with standard antiviral agents such as ganciclovir

has led to serious side effects including granulocyte disorder clinically known as neutropenia [97]. Searching for antiviral drugs from natural occurring anthraquinones and their derivatives has been an interesting topic in scientific researches [98,99]. In Human lung fibroblast (MRC-5 cells), the antiviral activity of rhein along with other natural bioactive compounds was evaluated against HCMV strain AD-169 and ganciclovir-resistant strain of HCMV [33]. The results showed rhein displayed distinguishable antiviral activity against the two HCMV strains cited above. Based on structure-activity relationship analysis, substitutions at the C-3 position of the basic C-1,8 dihydroxyanthraquinone structure appeared to enhance antiviral activity. In addition, substitution with carboxyl or hydroxyl group resulted in antiviral activity while substitution with methyl, methyl alcohol or methoxyl group led to loss the antiviral activity [33]. The structure of the molecule rhein fits the structure-activity relationship analysis, therefore was significantly proven to be an important antiviral biomolecule.

4.5. Anticancer, antitumor and anti-genotoxic activity

For many years, traditional Chinese medicines (TCM) have been applied for the treatment of cancers in China and beyond. One of its major principles is the emphasis on an individual therapy, meaning for the same type of cancer in different persons, the diagnosis and treatment schemes could be very different [100]. However, due to various factors – including inconsistency in treatment schemes, the limited sampling sizes, and lack of quality assurance of the herbal products - well-designed randomized controlled trials (RCT) to prove the effectiveness of TCM as adjuvant therapy for cancer are scarce [100]. Along with two other hydroxyanthraquinones (Danthron and Alizarin), rhein was shown to be a tumor promoter in C3H/M2 mouse fibroblasts in in vitro study model [101]. However proponents of Chinese traditional medicines point out that TCM-derived pure compound have gained increasing acceptance worldwide and pursued by pharmaceutical companies as rich resources for drug discovery in chemotherapy [100].

To assess TCM pharmacological activities and mechanisms induced in tumorigenic cell death various studies were conducted. Rhein has shown significant anticancer and anti-genotoxic activities in several tumor cells, both in vivo and in vitro pre-clinical animal models. The mechanism of the bioactivity of rhein on carcinoma cell lines implicates transduction cascades of various signaling pathways. In human cervical epidermoid carcinoma cell line (Ca Ski), rhein induced apoptosis via caspase-dependent and mitochondria dependent [102]. This apoptosis was characterized with an increase in levels of Fas, p53, p21 and Bax, and a

decrease in level of Bcl-2. Rhein also enhanced the activities of caspase-8 and -9, and induced an increase in level of cytoplasmic Ca^{2+}, which was inhabited by BAPTA (a calcium chelator) [102]. The same effects were also noticed on human hepatocellular carcinoma Hep-G2 [103].

Nasopharyngeal carcinoma (NPC), also known as "Canton tumor" is a rare tumor arising from the epithelium of the nasopharynx [104], occurs with high frequency in Asian populations, especially among Chinese people [105]. The endoplasminc reticulum (ER) stress pathway, shown by an increase in the levels of glucose-regulated protein 78 (GRP 78), PKR-like ER Kinase (PERK), activating transcription factor 6 (ATF6) and CCAAT/enhancer-binding protein homologous protein (CHOP), accumulation of Ca^{2+} as well as the activation of caspase-3, -8, -9 and -12 was demonstrated to be significantly involved in rhein-induced apoptosis mechanism in NPC-039 cells [28].

Resulting from gene amplification and/or overexpression of some oncogenes like HER2/neu (also known as ErbB2) and oestrogen receptors, breast cancer is one of the most common cancers in women and the second most common etiologic cause malignant pleural effusions (MPE) [106,107]. Rhein showed antiproliferative and apoptotic effect on both HER2-overexpressing MCF-7 (MCF-7/HER2) and control vector MCF-7 (MCF-7/VEC) cells, mechanism done in dose- and time-dependent manner with activation of caspase-9, ROS-mediated activated of NF-κB and p53-signaling pathway [29]. Significant similar anticancer bioactivities have been also emphasized in human tongue Cancer SCC-4 cells [27]; human myeloid and lymphoid leukemia cell lines [108]. Furthermore, in a study conducted to assess the anti-angiogenic effects of rhubarb and its anthraquinone derivatives, rhein showed the strongest anti-angiogenic activity [109]. This strong potency is due to its planar chemical structure with its carboxylic group substitution at C-3 position.

4.6. Other bioactivities

4.6.1. Purgative action

Anthraquinone compounds are famous for their laxative property and their laxative effect is caused by two independent mechanisms [110]. While the first is the changing in the colonic motility which leads to an accelerated large intestine transit, the second one is alteration in colonic absorption and secretion, resulting in fluid accumulation which causes diarrhea [111]. Feng et al. (2013) discussed the *in vitro* effects of rhubarbs components, as adrenergic receptor inhibitors linked with glucose carrier, on isolated male ICR mice intestine. Moreover,

comparing the sequence of mice β_2-adrenergic receptor and human β_2-adrenergic was also conducted in order to analyze the binding and to build the interaction model of anthraquinone derivatives and adrenergic receptor, based on computer docking model [112]. Their findings suggested that after injection of 10µl of 25mg/mg drug solution into the intestine, anthraquinone derivatives can antagonize the adrenaline effectively, thus can be used for development of new purgative drugs. Furthermore, rhein (1.3 – 10.7 mg/kg) also accelerated the purgative activity of sennoside A (Fig.2) in male ddY mice [37].

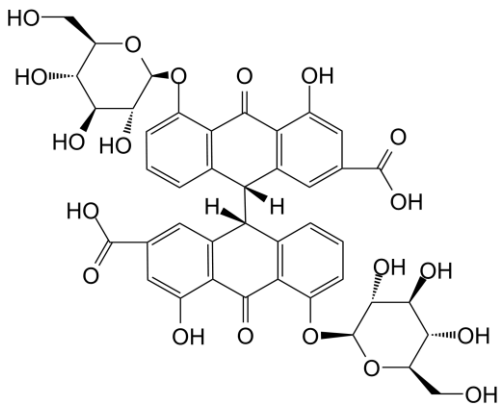


Fig.2: Chemical structure of Sennoside A

4.6.2. Anti-feedant activity

Antifeedants can be described as allomone substances which inhibit feeding and do not kill the pest directly, but rather limit its development potential considerably and act as a phagodetergent or phagorepellent over test as well as permanent pest feeding on the plant [113]. Few authors published studies of antifeedant effect of plant extracts [114,115]). Duraipandiyan et al. [8], using a leaf disc no choice method, assessed the antifeedant activity of the crude ethyl acetate extract and rhein, from Cassia fistula flowers. The study findings showed that the isolated compound rhein exhibited a significant antifeedant activity against *H. armigera*, it also had the potential to act as feeding deterrent against *H. armigera* [8]. The great advantage of antifeedant is their selective action against parasites and pest predators, as well as pollinators [113].

5. CONCLUSION

The above mentioned scientific reports reviewed in detail the therapeutic potential of Herbal medicinal plant component in various diseases. The substantial popularity of herbal medicinal

drugs in different countries and patient populations has been well demonstrated by a number of studies. Rhein, an anthraquinone derivative, is an herbal medicinal bioactive drug of immense importance with a diverse pharmacological spectrum. This review summarizes the evidence which stresses out that the bioactive molecule rhein can modulate a diverse array of molecular targets and thus has a great significant potential to be used as a therapeutic drug for various diseases and disorders. Rhein has a pleiotropic effect, capable to interact with various targets signaling pathways including IL-1, p53, TNF-α, IFNγ, TGF-β and PPAR-γ. It also affects LRX, NF-κB, HER2/neu, LOX and p38 MAPK. Clear evidence provided also strongly emphasizes the ability of rhein to alter or inhibit various signaling events involved in tumor and inflammatory diseases. However, to date only limited data related to the pharmacokinetics and pharmacodynamics of rhein is available and requires much study to fully adapt the observed pharmacological findings of natural plant drugs into proper designed human clinical trials.

CONFLICT OF INTEREST

We declare that none of the authors have financial interest related to this work.

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