

Ginsenoside Rh1: A Systematic Review of Its Pharmacological Properties

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ABSTRACT

Ginsenoside Rh1 is one of major bioactive compounds extracted from red ginseng, which has been increasingly used for enhancing cognition and physical health worldwide. The objective of this study was to review the pharmacological effects of ginsenoside Rh1 in a systematic manner. We performed searches on eight electronic databases including MEDLINE (Pubmed), Scopus, Google Scholar, POPLINE, Global Health Library, Virtual Health Library, the System for Information on Grey Literature in Europe, and the New York Academy of Medicine Grey Literature Report to select the original research publications reporting the biological and pharmacological effects of ginsenoside Rh1 from *in vitro* and *in vivo* studies regardless of publication language and study design. Upon applying the inclusion and exclusion criteria, we included a total of 57 studies for our systemic review. Ginsenoside Rh1 exhibited the potent characteristics of anti-inflammatory, antioxidant, immunomodulatory effects, and positive effects on the nervous system. The cytotoxic effects of ginsenoside Rh1 were dependent on different types of cell lines. Other pharmacological effects including estrogenic, enzymatic, anti-microorganism activities, and cardiovascular effects have been mentioned, but the results were considerably diverged. A higher quality of evidence on clinical trial studies is highly recommended to confirm the consistent efficacy of ginsenoside Rh1.

* Dao Ngoc Hien Tam and Duy Hieu Truong contributed equally to this work.

ABBREVIATIONS

AAPH	2,2'-azobis(2-amidinopropane hydrochloride)
ADP	adenosine 5'-diphosphate
aFABP	adipocyte fatty acid-binding protein
AP-1	activator protein 1
APV	(2R)-amino-5-phosphonovaleric acid
C/EBP	CCAAT/enhancer-binding protein
CCR2	chemokine receptor type 2
COX-2	cyclooxygenase
CXCL-10	C-X-C motif chemokine-10
CYPs	cytochromes
DMI	1 μ M dexamethasone, 0.5 mM methyl isobutyl-xanthine, and 0.28 unit/mL insulin
DPPH	2,2-diphenyl-1-picrylhydrazyl
DUSP1	dual specificity protein phosphatase 1
ERK	extracellular regulated protein kinases
fEPSPs	field excitatory postsynaptic potentials
fMLP	N-formylmethionyl-leucylphenylalanine
GJIC	gap junction-mediated intercellular communication
GRE	glucocorticoid response element
G-Rh1	ginsenoside Rh1
HEK	human embryonic kidney
HIV	human immunodeficiency virus
HP	<i>Helicobacter pylori</i>
HUVEC	human umbilical vein endothelial cells
IFN- γ	interferon- γ
Ig	immunoglobulin
IL	interleukin
JAK/STAT	Janus kinase/signal transducer and activator of transcription
JNK	c-Jun N-terminal kinase
LPAK	lymphokine and phytohemagglutinin activated killer
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MCP	monocyte chemoattractant protein
MMP	matrix metalloproteinase
m-TOR	mechanistic target of rapamycin
NF- κ B	nuclear factor- κ B
NMDA	N-methyl-D-aspartate
NO	nitric oxide
OVA	ovalbumin
PDK-1	phosphoinositide-dependent kinase-1
PI3K	phosphoinositide 3-kinase
PKB/Akt	protein kinase B
PMA	phorbol 12-myristate 13-acetate
PPAR- γ	peroxisome proliferator-activated receptor
ROS	reactive oxygen species
TNBS	2,4,6-trinitrobenzene sulfonic acid

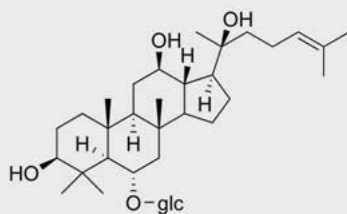
Introduction

For thousands of years, ginseng has been used as a tonic to invigorate the body and promote longevity, and as an adaptogen for mental and physical stress as well as fatigue in traditional herbal medicine [1,2]. It has increasingly achieved more popularity to become one of the most consumed herbal nutritional products worldwide with a total production volume of over 80 thousand tons and total sales of approximately \$2.1 billion in 2013 [3]. The global ginseng market is also predicted to grow at a compound annual growth rate of around 11.9% over the next decade and reach approximately \$7.51 billion by 2025 [4]. Ginseng is a common name for perennial plants of several species belonging to the genus *Panax* of the family Araliaceae, such as *Panax ginseng* C. A. Mey., Araliaceae (Asian ginseng), *Panax quinquefolius* L., Araliaceae (American ginseng), and *Panax vietnamensis* Ha et Grushv., Araliaceae (Vietnamese ginseng) [5]. With the intention of improving the stability and efficacy reasons, fresh ginseng is often processed by simple drying into white ginseng or steaming and drying into red ginseng. Red ginseng is considered to have a notably higher commercial value than fresh and white ginseng owing to significantly fewer side effects and higher biological effects [6, 7]. According to a clinical trial in premenopausal women, red ginseng has no significant side effect but one adverse event of mild gastric discomfort [8].

Ginsenosides have been found to be the major components of ginseng, which significantly contribute to its precious effects [9–11]. Based on their chemical structures, ginsenosides can be classified into three principal structural types, namely, protopanaxadiol, protopanaxatriol, and oleanane. More than 40 ginsenosides have been identified and isolated so far [12, 13]. Their nomenclature is based on the designation of Rx and Fx, where R and F refer to the origin of ginsenosides, namely, roots and leaves (“folia”) of the species, respectively, and x ($x = 0, a1, a2, b1, \text{etc.}$) denotes the order of ginsenosides eluted on thin-layer chromatograms starting with the most hydrophilic ones [14]. Among these compounds, G-Rh1 (► Fig. 1) has been found to be one of the main ginsenosides of red ginseng, compared to trace amounts in unprocessed ginseng [6, 15, 16]. Moreover, it has been found that protopanaxatriol-type ginsenosides are mainly hydrolyzed or metabolized to G-Rh1 in the gastrointestinal tract after oral administration of ginseng extracts [17–19]. This compound has been additionally reported to effectively stimulate the central nervous system and enhance mental acuity and intellectual performance [20]. Several studies have shown that G-Rh1 possesses neuroprotective effects, potential antineoplastic effects, and the ability to be adjuvant therapy in chronic inflammatory diseases to dexamethasone [21–23]. Nonetheless, there is no critically evaluated review of the pharmacological effects of G-Rh1 based on summarizing the current reliable evidence. Therefore, the purpose of our study was to systematically review the pharmacological actions of G-Rh1 in the published literature.

Search Strategy

We performed searches on eight electronic databases including MEDLINE (Pubmed), Scopus, Google Scholar, POPLINE, Global



► **Fig. 1** Chemical structure of 20(S)-G-Rh1.

Health Library (GHL), Virtual Health Library (VHL), the System for Information on Grey Literature in Europe (SIGLE), and the New York Academy of Medicine Grey Literature Report (NYAM) for original studies published up to August 22, 2015 using the defined search term for each database listed in **Table S1**, Supporting Information. The inclusion criteria were (i) G-Rh1 as a pure chemical compound alone or combined with other interventions, (ii) *in vitro* and *in vivo* studies on human and animal subjects, and (iii) no restriction on language, publication year, and study design. The exclusion criteria were (i) the whole ginseng extract, which contains many different ginsenosides and other components, as the intervention, (ii) book chapters, posters, conference papers, papers without full-text, review articles, and theses, and (iii) overlapped data sets or unreliable extracted data.

We also performed a manual search to obtain relevant papers that were not automatically found in selected databases. In this step, we screened references of full-text papers in the previous step in order to look for potential papers. These papers would be downloaded, read, and data extracted the same as the other papers. Through several steps, as illustrated in ► **Fig. 2**, we included 57 studies for our systematic review.

Anticancer Effects

Eleven studies mentioned the effects of G-Rh1 on anticancer activity *in vitro* (► **Table 1**). Almost all studies used MTT assays to identify cell viability (%). Kim et al. [24] examined the cytotoxic activity of G-Rh1 in mouse lymphoid neoplasma cell line (P388), human lung carcinoma (A549), and human cervix uterine adenocarcinoma (HeLa). The IC_{50} of G-Rh1 on P388 was 0.037 mM, indicating a potent cytotoxic effect on this cell line; meanwhile, these values were higher than 0.1 mM on the other two cell lines, signifying low cytotoxicity. In another study, Wang et al. [25] demonstrated that G-Rh1 could act as an adjuvant to enhance the ability of dendritic cells in stimulating the cytotoxic effects of LPAK in a papilla tumor cell line and L929 cell line through induction of the secretion of IL-12 p40 and transcription of IL-12 mRNA. In the papilla tumor cell line, G-Rh1 significantly improved the cytotoxicity effects of LPAK when the ratio of LPAK and tumor cells was 10:1, even at a concentration as low as 1 mg/L.

Regarding the antiproliferative activity, G-Rh1 was reported to have significant effects on NIH 3T3 mouse fibroblast cells by inhibiting phospholipase C and decreasing the intracellular level of diacylglycerol, which is an endogenous activator of protein

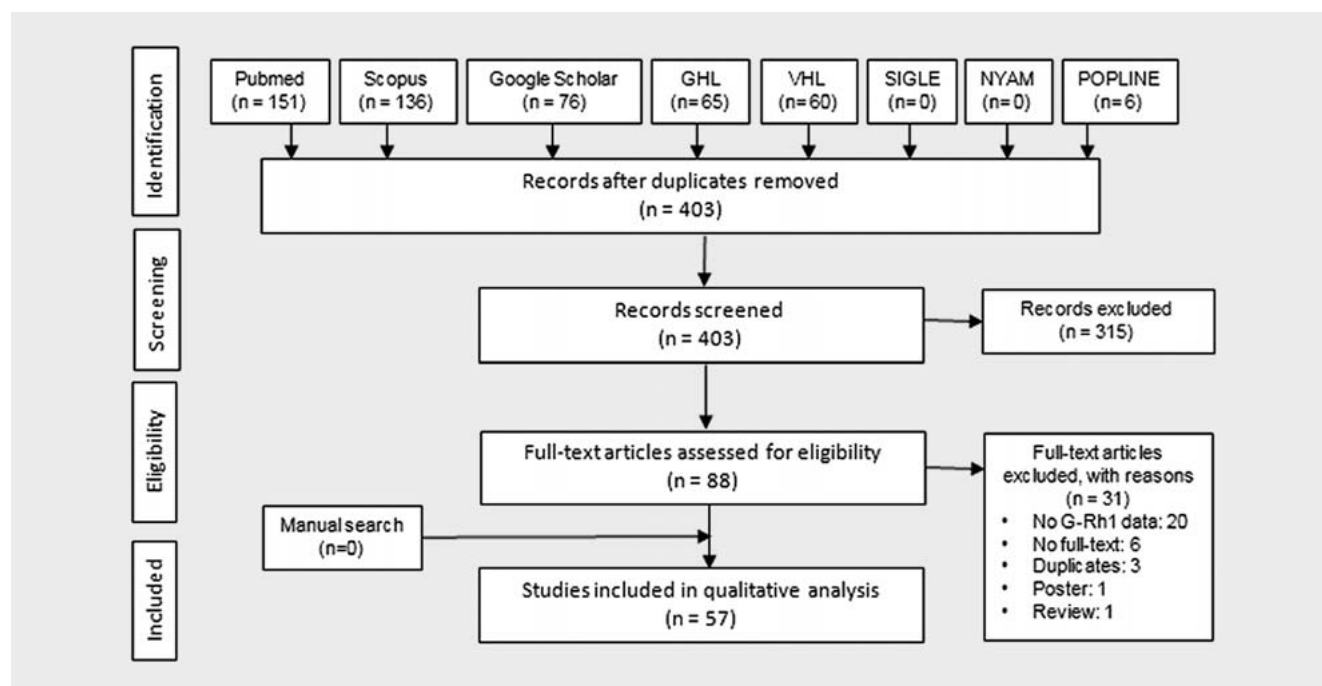
kinase C [26], and on a human acute monocytic leukemia suspension cell line (THP-1) by increasing apoptosis [27]. In contrast, this compound did not exert pronounced effects on the proliferation of human colon carcinoma (HCT-116), human liver carcinoma (HepG2), HeLa, human breast adenocarcinoma (MCF-7), human pancreatic cancer (PANC-1), human lung carcinoma (A549) [28], and B16 melanoma cells [29], even at the high concentration of 100 μ M. The antiproliferative effects of two stereoisomers were shown to be similar to each other [28]. Consistent with the previous study [24], G-Rh1 exhibited a weak cytotoxic effect on A549 and HeLa cell lines.

Yoon et al. [23] showed that G-Rh1 at concentrations of 50 and 100 μ M possessed significant antimetastatic properties in HepG2 cells stimulated by PMA via inhibiting MMP-1 transcriptional activity, reducing expression and stability of the AP-1 dimer, c-Fos and c-Jun, and inhibiting MAPK signaling pathways. The mentioned molecular mechanism is somewhat different from that in human astrogloma U87MG and U373MG cells in which G-Rh1 inhibits the invasion and migration of PMA-simulated U87MG cells by suppression of all three types of MAPKs (ERK, JNK, and p38) and DNA binding activities of transcription factors such as NF- κ B and AP-1 [30]. In this study, the authors observed a reduction by more than 90% in the invasiveness after 24 h treatment with 300 μ M G-Rh1. In the highly metastatic human fibrosarcoma cell line HT1080, G-Rh1 markedly inhibited the expression of MMP-9, but not MMP-2, the plasminogen activator inhibitor, and the urokinase-type plasminogen activator, suggesting that downregulation of MMP-9 significantly contributed to the anti-invasive effects of this compound [31]. The difference in mechanisms of anti-metastasis of G-Rh1 was supposed to be dependent on the cell type.

Lee et al. [32, 33] used F9 teratocarcinoma cells as a model to investigate the ability of ginsenosides to induce differentiation *in vitro* and the mechanism involved. They found that G-Rh1, along with ginsenoside Rh2, was the most effective differentiation agent in F9 teratocarcinoma cells regarding the induction of morphological change and marker gene expressions (i.e., laminin B1, type IV collagen) [32]. This was in agreement with a previous work of Odashima et al. [29] in B16 melanoma cells in which G-Rh1 stimulated the expression of the melanotic phenotype. In the later work of Lee et al. [33], they experimentally confirmed the involvement of a glucocorticoid receptor in the G-Rh1-induced differentiation process by inducing the nuclear translocation of the glucocorticoid receptor to the nucleus and inducing the GRE transactivation.

In brief, G-Rh1 exerted its cytotoxic effect on three specific cell lines, namely, P388, THP-1, and NIH 3T3 fibroblast cells in a concentration-dependent manner. With regard to the HepG2 cell line, G-Rh1 had no significant antiproliferation ability even with a very high concentration (80–200 μ M) compared with the other cell lines. The slight cytotoxic effect was seen only with 200 μ M and 48 h for incubation.

No study presents the cytotoxicity of G-Rh1 on non-tumoral cell lines, therefore, we could not draw any conclusion about its effects on these cell lines.



► **Fig. 2** The flowchart explaining the process of searching in eight databases, removing duplicates, screening, and qualitative analysis. GHL: Global Health Library, VHL: Virtual Health Library, SIGLE: Systems for Information of Grey Literature in Europe, NYAM: New York Academy of Medicine.

Immunomodulatory Effects

G-Rh1 has been proven to possess immunomodulatory effects mainly on the inflammatory response on a variety of cell lines *in vitro* by different mechanisms (► **Table 2**). He et al. [34] reported no significant effect of G-Rh1 in the range of 0.1–10 μM on the activity of NF- κB luciferase reporter activity in human embryonic kidney 293 (HEK 293) cells induced by TNF- α (10 ng/mL). However, significant inhibition of NF- κB activation by G-Rh1 was also reported in HEK 293 cells pretreated with TNF- α [35], in BV2 glioma cells induced by LPS [36] or IFN- γ [37], in macrophages stimulated by LPS or TNBS [38], and in murine macrophage RAW 264.7 cells treated with LPS [39] or TNF- α [22]. Both He et al. [34] and Xing et al. [35] assessed the protective effect of G-Rh1 on the activation of NF- κB on HEK 293 cells induced by TNF- α . However, G-Rh1 seemed not to inhibit NF- κB activation effectively when the higher concentration of TNF- α was used as the stimuli. In addition, G-Rh1 markedly suppressed the CXCL-10 expression in TNF- α -induced human promonocytic U937 cells, which may be related to the inactivation of the ERK1/2 signaling pathway [40]. In THP-1 cells, G-Rh1 did not only inactivate ERK1/2, but also attenuated the phosphorylation and activation of MAPK signaling, p38MAPK and JNK activation of PKB/Akt through lowering the expression of MCP-1 and CCR2, and deactivating integrins, the fibronectin receptor VLA-5 and CD29 [21]. Li et al. [22] found that after long-term dexamethasone treatment in RAW264.7 cells, it also induced the activation of DUSP1, resulting in the downregulation of proinflammatory cytokines (IL-6, IL-17, MMP-1, TNF- α). On THP-1 cells, the effects seemed to depend on the concentrations of G-Rh1 and stimuli, namely, LPS and PMA [41]. G-Rh1 significantly

increased the production of TNF- α and its mRNA expression in the presence of LPS at the high dose of 100 mg/L. In contrast, the production of IL-8 and IL-1 α only increased at the low dose of LPS (10 mg/L) with 1 mg/L and 100 mg/L of G-Rh1, respectively [41]. Gu et al. [42] showed that G-Rh1 dose-dependently inhibited the expression of PPAR- γ , C/EBP- α , and aFABP in DMI-stimulated 3T3-L1 adipocytes, thereby, inhibiting adipocyte differentiation and inflammation. In LPS-stimulated RAW 264.7 cells, Park et al. [39] observed a significant reduction in NO synthesis, inducible NO synthase activity, and prostaglandin E2 production at the concentrations of 50 μM and 100 μM of G-Rh1. G-Rh1 was also found to markedly inhibit NO production in rIFN- γ plus LPS-stimulated macrophages [43].

The immunomodulatory effects were also demonstrated by *in vivo* experiments on various animal models with chemical-induced inflammatory diseases. After oral administration at a dose of 20 mg/kg, G-Rh1 inhibited colon shortening, lowered myeloperoxidase activity, and suppressed the expression of IL-1 β , IL-17, and TNF- α in mice with TNBS-induced colitis [38], suppressed body weight gain, epididymal fat weight, and plasma triglyceride levels in high-fat diet-induced obese mice [42], as well as suppressed ear swellings and ear weight, and decreased IL-6 in the serum of hairless mice with atopic dermatitis-like skin lesions [44]. Gu et al. [42] indicated the effective attenuation of body weight gain, epididymal fat weight, and plasma triglyceride levels in obese mice. Nevertheless, relevant baseline characteristics (e.g., initial body weight, triglyceride level) or equivalent characteristics between control groups and intervention groups were not presented. Oral administration of G-Rh1 at different doses of 10 mg/kg and 25 mg/kg markedly suppressed the expression of cytokines

► **Table 1** Summary of anticancer activities of G-Rh1.

Experimental model	Method	Effects	Mechanism	Ref.
HepG2 human hepatocellular carcinoma cells	XTT assay	Slight cytotoxicity (at 200 mM for 48 h)		Yoon et al. [23]
	Cell-matrix adhesion assay	Significant inhibition of invasion and migration	Inhibit MMP-1 transcriptional activity, reduce expression and stability of the AP-1 dimer, c-Fos and c-Jun, and inhibit MAPK signaling pathways	
P388 mouse lymphoid neoplasma cells, A549 human lung carcinoma, HeLa human cervix uterine adenocarcinoma cells	MTT assay	Potent cytotoxic effect on P388 cells; low cytotoxic effect on A549 and HeLa cells		Kim et al. [24]
Papilla tumor cell line, L929 cell line	Neural red staining assay	Enhance the ability of dendritic cells (DC) to stimulate the cytotoxic activity of dendritic cell lymphokine- and phytohemagglutinin-activated killer (DC-LPAK)		Wang et al. [25]
NIH 3T3 fibroblast cells	Measurement of replicative DNA synthesis and intracellular DAG contents, protein kinase C assay, phospholipase C assay	Antiproliferative effect	Inhibit phospholipase C, which produces second messengers necessary for the activation of protein kinase C	Byun et al. [26]
THP-1 human leukemia cells	MTT assay; flow cytometry	Antiproliferative effect, proapoptotic effect		Popovich and Kitts [27]
HCT-116 human colon carcinoma cells, HepG2 human hepatocellular carcinoma cells, HeLa cervical cancer cells, MCF-7 breast cancer cells, A549 human lung carcinoma cells, PANC-1 human pancreatic carcinoma cells	MTT assay	Did not show remarkably antiproliferation ability in these cell lines		Quan et al. [28]
B16 melanoma cells	Assay of growth inhibition	Did not show remarkably antiproliferation ability in these cell lines		Odashima et al. [29]
HT1080 fibrosarcoma cells	<i>In vitro</i> invasion assay	Anti-invasive effect		Park et al. [31]
F9 teratocarcinoma cells	Phase-contrast microscopy, electrophoretic mobility shift assay, transient transfection assay	Cause the differentiation of F9 cells	Form a complex with steroid receptors, which might regulate the expression of proteins that have an important role in the differentiation process of F9 cells	Lee et al. [32]
F9 teratocarcinoma cells	Immunolocalization	Induce the differentiation of F9 cells	Stimulating the nuclear translocation of GR	Lee et al. [33]
U87MG and U373MG human astrogloma cells	Matrigel invasion assay, wound healing assay	Inhibit the invasion and migration of U87MG and U373MG glioma cells	Suppress all three types of MAPKs (ERK, JNK, and p38) and DNA-binding activities of transcription factors such as NF- κ B and AP-1	Jung et al. [30]

such as TNF- α and IL-4 compared to a histamine-induced control in mice by inhibiting the activation of transcription factors NF- κ B and c-jun (AP-1) in histamine-induced skin tissues [45]. It remains ambiguous on the role of G-Rh1 in the regulation of the Th1/Th2 balance through controlling the expressions of IFN- γ and IL-4. Zheng et al. [44] showed that oral administration of 20 mg/kg/

day G-Rh1 in mice upregulated the expression of IFN- γ and Foxp3, but did not reduce IL-4 levels. These results suggest that the improved symptoms of skin lesions were partly related to the reduction of IgE, which might have come from the upregulation of IFN- γ expression. However, this contradicted to the later work of Feng et al. [46] in which they reported that G-Rh1 decreased IFN- γ mRNA

► **Table 2** Summary of anti-inflammatory activities of G-Rh1.

Experimental model	Method	Mechanism	Ref.
THP-1 cells	ELISA		Choi et al. [21]
RAW 264.7 cells	Proinflammatory mediators determination assays	Repressed the expression of IL-6, IL-17, MMP-1, and TNF- α	Li et al. [22]
HEK293 Human embryonic kidney cells	NF- κ B activity luciferase reporter assay		Xing et al. [35]
RAW 264.7 cells	Western blot analysis	Suppressed the factors that were associated with the inflammatory processes (NO production, COX-2 protein, PEG2 expression, iNOS protein, iNOS activity, and the activation of NF- κ B transcription factor)	Park et al. [39]
U937 Human promonocytic cells	RT-PCR	Significantly inhibited TNF- α -induced CXCL-10 expression	Lee et al. [40]
THP-1 cells	Radioimmunoassay, RT-PCR		Wang et al. [41]
Male C57BL/6J mice	RT-PCR	Rh1 suppressed inflammatory factors (TNF- α , IL-1 β , IL-6) in the blood of obese mice	Gu et al. [42]
Thioglycollate-elicited macrophage	Griess	Markedly reduced the production of NO	Park et al. [43]
Female hairless mice	ELISA, RT-PCR	Reduced IgE and IL-6 levels in peripheral blood, increased Foxp3 expression, and inhibited the inflammation in skin regions	Zheng et al. [44]
Male ICR mice	Ear thickness	Potently suppressed TPA- and oxazolone-induced swelling	Shin et al. [49]
Murine BV2 microglia cells	RT-PCR	Suppressed proinflammatory cytokines (TNF- α , IL-6, MCP-1), COX-2, MCP-1, MMP-3, and MMP-9, and enhanced anti-inflammatory cytokines (IL-10)	Jung et al. [50]
Male C57BL/6 mice	Immunofluorescence	G-Rh1 markedly decreased the number of OX-42-positive activated cells in the cortex, hippocampus, and substantia nigra of the brain that was inflamed by LPS	

and increased IL-4 mRNA levels. In this study, they also found that potential G-Rh1 (25 mg/kg/day intraperitoneally) in combination with dexamethasone (1 mg/kg/day) significantly decreased proteinuria levels and the levels of anti-dsDNA and anti-ANA autoantibodies compared to those of dexamethasone alone in MRL/lpr mice, suggesting that G-Rh1 might potentiate the effects of dexamethasone in the treatment of systemic lupus erythematosus [46]. As for other immunoglobulins, G-Rh1 was shown to significantly enhance OVA-specific IgG, IgG1, IgG2a, and IgG2b antibody levels in OVA-immunized mice compared to the OVA group [47]. Besides, G-Rh1 was shown to significantly inhibit the IgE-dependent passive cutaneous anaphylaxis reaction in mice after administered 25 mg/kg orally or intraperitoneally [39]. In systemic anaphylactic shock model induced by 8 mg/kg of compound 48/80, pretreatment of G-Rh1 (5 mg/kg) considerably lowered the overall mortality rate [48]. Intraperitoneal administration of a higher dose of 50 mg/kg could significantly reduce the number of activated OX-42-positive cells in the cortex, hippocampus, and substantia nigra of the brain, as well as lower the immune reactivity of Iba1 and IL-1 β expression in the cortex [36]. Topically applied 0.01 and

0.05% G-Rh1 potently reduced the ear thickness in 12-O-tetradecanoylphorbol-13-acetate- and oxazolone-induced mouse dermatitis models and lowered the expression levels of COX-2, IL-1 β , and TNF- α [49]. When combined with dexamethasone, G-Rh1 (10 mg/kg) showed superior anti-inflammatory potential with a lower mean clinical severity in comparison with dexamethasone alone in collagen-induced arthritis mouse model [22].

Antioxidant Effects

The antioxidant effects of G-Rh1 are summarized in ► **Table 3**. It was shown that pretreatment with G-Rh1 at the concentrations of 100 μ M and 300 μ M for 1 h significantly suppressed ROS production in BV2 cells stimulated by LPS (0.1 μ g/mL) [50] or IFN- γ (100 U/mL) [37]. On cardiomyocytes from Wistar rats, G-Rh1 (30 μ M) decreased the free radical content of cardiomyocytes by more than 50% after incubation with xanthine-xanthine oxidase for 5 days [51]. Using the 2',7'-dichlorofluorescein diacetate assay, the intracellular ROS scavenging activity of G-Rh1 was determined to be 45% at 10 μ M compared to 90% in the case of the same dose

► **Table 3** Summary of antioxidant activities of G-Rh1.

Experimental model	Method	Effects	Mechanism	Ref.
BV2 microglial cell	iNOS reporter gene assay	Antioxidant	Significantly suppressed IFN- γ -induced iNOS promoter activity by inhibiting DNA binding of several transcription factors, such as NF- κ B, IRF-1, and STAT1; inhibited the phosphorylation of JAK1, STAT1, STAT3, and ERK	Jung et al. [36]
BV2 microglial cell	Western blot analysis	Antioxidant	Induced activation of the cAMP response element binding protein (CREB)	Jung et al. [50]
Myocardocyte	Spin number	Antioxidant	Antagonized the increase of free radical content induced by xanthine or xanthine oxidase	Jiang et al. [51]
V79-4 Chinese hamster lung fibroblast cells	2', 7', -Dichlorofluorescein diacetate method	Antioxidant	Scavenged reactive oxygen species	Chae et al. [52]
Human polymorphonuclear leukocytes	Superoxide generation	Antioxidant	Suppressed stimulus-induced superoxide generation of neutrophils	He et al. [53]
Human erythrocyte	Hemolysis assay	Prooxidant, but synergistic antioxidative interaction with α -tocopherol	Not defined	Liu et al. [54]
Human erythrocyte	Hemolysis assay	Antioxidant	Not defined	Liu et al. [55]
Human erythrocyte	Hemolysis assay	Antioxidant	Not defined	Li et al. [56]
Human erythrocyte	SH group content in erythrocyte membrane	Antioxidant	Not defined	Samukawa et al. [57]

of N-acetylcysteine as a positive control [52]. He et al. [53] compared the inhibitory effects of five ginsenosides (Rb1, Rb2, Rd, Rh1, Rh2) on superoxide generation induced by fMLP, PMA, and arachidonic acid in human neutrophils. They found that G-Rh1 slightly suppressed PMA- and arachidonic acid-induced superoxide generation, but strongly inhibited the fMLP-stimulated process at a higher extent than the four other ginsenosides. Moreover, it exhibited almost no effect on the lipid peroxidation level concentrations up to 200 μ M, suggesting the mechanism of action is to suppress stimulus-induced superoxide generation of neutrophils rather than scavenge generated free radicals. They also performed experiments with the DPPH assay and confirmed that G-Rh1 did not possess a scavenging effect. To explain this, Chae et al. [52] supports that the DDPH assay is not an appropriate method to identify antioxidant effects of ginsenosides because they are not electron-rich compounds to donate their electron to DPPH. Their antioxidant ability should be examined through measuring the activity of free radicals, hydroxyl, ROS, etc.

Although Jung et al. [37] measured intracellular ROS production, the used concentration was extremely higher than in the other ones. This is because they aimed to investigate a neuroprotective effect of G-Rh1 and its mechanism of action. In addition, ROS plays a role as a messenger of the inflammatory process. Therefore, they chose concentrations of G-Rh1 according to the minimum effective concentrations causing the other effects.

Interestingly, Liu et al. [54] found that G-Rh1 was a prooxidant when the lag time of hemolysis decreased at high concentrations of G-Rh1 (i.e., 10–25 μ M), but they observed its synergistic antioxidative properties with α -tocopherol using an AAPH-induced

hemolysis model on human erythrocytes. The same group of authors, however, concluded that G-Rh1 was an antioxidant when it decreased the hemolysis percentages in another study [55]. They supposed that this difference might come from whether glucose was present in the phosphate buffered saline systems or not. Using the hemolysis test, its antioxidative activity could be expressed by the linear equation $H = -0.703C + 83.1$, in which H and C referred to the hemolysis percentage and its concentration, respectively [55]. Its antioxidative activity was further confirmed by two other researches [56, 57]. Li et al. [56] showed the hemolysis percentage reduced to $74 \pm 11\%$ at 20 μ M G-Rh1 compared to 100% in the case of the control in hemin-induced hemolysis. One study stated that G-Rh1 significantly attenuated the decrease in thiol groups of band 3 protein in the erythrocyte membrane by inhibiting the oxidation of these groups in the cysteine residues [57]. Considering band 3 protein as a structural protein determining the stability and flexibility of erythrocytes, treatment with G-Rh1 could protect the rheological functions of erythrocytes. Unexpectedly, the authors showed that G-Rh1 did not exhibit the antioxidant effect using 2-methyl-6-methoxy phenylethynylimidazopyrazynone. The reason was probably due to the liposolubility of G-Rh1 as it could not display the effect in the aqueous phase of the 2-methyl-6-methoxy phenylethynylimidazopyrazynone reaction kit.

Effect on Nervous Systems

By the passive avoidance test, pretreatment of G-Rh1 at doses of 5 and 10 mg/kg was observed to significantly improve the learning

and memory in mice with scopolamine-induced memory impairment [58] or saline-treated mice [59]. In the Morris water maze test, G-Rh1 (10 mg/kg) markedly decreased the escape latency, and increased the number of crosses and the time spent in the platform, thereby improving the spatial learning ability [59]. Moreover, Wang et al. [58] found that the pain threshold was not affected in the tail-flick test, confirming the nootropic effect of G-Rh1.

Lee et al. [60] compared the effects of seven ginsenosides on neural activity by measuring changes in the slope of fEPSPs. At the concentration of 100 $\mu\text{g}/\text{mL}$, G-Rh1 was moderately effective ($46.1 \pm 6.7\%$) in suppressing fEPSPs. The complete abolition of fEPSPs could be observed in the case of the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione, but not the NMDA receptor antagonist (2R)-amino-5-phosphonovaleric acid. This might suggest that G-Rh1 exerted its effects by regulating the non-NMDA receptor-mediated synaptic activity.

The neuroprotective effects of G-Rh1 were reported on neuroblastoma SH-SY5Y cells and pheochromocytoma PC-12 cells [20]. G-Rh1 at the concentrations of 10 and 20 μM significantly attenuated the toxicity on SH-SY5Y cells exposed to 60 μM of six-hydroxydopamine. This was partially because of the reduction of ERK1/2 phosphorylation by G-Rh1 pretreatment, as could be seen in other cell lines such as HepG2, U87MG, U937, and THP-1 cells [21, 23, 30, 40]. G-Rh1 increased the percentage of PC-12 cells with neurites compared to that of the control. However, no apparent difference was observed between the two stereoisomers 20(S)-G-Rh1 and 20(R)-G-Rh1, suggesting the C-20 stereochemistry may not have a role in the neuroprotective effect.

Estrogenic Activity

Three papers reported estrogenic activity of G-Rh1 *in vitro* using the human breast cancer MCF-7 cell line. Dong et al. [61] observed that G-Rh1 stimulated cell proliferation in a dose-dependent manner, reaching a significant level at 100 μM with a significant correlation ($r = 0.218$, $p < 0.05$) between G-Rh1 and 17 β -estradiol in the expression profiles after treatment of MCF-7 cells with these two compounds. Lee et al. [62] demonstrated that G-Rh1 bound to and activated the estrogen receptor at an extent of 5000- to 10 000-fold weaker than that of 17 β -estradiol. In the work of Bae et al. [63], the proliferation of MCF-7 cells increased 2.1-fold at the concentration of 1 μM of G-Rh1 to reach the same level as 1 nM of 17 β -estradiol. The expressions of *c-fos* and *pS2* genes were also slightly induced in the presence of G-Rh1, as previously reported [62]. Taken together, these data consistently demonstrated G-Rh1 as a weak estrogenic compound.

Other Pharmacological Effects

Four studies investigating the enzymatic activities of G-Rh1 have been performed. Liu et al. [64] examined the effects of G-Rh1 on human CYPs. They witnessed the moderate inhibition on CYP3A4 (IC_{50} of $76.9 \pm 6.8 \mu\text{M}$) by competitive inhibition of testosterone 6 β -hydroxylation in cDNA-expressed CYP3A4 and weak stimulation on CYP2E1. However, no significant effects were observed on CYP1A2, CYP2A6, CYP2A9, and CYP2D6, even at 100 μM .

Etheridge et al. [65] found G-Rh1 ranging from 1 to 10 μM did not affect the activities of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and testosterone 6 β -hydroxylase. At the concentration of 10 μM , G-Rh1 suppressed midazolam 1-hydroxylase activity by 54% and increased P-glycoprotein ATPase activity to 17.2 nmol P_i/mg protein/min, but it exerted negligible effects on these at the low concentration of 1 μM . In contrast, Li et al. [66] found weak inhibitory activity of G-Rh1 on P-glycoprotein by 8.94% at 75 μM using a transport assay on Caco-2 cells. In agreement with the two abovementioned works, G-Rh1 was confirmed as a CYP3A4 inhibitor owing to 15% suppression of midazolam metabolism at 10 μM . In another enzymatic system in 3T3-L1 adipocytes, G-Rh1 at the concentration of 100 $\mu\text{g}/\text{mL}$ enhanced the lipoprotein lipase activity in the medium by 32%, whereas it exerted no effect on cellular lipase activity [67].

The anti-microorganism activity of Rh1 was mentioned in two *in vitro* studies. Bae et al. [68] showed that G-Rh1 did not inhibit the growth of four different strains of HP, even at the high concentration of 100 $\mu\text{g}/\text{mL}$. However, G-Rh1 (1 mM) did show weak inhibition of HP urease and stomach H^+/K^+ ATPase by 5 and 15%, respectively. Jeong et al. [69] examined the anti-HIV activity of G-Rh1 by its effects against cytoprotection on Tat-expressing CHME5 cells and HIV infected macrophages. G-Rh1 increased cell death in the presence of LPS/cycloheximide dose-dependently, although G-Rh1 or LPS/cycloheximide alone did not affect it significantly. The mechanism was supposed to inhibit Akt, glycogen synthase kinase 3 β , m-TOR, PDK-1 phosphorylation, and BAD activation in the PI3K/Akt pathway. The synergistic anti-cytoprotective effects of G-Rh1 and miltefosine (5 or 10 μM), which is a PI3K/Akt phosphorylation inhibitor used for treatment of HIV-1 dementia, on Tat-transduced CHME5 cells were also observed.

The cardiovascular effects of G-Rh1 were reported in three studies. G-Rh1 (60 $\mu\text{mol}/\text{L}$) affected the basic functions of myocardiocytes by reducing the open times, increasing the close times, and reducing the open-state probabilities of all three types (i.e., B, L, and T) of calcium channels [51]. These effects were slightly different to those of two calcium antagonists, verapamil and Bay K 8644, in which these positive controls showed no activity on the T-type calcium channel. In the work of Gai et al. [70], pretreatment of G-Rh1 at the dose of 10 mg/kg for 7 days decreased the activity of CK-MB and troponin T level, and significantly alleviated the increase of left ventricular end-diastolic pressure and the decrease of left ventricular systolic pressure and $\pm \text{dp}/\text{dt}$ in isoproterenol-induced myocardial infarcted rats. This might suggest that G-Rh1 could ameliorate heart function impairment. Lee et al. [71] reported that G-Rh1 at the nontoxic dose of 25 μM exhibited marked attenuation of the monocyte adhesion of HUVEC exposed to 10 ng/mL of TNF- α . G-Rh1 also substantially inhibited adhesion molecules such as VCAM-1, ICAM-1, and E-selectin in TNF- α -stimulated HUVEC, and suppressed the induction of $\alpha 4/\beta 1$ integrin VLA-4 and $\alpha 4/\beta 2$ integrin LFA-1 in TNF- α -stimulated THP-1 monocytes. However, G-Rh1 did not disturb the NF- κB signaling pathway; therefore, the authors supposed that the anti-atherogenic activity of G-Rh1 related to the monocyte adhesion to activated endothelium may be JAK/STAT and/or ERK responsive as Jung et al. [37] mentioned previously.

The cholesterol-like structure of G-Rh1 inspired Lee et al. [72] to explore whether G-Rh1 could substitute cholesterol in the growth of *Caenorhabditis elegans*. These nematodes were fed in medium with cholesterol, without cholesterol, or cholesterol-deprived medium supplemented G-Rh1, and the growth rate (i.e., time for eggs to reach adults) was appraised. In the absence of cholesterol, worms grew slowly, expressing that the growth rate of worms from eggs to young adults or from young adults to adults were greatly retarded in both the F1 and F2 generations. Nevertheless, adding G-Rh1 into cholesterol-deprived medium could improve in F1 generation, not only the growth rate of all stages but also the worm length. In F2 generation, the addition of G-Rh1 into the cholesterol abolished medium could not recover the growth rate, only the worm length was recovered.

Tachikawa et al. [73] studied the inhibitory effect of ginsenosides on catecholamine secretion from bovine adrenal chromaffin cells. Upon stimulation by 50 μM of acetylcholine, G-Rh1 showed intermediate inhibition of 39% in comparison with the most potent inhibitor of ginsenoside Rg2 with 72% at the same concentration of 10 μM .

Using mouse osteoblastic MC3T3-E1 cell line as an *in vitro* model, Siddiqi et al. [74] showed that this compound possessed osteoblast differentiation and osteogenic stimulatory activity. G-Rh1 in the range of 1–300 μM significantly stimulated the osteoblastic MC3T3-E1 cell viability, and improved cell differentiation and mineralization, which was extended for up to 28 days. The mechanism of action was determined to be due to the upregulation of osteogenic markers such as alkaline phosphatase, type-I collagen, and osteocalcin via bone morphogenetic protein 2/Runt-related gene 2 signaling pathways.

Matsuda and colleagues [75] tested the effects of two stereoisomers (20S, 20R) of G-Rh1 on blood platelet aggregation induced by collagen or ADP and conversion of fibrinogen to fibrin. They found that 20(R)-G-Rh1 showed a higher inhibitory activity on blood platelet aggregation than the 20(S)-G-Rh1 counterpart. In contrast, the trend was reversed on conversion of fibrinogen to fibrin when 20(S)-G-Rh1 required a longer time to clot. The effects of two abovementioned isomers on GJIC, which plays a crucial role in the many complex cellular processes, was investigated by Zhang et al. [76]. Both isomers significantly suppressed the GJIC function at a concentration of 10 μM , but the mechanism seemed to be different. 20(S)-G-Rh1 inhibited GJIC via tyrosine kinase activation, whilst GJIC reductions induced by 20(R)-G-Rh1 were owing to both tyrosine kinase and protein kinase C.

Methodological Quality of Studies

The methodological quality of included animal studies was assessed (► **Table 4**) by using criteria from SYRCLE's tool [77]. This tool contains 10 items: sequence generation (selection bias), baseline characteristics (selection bias), allocation concealment (selection bias), random housing (performance bias), blinding (performance bias), random outcome assessment (detection bias), blinding (detection bias), incomplete outcome data (attrition bias), selective outcome reporting (reporting bias), and other sources of bias (other bias). Risks of bias of each item were detected by answering some questions in relation to the method.

The symbol “√” shows a low risk of bias, symbol “×” shows a high risk of bias, and symbol “?” shows an unclear risk of bias. We only judged and reported the levels of risks of bias for each item through the response of individual questions. There was no general conclusion for quality of the whole study. There are six studies that claimed the animals were divided randomly [22,44,50,58,59,70]. However, no study presented any method to generate the allocation sequence.

Regarding the baseline characteristic item, seven studies showed the balance of relevant baseline characteristics between the control group and the intervention group [38,45,47,48,50,58,59]. No study noted any adjustment if the unequal distribution of some relevant baseline characteristics in analysis had existed. In addition, the time to induce disease of all studies was inadequate.

Some signals indicated an unclear risk of bias, such as no report of the allocation concealment and the random outcome assessment. Moreover, only one study reported the number of animals in analysis while the rest did not mention this [46]. In consequence, attrition bias of almost studies are unclear.

The blinding process for investigators and assessors was even not ensured. Nevertheless, only one study showed that its outcome would be biased due to not complying with this process [58]. We determined this outcome was due to their measurement being dependent on a subjective awareness of the assessors.

Although all of the authors did not give information whether they placed the cages or animals randomly in the room/facility, we made a consensus that the results were not skewed by the non-randomization because all studies followed a standard guideline. There is no protocol available, but all outcomes mentioned in the results part are the same compared with the methods in the publication.

With regard to other biases, all studies presented that contamination and design-specific risks of bias were absent. Four trials provided information about conflicts of interest [38,45,46,70]. Finally, we have no details to verify unit of analysis errors and whether new animals had been added to replace dropouts from the original population.

Discussion

The present study demonstrated that G-Rh1 exhibited a wide range of pharmacological effects including anti-inflammation, antioxidation, immunomodulation, and positive effects on cognitive functions. This could partly elucidate some of the precious activities of ginseng on cardiovascular risk factors [78], hypertension [79], Alzheimer's disease [80], and cognitive function [81]. Together with the low toxicity in normal cells or in animal models [47,53,82] as well as high liposolubility and high permeability through the blood brain barrier, G-Rh1 could be a compound of interest when developing a therapeutic regime for treatment of central nervous system diseases [20,50,58,59].

G-Rh1 expressed its antioxidant effects by reducing ROS production or suppressing superoxide generation in neutrophils stimulated by H_2O_2 , LPS, IFN- γ , or xanthine-xanthine oxidase [37,50–53]. Nevertheless, there have been some inconsistencies in the reporting of its effects on erythrocytes, even from the same group of authors [39,54–56]. Only one out of four demonstrated G-Rh1

► **Table 4** Risk of bias for animal studies assessed by the SYRCLÉ's tool.

References	1		2		3		4		5		6		7		8			9			10				
	Selection bias 1		Selection bias 2		Selection bias 3		Performance bias 1		Performance bias 2		Detection bias 1		Detection bias 2		Attrition bias			Reporting bias			Other sources of bias				
	a	b	c	a	b	a	b	a	b	a	b	a	b	a	b	c	d	a	b	c	a	b	c	d	e
Jang et al. [45]	x	?	√	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Lee et al. [38]	x	?	√	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Jung et al. [50]	?	?	√	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Gai et al. [70]	?	x	√	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Gu et al. [42]	x	x	√	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Feng et al. [46]	x	x	√	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Park et al. [39]	x	x	√	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Li et al. [22]	?	x	√	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Wang et al. [58]	?	√	?	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Kim et al. [48]	x	√	?	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Hou et al. [59]	?	√	?	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Zheng et al. [44]	?	x	√	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?
Sun et al. [47]	x	√	?	?	?	?	?	?	x	?	?	?	x	?	?	?	?	x	?	?	?	?	?	?	?

1. x: No evidence of randomization. ? : Randomization without details; 2.a.√: Relevant baseline characteristics were balanced out in the control and investigation groups. x: Relevant baseline characteristics were not given; 2.b. x: Relevant baseline characteristics not given. ? : No report in the analysis about adjustments for the unequal distribution of some relevant baseline characteristics; 2.c. √: Adequate time to induce disease, 3. ? : No report of the adequately concealment of groups, 4.a. ? : No report of cage or animal arrangements; 4.b. √: The outcome measurement was not influenced by the randomization of cages or animals; 5. x: No insurance for blinding caregivers and investigators; 6. ? : No report about whether selected animals were picked randomly for the outcome assessment; 7.a. x: No insurance for blinding the outcome assessor; 7.b. √: Consensus that the outcomes were not influenced by lack of blinding. x: Consensus that the outcomes were influenced by lack of blinding; 8.a. √: The number of included animals for analysis was reported the same as for the investigation. ? : No report for the number of included animals for analysis; 8.b. √: No missing outcome data. ? : No report whether missing outcome data was imputed or not; 8.d. √: No missing outcome data. ? : No report whether missing outcome data was imputed using appropriate methods; 9.a. x: No available protocol; 9.b. √: Presented all of the outcomes mentioned in the methods part; 10.a. √: No contamination existed; 10.b. √: Conflict of interest was claimed. ? : No report of conflict of interest; 10.c. ? : No details to identify unit of analysis errors; 10.d. √: No design-specific risks of bias; 10.e. ? : No report of new animals addition to replace dropouts from the original population.

as a prooxidant [54], which was explained later as the presence of glucose in the phosphate buffered saline somehow affected the prooxidant or antioxidant activities of G-Rh1 on AAPH-induced hemolysis [55].

Some studies showed that different stereoisomers of the same ginsenoside, i.e., 20(R) and 20(S) ginsenoside, may have different pharmacological effects [83,84]. Our study showed that the stereochemistry at C-20 of ginsenoside Rh1 did not affect the neuroprotective and antiproliferative activities [20,60], but might have some effects on blood platelet aggregation and GJIC [75,76].

Regarding the significant role of G-Rh1, knowledge of its pharmacokinetic profile and bioavailability would be a topic of interest to understand the pharmacology of ginseng and develop carrier delivery systems for bioavailability enhancement. By using the ADMET (absorption, distribution, metabolism, excretion, and toxicity) model, G-Rh1 was predicted to have favorable aqueous solubility and oral absorption in the human gastrointestinal tract [82]. However, this contradicted the results of a previous study by Lai et al. [85], which investigated the pharmacokinetic parameters and bioavailability of G-Rh1 using intravenous and intragastrical administrations in Sprague-Dawley rats. They found that this compound exhibited extremely poor absolute bioavailability of about 1% and rapid clearance with a short elimination half-life as can be seen in other protopanaxatriol ginsenosides [86]. The phenomenon might be partly explained by its main metabolic pathways, including CYP450-catalyzed mono-oxygenation, the intestinal bacteria mediated deglycosylation, and the gastric acid mediated hydration reaction [85]. To overcome this problem, Yang et al. [87] encapsulated G-Rh1 into self-microemulsions comprising P-pg and/or CYP450 inhibitory excipients. They found that the bioavailability of the formulation containing both excipients was significantly higher than those of the formulation containing P-gp inhibitory excipient alone or the free drug, confirming the CYP450-mediated metabolism of G-Rh1. Additionally, in order to treat various chronic diseases, many nanodelivery platforms have been fabricated to improve the bioavailability of ginsenosides from ginseng, such as liposomes, mixed micelles, and poly(lactic-co-glycolic acid) nanoparticles [9], which might also be applied to G-Rh1.

We choose SYRCL's risk of bias tool to assess quality of included studies' methods because of various benefits. To reduce risks of bias for these studies, the randomization of the allocation sequence should be applied. A method of generation apparently needs to be reported. The blinding of investigators and assessors and allocation concealment also need to be guaranteed to reach reliable outcomes. Moreover, we realize that relevant baseline characteristics were not identified before starting experiments. The balance of baseline characteristics between the control and experimented groups, thus, was not verified. Consequently, it would influence the results. We recommend that the authors should report clearly the numbers of included animals in experiments and the number of animals in analysis. If there is any addition or exclusion, it should be noted with suitable reasons. On the other hand, we also agree with Hooijmans et al. [77] that the registration of all animal studies should be more common and their protocols should be published in accessible databases with the hope of improving the standards of these studies. Thereby, further studies in humans would be more accurate.

One of our study's limitations is that we missed data from other popular databases. Nevertheless, we searched many reliable databases and included a large number of articles for the qualitative analysis. We strongly believe, therefore, that our results cover nearly sufficient biological properties of G-Rh1 as above. An updated version, in the future, is obviously necessary when more studies are published.

In general, G-Rh1 has significant potential activity for anti-inflammatory and antioxidant effects. Besides, G-Rh1 has been reported to exert several positive effects on the nervous system, which potentiate the clinical application of G-Rh1 in the treatment of neurodegenerative diseases. However, the cytotoxic effects of G-Rh1 varied depending on the cell lines. There are some additional studies dealing with other separated pharmacological properties, such as estrogenic, enzymatic, anti-microorganism activities, and cardiovascular effects, but results were considerably diverged. Further investigations in randomized clinical trials are highly recommended to provide more reliable evidence for the efficacy of G-Rh1.

Supporting information

The search strategy of the systemic review is available as Supporting Information.

Author Contributions

D. N. H. T. and D. H. T. organized and wrote the manuscript. Others were involved in searching, screening the search results, translation, data collection, and making tables and figures under the supervision of Prof. K. H. and N. T. H. Each step of this study (screening title and abstract, screening full texts, and extraction of data for analysis) was performed by three independent reviewers.

Conflict of Interest

All contributing authors declare no conflicts of interest.

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