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Ginsenoside Rk1 bioactivity: A systematic review

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Abstract  Ginsenoside Rk1 (G-Rk1) is one of the unique components created by processing ginseng at high temperature, in particular of Sun Ginseng (SG). The aim of our study was to systematically review the pharmacological effects of G-Rk1. We conducted our search in eight databases and searched manually to select in vivo and in vitro original studies that provided information about biological pharmaceutical effects of G-Rk1 and were published up to August 2015 with no restriction in language or study design. We retrieved 21 eligible papers out of 121 identified ones. Some studies confirmed the G-Rk1 anticancer effects by investigating “cell viability”, “cell proliferation inhibition”, “apoptotic activity”, “anticancer activity” and “effects of G-Rk1 on G1 phase and autophagy in tumor cells” either alone or in combination with G-Rg5. Others proved that it has anti-platelet aggregation activities and anti-inflammatory effects, enhances cognitive function, reduces lipid accumulation and prevents osteoporosis. In conclusion, G-Rk1 has a significant anti-tumor effect of liver cancer, melanoma, and gastric cancer. Additionally, G-Rk1 has demonstrated as anti-platelet aggregation, anti-inflammatory, and anti-lipid accumulation. All these results corroborate the clinical effects of G-Rk1 and demonstrate the potential possibility to develop G-Rk1-based treatments, either alone or in combination with G-Rg5, with previously mentioned conditions.
Ginsenoside Rk1 bioactivity: A systematic review.

Running title: A systematic review of ginsenoside Rk1 bioactivity.

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Ginsenoside Rk1 (G-Rk1) is one of the unique components created by processing ginseng at high temperature, in particular of Sun Ginseng (SG). The aim of our study was to systematically review the pharmacological effects of G-Rk1. We conducted our search in eight databases and searched manually to select in vivo and in vitro original studies that provided information about biological pharmaceutical effects of G-Rk1 and were published up to August 2015 with no restriction in language or study design. We retrieved 21 eligible papers out of 121 identified ones. Some studies confirmed the G-Rk1 anticancer effects by investigating “cell viability”, “cell proliferation inhibition”, “apoptotic activity”, “anticancer activity” and “effects of G-Rk1 on G1 phase and autophagy in tumor cells” either alone or in combination with G-Rg5. Others proved that it has anti-platelet aggregation activities and anti-inflammatory effects, enhances cognitive function, reduces lipid accumulation and prevents osteoporosis. In conclusion, G-Rk1 has a significant anti-tumor effect of liver cancer, melanoma, and gastric cancer. Additionally, G-Rk1 has demonstrated as anti-platelet aggregation, anti-inflammatory, and anti-lipid accumulation.

All these results corroborate the clinical effects of G-Rk1 and demonstrate the potential possibility to develop G-Rk1-based treatments, either alone or in combination with G-Rg5, with previously mentioned conditions.

**Keywords:** Ginsenoside, Rk1, Systematic review, Clinical pharmacology
Introduction

Ginseng originally refers to the medicinal herbs, which were extracted from the genus *Panax*‘s roots (Shin et al. 2015). The genus *Panax* belongs to one of the most ancient herbs in traditional Chinese medicines and is currently used around the world (Choi et al. 2013). The active principles evolved to be the triterpenoid saponin glycosides called ginsenosides or panaxosides. Over 30 ginsenosides have been classified into two categories including the 20(S)-protopanaxadiol and the 20(S)-protopanaxatriol according to the presence or absence of a carboxyl group at the C-6 position. Although two forms of ginsenosides have been identified stereocytochemistrially, the relevance of its effects needs more investigations until now (Li et al. 2011). Ginsenosides are widely known to have many pharmacological activities (Choi 2008; Ernst 2010) such as anti-tumor, anti-inflammatory (Chen et al. 2007), anti-fatigue (Tang et al. 2008) and analgesic effects (Nemmani & Ramarao 2003).

*Panax ginseng* Meyer is commonly harvested after four to six years of cultivation and is characterized into three types based on the processing methods: (1) fresh ginseng (less than four years old, consumed in fresh state), (2) white ginseng (four to six years old, typically air or oven dried after peeling), (3) red ginseng (six years old, steamed prior to drying, without peeling). These processing methods aim to improve the efficacy, safety, and preservation (Yun 2001). Sun ginseng (SG), was recently developed by heat-treatment at high temperature and pressure, which were higher than those applied to the conventional preparation of red ginseng. This was observed to have higher concentrations of less polar ginsenosides, which were either entirely absent or present in trace amounts in conventional red ginseng (Keum et al. 2000; Kwon et al. 2001).

The ginsenoside Rk1 (G-Rk1) is one amongst the main elements of SG (Kim et al. 2008). Various studies have confirmed the anti-cancer effects of G-Rk1 on different neoplastic cells
including hepatocellular carcinoma and melanoma cells (Kim et al. 2012; Kim et al. 2008). In recent studies, G-Rk1 has been confirmed as a new endothelial barrier enhancer, which is capable of preventing or even reversing the vascular endothelial growth factor (VEGF)-induced vasopermeability in the endothelial cells, thus highly valuable for the pharmaceuticals development, which may effectively control the pathologic vascular leakage (Maeng et al. 2013). Therefore, we aimed to systematically review the bioactivities of G-Rk1 in both human and animals.

Methods

Protocol and registration

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) Checklist (Moher D 2009) was followed in this systematic review. Our protocol was registered at PROSPERO CRD42016029129 in January 2016.

Eligibility criteria

We selected only original studies published up to August 2015 that provided information on the biological and pharmaceutical effects of G-Rk1. We included articles with G-Rk1 biological effects on human and animals either in vivo or in vitro with no restriction regarding publication language, publication date, and study design.

We excluded three main types of studies, including 1-Studies with unreliably extracted data or overlapped data set; 2- Studies with only abstract available or no full-text available; 3- Books, reviews, meta-analysis studies, conference papers, and thesis. Any disagreement was discussed carefully among three reviewers to get a final decision.

Information sources and search strategies
We conducted electronic searches in eight databases including: PubMed, Scopus, ISI Web of Science, Google Scholar, SIGLE (System for Information on Grey Literature in Europe), Virtual Health Library (VHL), World Health Organization Global Health Library (GHL) and POPLINE and then performed manual search using reference lists of studies to find more relevant studies. The search strategy was performed by (AE, NXT, SS, YSS, EBO, MTE, ARK) and more information on search strategy was provided in S1 Table.

Study selection

We selected articles in two phases: 1. Title and abstract screening of all searched articles; 2. Full-text screening and the articles, which were not in agreement with our inclusion and exclusion criteria, were excluded. Three independent reviewers finished these two selecting phases. When disagreement occurred, a consensus decision was made following a discussion with supervisor (NTH).

Data collection process and data items

We made our primary extraction form; extracted three papers with it one by one, modified our form after each paper extraction and finally developed the extraction sheet that we used on the remaining articles. Three independent reviewers extracted the data from each paper. When there was a disagreement in any information retrieved, a discussion among the three reviewers was held to reach a consensus. If three reviewers could not reach an agreement, the supervisor (NTH) was consulted.

The extracted data items including first author last name, year of publication, year of subject recruitment, journal name, study design, country and city of origin of cell lines, the name of the plant, and method of extraction of our targeted material (G-Rk1). If the study had been done in animals, we extracted their species, sex, age and weight and if it had been done in vivo, we extracted the name of the cell line, its origin, the main medium used in terms of either
primary (isolated by authors) or commercial cell lines. In addition, we extracted the name of the measured parameter, an assay for its measurement, time effect, administration time, active substance name, its concentration, mean, standard deviation, standard error, a $P$ value of results and the statistical test. When the data were presented as graphs, we used Web blot digitizer software and the average of the results from three reviewers was calculated to obtain one result.

**Risk of bias in individual studies**

Two independent reviewers appraised all of the selected studies according to the GRADE method (Guyatt et al. 2011) to judge the quality of evidence and any disagreement was resolved by discussion between them. Items as a limitation, inconsistency, indirectness, imprecision, publication bias, and moderate or large effect size were to be scored as “1” if there is no serious limitation or “0” if there is a serious limitation that has been defined according to GRADE criteria. Then the overall quality was to be scored as “high”, “moderate”, “low” or “very low” quality, according to their analysis of each study. The supervisor (NTH) was consulted when a disagreement occurs.

**Summary measures**

Inhibition of cell proliferation, apoptosis, and regulation of protein expression were the main evaluated outcomes.

**Results**

**Study selection**

We identified 389 citations using the search strategy. From these, we included 121 articles after removing the duplicates. After that, we examined the title, abstract and excluded additional 86 articles. We retrieved and evaluated the full-text of the remained 35 articles, of which 18 articles were excluded, leaving 17 articles that were eligible, in addition to four articles.
were retrieved from manual searching of included references. A flowchart described in details
the process of identification, inclusion, and exclusion of articles was presented in Figure-1.

**Study characteristics**

Out of the 21 studies included, 16 studies were related to the effectiveness of G-Rk1 only
and five studies were reported on the combined effects of G-Rk1 and G-Rg5. The most common
study design was *in vitro* study with 16 studies (Kim et al. 2008; Ko et al. 2009; Lee et al. 2010;
2005; Ju et al. 2012; Liu et al. 2007; Park et al. 2015; Kim et al. 2010; Kang et al. 2006; Siddiqi
et al. 2014; Kang et al. 2007), while *in vivo* study was less common with only two studies (Toh
et al. 2011; Erb et al. 2005). The remaining three articles were both *in vitro* and *in vivo* study
(Lee. 2014; Lee et al. 2009; Maeng, 2013). A summary of the studies was presented in Table 1.

**Risk of bias across studies**

We used the GRADE method (Guyatt et al. 2011) to assess the quality of the included
2012; Kim et al. 2010; Lee et al. 2010; Park et al. 2015; Siddiqi et al. 2014) were categorized as moderate quality. Five of them (Bao et al. 2005; Jing et al. 2006; Kim et al.
2012; Park et al. 2015; Siddiqi et al. 2014) were focusing on the effectiveness of combined (G-
Rk1, G-Rg5), thus, they were downgraded in indirectness item of GRADE factors. Three studies
(Kang et al. 2007; Kim et al. 2010; Lee et al. 2010) were not completely pertained to our main
outcome since they were not focused mainly on G-Rk1. Four studies (Kang et al. 2006; Lim et
al. 2009; Liu et al. 2007; Park et al. 2002) were not downgraded in spite of having insufficient
data regarding dose effect factor as this factor does not belong to the downgraded factors of
GRADE method that include (limitation, inconsistency, indirectness, and Imprecision). In
contrast, one study (Lee et al. 2010) that was downgraded since it focused on combined (G-Rk1
and G-Rg5) not because of dose effect insufficient data. Another study (Kim et al. 2012) was
downgraded because it was used to compare the anti-tumor activity of G-Rk1 versus G-Rk3.
However, G-Rk3 has been proven to have a potential antitumor activity. One study (Ju et al.
2012) was downgraded as it has statistical typing mistake of one of its values (S2 Table).

Synthesis of results

Anti-cancer activity

Cell viability was measured by different assays through the studies including four studies
used Cell Counting Kit-8 (CCK8) assay (Kim et al. 2012; Kim et al. 2008; Kim et al. 2013; Ko et
al. 2009), three studies used 3-(4,5-dimethyl-thiazol-2yl) -2,5-diphenyl tetrazolium bromide
(MTT) assay (Lee et al. 2010; Park et al. 2002; Siddiqi et al. 2014), one study used WST-1 assay
(Toh et al. 2011) while the final one used EZ-CytoTox cell assay kit (Park et al. 2015). (Table 2)

Liver cancer

Toh et al. in 2011 (Toh et al. 2011) evaluated the inhibitory effects of G-Rk1 (0.25µg/ml)
on liver cancer cell growth in the cell lines (human hepatocellular carcinoma cells (HepG2),
SNU449, and SNU182). While SNU449 and SNU182 were incubated for 48 h, the remaining
cell lines HepG2 was incubated for 72 h. Then, they found that a significant reduction in cell
viability caused by G-Rk1. This result suggested that G-Rk1 has a potent effect on liver cancer
cell growth inhibition. In this study, they also evaluated the inhibition concentration (IC$_{50}$) value
of G-Rk1 for inhibiting growth in the SNU449 cell line for 48 h was 0.08 mg/ml (100 µM) by
using the WST-1 assay. They also indicated that G-Rk1 is one of the most anti-proliferative
ginsenosides of raw and steamed P. notoginseng.

Ko et al. in 2009 (Ko et al. 2009) evaluated the effect of G-Rk1 on cell viability of
HepG2 cells after 24 h incubation in concentrations of 50, 75, 100 µM in the presence of 0.1 µM
taxol used as a positive control. By using CCK8 assay, the results from this study also illustrated
that the inhibition of cell viability in a dose-dependent manner. The cell viability was also tested in the addition of G-Rk1 (100 μM) to bafilomycin A1. Then, three independent experiments showed that this co-treatment enhanced cell death of HepG2 cells more than the cells that were treated with 100 μM of G-Rk1 alone. To verify the effects of this combination and exclude cytotoxicity of bafilomycin A1, cytotoxicity was measured after 24 h and no cytotoxicity was detected. The effects of G-Rk1 on cell proliferation inhibition is also assessed by using these measures. Their finding show that G-Rk1 slightly reduced the cell growth rate. Compared with the vehicle control, G-Rk1 (at a dose of 100 μM) inhibited HepG2 cell proliferation by about 40%. Treating HepG2 cells for 24 h with various G-Rk1 concentrations (from 50 to 100 μM), the cell proliferation inhibition raises significantly, from 8% to 37.5%, with a dose-dependent manner. They also investigated whether G-Rk1-induced anti-proliferation was related to apoptosis or not. Their findings suggested that in the early stage of G-Rk1-induced apoptosis in HepG2 cells, G-Rk1 inhibits cell proliferation.

In the study of Kim et al. in 2008 (Kim et al. 2008), they assessed the effects of G-Rk1 on cell viability of HepG2 cells. The concentrations of G-Rk1 ranging from 12.5 to 100 μM were used in this study. They also added 0.5 (v/v) dimethyl sulphoxide as control and incubated the cells for 48 h. By using CCK8 analysis, they showed that when exposing to G-Rk1, the suppression of HepG2 cell growth took place. At 75 and 100 μM of G-Rk1, the effect of G-Rk1 induced cell death was maximized in 55% and 95% cell death respectively. They also evaluated the effects of G-Rk1 on apoptotic cell death in HepG2 cells. Their finding pointed out that with the treatment of HepG2 cells with 100 μM G-Rk1, the fraction of early apoptotic cells increased from 0.46 to 16.23%. Moreover, they also carried out further to determine the mechanisms of this effect. They suggested that the underlying mechanism behinds G-Rk1 induces the mitochondria-independent apoptosis can be the activation of capspase-8, the signaling cascade of
the one not associated with Fas-associated death domain expression.

To increase their cytotoxicity against Sk-Hep-1 hepatoma cancer cells, Park et al. in 2002 (Park et al. 2002) carried out the study used steamed ginseng. Steamed ginseng was separated by HPLC and tested with MTT assay to produce many active ginsenosides including G-Rk1. In this study, they found that isolated G-Rk1 showed the inhibition of cell viability of Sk-Hep1 cells. The growth inhibition concentration of G-Rk1 was 13 µM.

**Melanoma**

To evaluated the inhibition of cell viability of SK-Mel-2 human melanoma cells, Kim et al. in 2012 (Kim et al. 2012) incubated this cells with G-Rk1 for 24 and 48 h at different concentrations (0, 10, 25, 50, 75, 100 µM) in a dose-dependent manner. Erb et al. (Erb et al. 2005) in 2005 provoked a controversy with the role of FAS and/or FASL in human malignant melanoma. Therefore, the effect of FAS and/or FASL on cell viability was evaluated by Kim et al. (Kim et al. 2012) by adding Fas/FasL antagonist Kp 7-6 of concentration 1mM and incubated it for 1 h. Then, the cells were treated with various concentrations of G-Rk1 (1, 5, 10, 50 and 100 µM). The results showed that Kp 7-6 treatment alone did not induce cell death or cell proliferation. Therefore, they concluded that there is no effect on cell viability if Kp 7-6 used alone. However, when the cells treated by G-Rk1 (100 µM) that followed the Kp7-6 treatment, the reduction of the effect of G-Rk1 were 32 % compared to the control (no treatment of Kp7-6).

Moreover, they also assessed the induction of apoptosis by G-Rk1 in SK-MEL-2-Human Melanoma. The cells were treated with G-Rk1 at various concentrations (25, 50, 75, and 100 µM), with G-Rg3 (100 µM), and with a control for 24 h. After that, cells were stained with Annexin V/PI and analyzed by FACS analysis. Their findings showed that the concentration of G-Rk1 was increased, the number of apoptotic cells also increased. More importantly, the cell lines responded in a dose-dependent manner. The corresponding quantities of total cell apoptosis
were 15.72% at 100 µM G-Rg3 and 4.96%, 6.25%, 8.42%, and 78.97% at 25, 50, 75, and 100 µM G-Rk1, respectively. Therefore, they concluded that G-Rk1 induces apoptosis in a concentration-dependent manner.

**Gastric cancer**

Kim *et al.* (*Kim et al. 2013*) evaluated the effect of the combination of G-Rg5/G-Rk1 on cell viability of gastric cancer cells. After treatment with this combination at different concentrations (12.5, 25, 50 and 100 µM) for 24 h, the result is that in inhibition of cell viability and proliferation of these cells in a dose-dependent manner (99, 93.5, 37.5, 3 %) respectively.

**Anti-platelet aggregation activity**

Two studies evaluated the anti-aggregation effects of G-Rk1 both *in vivo* and *in vitro* (*Ju et al. 2012; Lee et al. 2009*) respectively. *Ju et al.* compared the antiplatelet aggregation activity of G-Rk1 and acetylsalicylic acid (ASA) (*Ju et al. 2012*). The results indicated that G-Rk1 exhibits a stronger antiplatelet aggregation activity than ASA and that the action of G-Rk1 in platelets might be related to arachidonic acid (AA) metabolism. In addition, in the same study, the alteration of (S) hydroxyl eicosatetraenoic acids and thromboxane B2 levels were determined using an immunoassay kit and UPLC/Q-TOF MS system, respectively. The 12-hydroxyleicosatetraenoic acid was remarkably decreased in the in the G-Rk1 group but increased in the ASA-treated group. The thromboxane B2 level in the washed platelets decreased significantly after treatment with 100 µM ASA and 10 µM G-Rk1, exhibiting 66% and 77% inhibitory activity, respectively (*Ju et al. 2012*). They used the colorimetric COX inhibitor screening assay to measure the inhibitory effects of G-Rk1 on COX-1 and COX-2. It was found that G-Rk1 inhibits both COX-1 and COX-2 activities. However, at a concentration of 20 µM, G-Rk1-derived inhibition was higher on COX-2 than on COX-1 (*Ju et al. 2012*).

In the other study (*Lee et al. 2009*), the effect of G-Rk1 on adenosine diphosphate (3-4
μM) induced platelet aggregation was monitored turbidimetrically by using ASA as a positive control. Both ASA and G-Rk1 showed the dose-dependent inhibitory effect to collagen, AA and U46619 (9,11-dideoxy-11a,9a-epoxy methanoprostaglandin F2a) (thromboxane A2 mimetic drug)-induced platelet aggregation. However, they showed a negligible effect on adenosine diphosphate-induced aggregation. G-Rk1 exhibited the strongest inhibitory effect on collagen, AA, and U46619-induced platelet aggregation. In particular, it presented a 22-fold activity of ASA on AA-induced aggregation (Lee et al. 2009). G-Rk1 was found to be a potential inhibitor of platelet aggregation induced by AA and U46619. (Table 3)

**Anti-inflammatory activity**

G-Rk1 was found to have an anti-inflammatory effect by inhibition of NF-κB levels in *in vitro* models (Lee 2014). These results were assessed using luciferase assay. HepG2 cells were seeded at 1 × 10^5 cells/well in a 12-well plate and grown for 24 h. While G-Rk1 was pretreated with dimethyl sulphoxide for 1 h and then it was treated with tumor necrosis factor-α (10 ng/mL), the sulfasalazine was used as positive control. Their data demonstrated the strong inhibitory activity of G-Rk1 on NF-κB expression with 50% (IC50) value from 0.75 μM. However, the results revealed that G-Rk1 has cytotoxic effects, which occur in concentrations higher than 10 μM. Another evaluation of G-Rk1 anti-inflammatory activity (Erb et al. 2005), was its suppressing effect on 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) induced mouse ear edema. The right ear of ICR mouse was treated with red ginseng saponin extract, G-Rg3, G-Rg5, and G-Rk1 of 10, and 50 mg/kg and after 30 minutes, ear edema in both ears was induced by topical application of TPA, which is a potent inflammatory agent so the left ear only was treated with TPA. They measured the extent of edema and noticed that the pretreatment with red ginseng saponin extract or G-Rk1 suppresses TPA-induced mouse ear edema, and when administering G-Rk1 orally, the formation of edema has been blocked. (Table 3)
Effect of G-Rk1 on vascular leakage

A study evaluated the G-Rk1 activity on VEGF (Maeng et al. 2013) through treating primary human retina microvascular endothelial cells with G-Rk1 at a concentration of (10 μg/ml) for 40 minutes then stimulating it with 20 μg/ml of VEGF to disrupt the cell membrane. Sucrose permeability assay was used to evaluate the endothelial permeability and the results showed that G-Rk1 inhibits VEGF-induced retinal endothelial permeability. They used reverse-transcription polymerase chain reaction and Densitometric analysis was used to assess translocation of tight junctions (TJ) proteins and immunostaining was used to evaluate disruption of TJ proteins after the cells were stained with anti-ZO-1, anti-ZO-2, and anti-occludin antibodies. The authors found that G-Rk1 inhibits VEGF effect on TJ protein localization but it does not affect the transcription of TJ proteins. (Table 3)

Effect of G-Rk1 on lipid accumulation

The Ginseng was known to have effects on obesity (Kim et al. 2009). In vitro treatment of mouse 3T3-L1 fibroblast cells with G-Rk1 resulted in reduction of lipid accumulation, in which these cells differentiated into adipocytes after been treated with Korean red ginseng extract and its digest, triol and diol with various G-Rk1 concentrations (10, 50, 100 μM) for 2 h at 490 nm optical absorbance (Kim et al. 2009) (Table 3).

Combined effect

The combination of G-Rg5/G-Rk1 had a pronounced effect on the excitotoxic and oxidative stress-induced neuronal cell damage that was tested in primary cultured rat cortical cells (Bao et al. 2005). These cells were cultured in vitro for 12-20 days, then exposed to 100 μM glutamate or N-methyl-D-aspartate for 15 min in the absence or presence of G-Rg5/G-Rk1. The cell damage was assessed after 20-24 h by measuring LDH activity in the culture media. Data was calculated from cells exposed to the respective excitotoxic insults without ginsenosides.
Data presented that approximately 70-80% of the cells were damaged by glutamate or N-methyl-D-aspartate compared to vehicle-treated control cells. The excitotoxic effect was significantly inhibited by G-Rg5/G-Rk1 in a concentration-dependent manner, in which 50% inhibition was achieved at 14.7 µg/mL of G-Rg5/G-Rk1.

In previous work, Bao et al. (Bao et al. 2005) used a passive avoidance test to evaluate the effect of G-Rg5/G-Rk1. The latency in seconds was used to measure the cognitive performance of ethanol-induced amnesia in mice. The mice were orally treated with saline as vehicle and ratio of G-Rg5/G-Rk1 equal 1:1 with a concentration of 10 mg/kg once a day during 4 days. The latency period of the mice administrated with ethanol was 24.9% less than the 1 of control mice (without ethanol-treatment), but it was significantly enhanced by the oral administration of G-Rg5/G-Rk1 with 1.2-fold increase more than that of the control. The same steps were done but this time after inducing amnesia with a single injection of scopolamine (3 mg/kg), also G-Rg5/G-Rk1 (10 mg/kg) provided the same enhancing significant result (p <0.01).

In another work, Jing et al. (Jing et al. 2006) did the same tests of ethanol-induced amnesia in mice, which were given water as the control and ratio of G-Rg5/G-Rk1 equal 1:1 in the concentration of 10 mg/kg. They found that G-Rg5/G-Rk1 could significantly prolong the latency period by 2.97 folds more than that of the control. These two studies presented that G-Rg5/G-Rk1 would give beneficial results in the memory function of the normal, ethanol or scopolamine-induced amnesia in brains.

Park et al. (Park et al. 2015) explored the effect of the G-Rg5/G-Rk1 combination on cisplatin-induced cytotoxicity in mice at cisplatin concentration 25 µM and G-Rg5/G-Rk1 concentrations of (0, 50, 100, 250 µg/ml). Results with EZ-cytotoxic cell viability assay kit showed a significant reduction in cisplatin and induced a reduction in cell viability. This effect was higher than that of Epigallocatechin gallate at same concentrations as G-Rk1.
Siddiqi et al. (Siddiqi et al. 2014) in 2014 evaluated the osteogenic activity of G-Rg5/G-Rk1. MC3T3-E1 cells were treated with differentiation medium (either with or without G-Rg5/G-Rk1) for 12 days at different concentrations in which different substances were added to the culture medium in order to evaluate various effects of G-Rg5/G-Rk1 on differentiated fibroblast. The extent of calcium deposition, which is an indicator of osteoblasts mineralization, was quantified by MTT assay. Data were expressed as a percentage of control, which showed that G-Rg5/G-Rk1 protected the extracellular matrix mineralization from antimycin A devastating effects. Besides, alkaline phosphatase (ALP) activity evaluated by Smart BCA protein assay kit and it turned out that ALP activity increased by two folds after treatment with G-Rg5/G-Rk1 (30–50 μg/mL).

The effect of G-Rg5/G-Rk1 on cellular collagen was measured using Sirius Red-based colorimetric assay. Results were similar to that of ALP activity in which cellular collagen was markedly increased. While glutathione contents of the cells were measured by glutathione assay kit after exposure to various concentrations of G-Rg5/G-Rk1, data showed that G-Rg5/G-Rk1 increase the level of glutathione in a dose-dependent manner. In order to evaluate gene expression levels, total RNA was isolated from cells, which were treated with G-Rg5/G-Rk1 and was amplified by reverse-transcription polymerase chain reaction. The results indicated that the maturation and the differentiation of MC3T3-E1 cells were induced by G-Rg5/G-Rk1 mediated BMP-2/Runx2 and the level of expression of Runx2 was increased by G-Rg5/G-Rk1.

Discussions

Ginsenosides are active compounds extracted from white or red ginseng (P. Ginseng Meyer). Ginsenosides have shown pharmacological effects in the cardiovascular system (Sun Y et al. 2016), the immune system (Song X et al. 2009), and the central nervous system (Zhou J et al. 2014), as well as anti-stress and antioxidant activity and antitumors. Moreover, ginsenosides
have shown good results in the treatment of diabetes disease by improving glucose and insulin control in type 2 diabetes in a clinical trial (Vuksan V et al. 2008). Antitumor inhibitory effects of ginsenosides have been demonstrated because of their cytotoxicities such as the suppression of tumor angiogenesis and metastasis by G-Rb2 (Sato K et al. 1994) and the enhancement of apoptosis by G-Rg3 in various cancer cell lines such as breast (Kim BM et al. 2013). Although G-Rk1 has a similar structure to G-Rg3, G-Rk1 could be formed by processing ginseng at high temperature and its antitumor activities have been limited. Its pharmacological activity has been assessed on antitumor activity in human hepatocellular carcinoma cells (Kim et al. 2008). Apart from these activities, G-Rk1 have been demonstrated to ameliorate impaired memory function, anti-platelet aggregation (Lee et al. 2009). Furthermore, G-Rg3, G-Rk1, and G-Rg5 exhibited a potential effect in the management of human arthritis (Kim et al. 2010).

In this systematic review, we found that 21 studies have been reported to show various pharmacological and therapeutic effects of G-Rk1 such as anti-cancer effects (Kim et al. 2008), anti-platelet aggregation activities (Ju et al. 2012; Lee et al. 2009), cognitive function enhancement (Bao et al. 2005), anti-inflammatory effects (Kim et al. 2010; Lee 2014), lipid accumulation reduction (Kim et al. 2009), antioxidant effects, and protection against human arthritis (Kim et al. 2010).

Anti-cancer activity is one of the most common bioactivities of G-Rk1. By assessing such studies in “cell viability”/“cell proliferation inhibition” and “apoptotic activity”, these studies confirmed the anti-cancer effects of G-Rk1 as well as a combination of G-Rg5/G-Rk1 (ratio equal 1:1). In terms of “cell viability”, the effects of G-Rk1 on cell viability of HepG2 cells, SNU449, SNU182, SK-Hep-1, SK-Mel-2, human malignant melanoma was found significantly in a dose-dependent manner (Kim et al. 2012; Kim et al. 2008; Ko et al. 2009). The concentrations of G-Rk1 vary from 0 to 100 µM and the cytotoxic effect was maximum at 75
and 100 µM (Kim et al. 2008). The effects of G-Rk1 were also evaluated in combination with other chemotherapeutics (Bafilomycin A1)(Ko et al. 2009).

It was found that the enhancement of HepG2 cell death is higher in G-Rg5/G-Rk1 combination than that of G-Rk1 alone. Furthermore, we also found three relevant studies (Kim et al. 2013; Park et al. 2015; Siddiqi et al. 2014) that evaluated the effects of G-Rg5/G-Rk1 co-treatment on cell viability of gastric cancer cells, mice, MC3T3-E1 cells. The authors demonstrated that G-Rg5/G-Rk1 had potential effects on inhibition in cell viability and proliferation with the inhibition in dose dependent. The combination of G-Rg5/G-Rk1 with others chemotherapies (cisplatin (Park et al. 2015), antimycin A (Siddiqi et al. 2014)) has a greater effect on cell death than using G-Rg5 or G-Rk1 alone. Besides, it was also proved that co-administration of G-Rg5/G-Rk1 with ratio equal 1:1 could have various effects such as improving the cognitive performance in ethanol-induced amnesia in mice (Bao et al. 2005; Jing et al. 2006), inhibiting the exotoxic effects and oxidative stress-induced neuronal cell damage (Bao et al. 2005), and stimulating the mineralization of the extracellular matrix of osteoblasts (Siddiqi et al. 2014).

In this systematic review, we found two studies presented the anti-platelet aggregation activities with the results indicated that G-Rk1 (10 µM) can be stronger than ASA (100 µM) in terms of antiplatelet aggregation (Ju et al. 2012). Lee et al. in 2009 also showed that G-Rk1 inhibited the effects of collagen, AA, and U46619-induced platelet aggregation (Lee et al. 2009). G-Rk1 was also indicated as one of the effective anti-inflammatory agents through the inhibition of both COX1 and COX2 activities and NF-κB levels (Ju et al. 2012; Lee 2014).

Several limitations were faced regarding the methodological approaches. One of them is that we could not find a clinical study that used G-Rk1 in healthy people or in patients. Out of 389 studies, we included 21 studies using our criteria and they were in vitro study and in vivo
animals. Based on GRADE method, there were remained five studies due to indirectness of
evidence (Bao et al. 2005; Jing et al. 2006; Kim et al. 2013; Park et al. 2015; Siddiqi et al. 2014)
and inability to explain heterogeneity in results (Bao et al. 2005). To date, there is a shortage of
literature concerning clinical studies and the clinical use of G-Rk1 to treat some diseases in
patients and it consequently prohibits the clinical analysis.

Conclusions
In general, G-Rk1 has a significant anti-tumor effect of liver cancer, melanoma, and gastric
cancer. Additionally, G-Rk1 has demonstrated as anti-platelet aggregation, anti-inflammatory, and
anti-lipid accumulation. All these results corroborate the clinical effects of G-Rk1 and demonstrate
the promising possibility to develop G-Rk1-based treatments, either alone or in combination with
G-Rg5, for the previously mentioned conditions.

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Competing financial interest
Authors (AE, NXO, SS, YSS, EBO, MTE, ARK, LT, LV) are members of Online
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interest

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Chen LW, Wang YQ, Wei LC, Shi M, and Chan YS. 2007. Chinese herbs and herbal extracts for neuroprotection of


Jing Z, Shi-rong W, Quan-cheng C, Long PH, and Kang JS. 2006. Effects of Ginsenosides Rg3(R), Rg3(S)and Rg5/Rk1 on Memory Improvement of Ethanol Treated Mice. Journal of Jilin Agricultural University 28.


**Figure legends**

**Figure 1.** Summary of how the systematic search was conducted and eligible studies was identified (PRISMA flow diagram). PRISMA = Preferred Reporting Items for Systematic reviews and Meta-Analyses.
Summary of how the systematic search was conducted and eligible studies was identified (PRISMA flow diagram). PRISMA = Preferred Reporting Items for Systematic reviews and Meta-Analyses.
<table>
<thead>
<tr>
<th>Author and Year</th>
<th>Country</th>
<th>Study design</th>
<th>Cell lines</th>
<th>Parameter Assessed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ko et al., 2009</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>HepG2</td>
<td>Cell viability, cell proliferation, inhibitory activity (IC50)</td>
</tr>
<tr>
<td>Lee et al., 2014</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>HepG2</td>
<td>Cell viability</td>
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<tr>
<td>Toh et al., 2011</td>
<td>Singapore</td>
<td><em>In vitro</em></td>
<td>SNU449 (CRL-2234), SNU182 (CRL-2235) and HepG2 (HB-8065)</td>
<td>Cell viability, cell proliferation</td>
</tr>
<tr>
<td>Kim et al., 2008</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>HepG2</td>
<td>Cell viability, telomerase activity</td>
</tr>
<tr>
<td>Park et al., 2002</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>SK-Hep-1 cells</td>
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<td>Lim et al., 2009</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>Junctional proteins (zo-1, occludin and plakoglobin)</td>
<td>ND</td>
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<tr>
<td>Kim et al., 2009</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>3T3-L1 fibroblast cells</td>
<td>Cell viability, lipid accumulation</td>
</tr>
<tr>
<td>Kim et al., 2012</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>SK-MEL-2 human melanoma</td>
<td>Cell viability</td>
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<td>Ju et al., 2012</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>Platelet</td>
<td>Anti-platelet aggregation activity</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Setting</td>
<td>Cell Type/Model/Experiment</td>
<td>Activity/Effect</td>
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<tr>
<td>Liu et al., 2007</td>
<td>France</td>
<td><em>In vitro</em></td>
<td>Embryonic neural stem cells (neurospheres)</td>
<td>Anti-platelet aggregation activity</td>
</tr>
<tr>
<td>Lee et al., 2009</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>Platelet</td>
<td>Collagen (3-4 μg/L) induced platelet aggregation</td>
</tr>
<tr>
<td>Kim et al., 2010</td>
<td>South Korea</td>
<td><em>In vivo</em></td>
<td>ND</td>
<td>TPA-induced mouse ear edema</td>
</tr>
<tr>
<td>Maeng et al., 2013</td>
<td>South Korea</td>
<td><em>In vitro and In vivo</em></td>
<td>HREC cells</td>
<td>VEGF-induced retinal endothelial permeability, VEGF-induced destabilization of TJ protein ZO-1, ZO-2 and occludin in membrane and cytosol</td>
</tr>
<tr>
<td>Kang et al., 2007</td>
<td>Japan</td>
<td><em>In vitro</em></td>
<td>ND</td>
<td>The OH scavenging inhibition</td>
</tr>
<tr>
<td>Kang et al., 2006</td>
<td>Japan</td>
<td><em>In vitro</em></td>
<td>ND</td>
<td>The OH scavenging activities</td>
</tr>
<tr>
<td>Lee et al., 2010</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>HUVECs</td>
<td>Cell viability</td>
</tr>
<tr>
<td>Kim et al., 2013</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>Gastric cancer AGS cell</td>
<td>Cell viability, the anticancer activity of ginsenosides after heat processing (IC50)</td>
</tr>
<tr>
<td>Authors</td>
<td>Country</td>
<td>Study Type</td>
<td>Cell Line/Model</td>
<td>Assays</td>
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<tr>
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<tr>
<td>Bao et al., 2005</td>
<td>South Korea</td>
<td><em>In vitro</em> and <em>In vivo</em></td>
<td>Cortical cell cultures containing neuronal and non-neuronal cells</td>
<td>Cognitive performance, excitotoxicity induced by NMDA and glutamate</td>
</tr>
<tr>
<td>Park et al., 2015</td>
<td>South Korea</td>
<td><em>In vitro</em> and <em>In vivo</em></td>
<td>LLC-PK1 cells</td>
<td>Cell viability</td>
</tr>
<tr>
<td>Siddiqi et al., 2014</td>
<td>South Korea</td>
<td><em>In vitro</em></td>
<td>The murine cell line, MC3T3-E1</td>
<td>Cell viability, mineralization, ALP activity, Collagen and glutathione</td>
</tr>
<tr>
<td>Jing et al., 2006</td>
<td>China</td>
<td><em>In vivo</em></td>
<td>ND</td>
<td>Cognitive performance</td>
</tr>
</tbody>
</table>

AMA: antimycin A; ALP: alkaline phosphatase; HepG2: human hepatocellular carcinoma cells; HUVEC: human umbilical vein endothelial cell; HRECs: Primary human retina microvascular endothelial cells; LLC-PK1: (pig kidney epithelium, CL-101); SNU449, SNU182: Human liver cancer cell lines; NMDA: *N*-methyl-D-aspartate; ND: not defined; VEGF: vascular endothelial growth factor; TPA: 12-*O*-Tetradecanoyl-phorbol-13-acetate; TJ: tight junctions.
Table 2. Summary of anti-cancer activity of G-Rk1.

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Cells / Cell lines</th>
<th>Methods/ Assays</th>
<th>Method/ Time</th>
<th>Positive control</th>
<th>Active compound</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ko et al., 2009, South Korea</td>
<td>HepG2</td>
<td>CCK-8</td>
<td>24h</td>
<td>Taxol 0.1 µM</td>
<td>G-Rk1 50, 75, 100 µM</td>
<td>92, 70, 62.5</td>
</tr>
<tr>
<td>Toh et al., 2011, China</td>
<td>SNU449, SNU182, HepG2</td>
<td>WST-1, (12h for SNU182, 48h for HepG2)</td>
<td>48h</td>
<td>ND</td>
<td>ND</td>
<td>G-Rk1 250 µg/ml</td>
</tr>
<tr>
<td>Kim et al., 2008, South Korea</td>
<td>HepG2</td>
<td>CCK-8, supplied</td>
<td>48h</td>
<td>Kit-supplied</td>
<td>G-Rk1 25, 50, 75, 100 µM</td>
<td>89.2, 68.6, 45.7, 5.4</td>
</tr>
<tr>
<td>Park et al., 2002</td>
<td>South Korea</td>
<td>SK-Hep-1 cells</td>
<td>MTT assay</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Kim et al., 2012</td>
<td>South Korea</td>
<td>SK-MEL-2 human melanoma cells</td>
<td>Cell viability assay</td>
<td>12h</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Lee et al.</td>
<td>ND</td>
<td>HUVEC</td>
<td>MTT assay</td>
<td>24h</td>
<td>ND</td>
<td>ND</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>48h</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Authors</td>
<td>Country</td>
<td>Tumor Type</td>
<td>Assay Type</td>
<td>Cell Line</td>
<td>Time (h)</td>
<td>EGCG Concentration (µg/ml)</td>
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<tr>
<td>Kim et al., 2010</td>
<td>South Korea</td>
<td>Gastric cancer</td>
<td>AGS cell CCK-8 assay</td>
<td>24h</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Park et al., 2015</td>
<td>South Korea</td>
<td></td>
<td>LLC-PK1 EZ-Cytox cell viability assay kit</td>
<td>24h</td>
<td>EGCG 0 µg/ml</td>
<td>G-Rg5/G-Rk1 100.04</td>
</tr>
<tr>
<td>Siddiqi et al., 2014</td>
<td>South Korea</td>
<td>Murine cell line, MC3T3-E1</td>
<td>MTT assay</td>
<td>24h+48h</td>
<td>AMA 60 µg/ml</td>
<td>G-Rg5/G-Rk1 109.21, 111.54, 123.43, 131.21, 140.05</td>
</tr>
<tr>
<td>Study</td>
<td>Country</td>
<td>Cell Line</td>
<td>Assay</td>
<td>Time (h)</td>
<td>Control</td>
<td>Test Concentration(s)</td>
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<tr>
<td>Ko et al., 2009</td>
<td>South Korea</td>
<td>HepG2</td>
<td>CCK-8</td>
<td>24</td>
<td>Taxol 0.1 µM</td>
<td>100 G-Rk1 50, 75, 100</td>
</tr>
<tr>
<td>Toh et al., 2011</td>
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<td>SNU449</td>
<td>WST-1</td>
<td>48</td>
<td>ND</td>
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<td>Apoptotic activity</td>
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<td>HepG2</td>
<td>CCK-8</td>
<td>48</td>
<td>ND</td>
</tr>
<tr>
<td>Kim et al., 2012</td>
<td>South Korea</td>
<td>SK-MEL-2</td>
<td>FAS/FAS</td>
<td>24</td>
<td>Fas/FasL 1 mM</td>
<td>ND</td>
</tr>
</tbody>
</table>
AMA: antimycin A; CCK-8: Cell Counting Kit-8; EGCG: Epigallocatechin gallate; HepG2: human hepatocellular carcinoma cells; HUVEC: human umbilical vein endothelial cell; LLC-PK1: (pig kidney epithelium, CL-101); SNU449, SNU182: Human liver cancer cell lines; MTT: 3-(4,5-dimethyl-thiazol-2-yl) -2,5-diphenyl tetrazolium bromide; MC3T3-E1: (RCB1126, an osteoblast-like cell line derived from C57BL/6 mouse calvarias); (a) measured by cell viability (%); (b) measured by cell proliferation inhibition (%).
Table 3. Summary of the effects of G-Rk1 on anti-platelet aggregation, anti-inflammatory, anti-vascular leakage and lipid accumulation.

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Cells origi</th>
<th>Method s/</th>
<th>Method s/</th>
<th>Method s/ Time</th>
<th>Positive control</th>
<th>Active compound</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ju et al., 2012</td>
<td>South Korea</td>
<td>Platelet A</td>
<td>ND</td>
<td>ASA</td>
<td>50 µM</td>
<td>ND</td>
<td>G-Rk1 50 µM</td>
</tr>
<tr>
<td>Lee et al., 2009</td>
<td>South Korea</td>
<td>Platelet turbidimetrically ND</td>
<td>ASA</td>
<td>66 µM</td>
<td>50</td>
<td>G-Rk1 3 µM</td>
<td>50a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anti-inflammatory activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee et</td>
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<tr>
<td>Authors</td>
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<tr>
<td>------------------</td>
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<tr>
<td>al., 2014</td>
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<tr>
<td>Kim et al., 2010</td>
</tr>
<tr>
<td>Maeng et al., 2013</td>
</tr>
<tr>
<td>Kim et al., 2009</td>
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<tr>
<td>t cells staining</td>
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</tbody>
</table>

ASA: acetylsalicylic acid; AA: arachidonic acid; HepG2: human hepatocellular carcinoma cells; HRECs: Primary human retina microvascular endothelial cells; VEGF: vascular endothelial growth factor. (a) 50% inhibition concentration (IC$_{50}$) values; (b) was indicated as the increase in weight of the right ear punch over that of the left (mg); (c) $[^3]$H] sucrose permeability (%); (d) measured by the optical absorbance at 490 nm.