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Review article

Molecular signaling of ginsenosides Rb1, Rg1, and Rg3 and their mode of actions

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ABSTRACT

Ginseng has gained its popularity as an adaptogen since ancient days because of its triterpenoid saponins, known as ginsenosides. These triterpenoid saponins are unique and classified as protopanaxatriol and protopanaxadiol saponins based on their glycosylation patterns. They play many protective roles in humans and are under intense research as various groups continue to study their efficacy at the molecular level in various disorders. Ginsenosides Rb1 and Rg1 are the most abundant ginsenosides present in ginseng roots, and they confer the pharmacological properties of the plant, whereas ginsenoside Rg3 is abundantly present in Korean Red Ginseng preparation, which is highly known for its anticancer effects. These ginsenosides have a unique mode of action in modulating various signaling cascades and networks in different tissues. Their effect depends on the bioavailability and the physiological status of the cell. Mostly they amplify the response by stimulating phosphotidylinositol-4,5-bisphosphate 3-kinase/protein kinase B pathway, caspase-3/caspase-9-mediated apoptotic pathway, adenosine monophosphate-activated protein kinase, and nuclear factor kappa-light-chain-enhancer of activated B cells signaling. Furthermore, they trigger receptors such as estrogen receptor, glucocorticoid receptor, and *N*-methyl-D-aspartate receptor. This review critically evaluates the signaling pathways attenuated by ginsenosides Rb1, Rg1, and Rg3 in various tissues with emphasis on cancer, diabetes, cardiovascular diseases, and neurodegenerative disorders.

1. Introduction

Ginseng, a traditional herbal adaptogen, is widely used in Southeast Asian countries, and continues to gain worldwide popularity because of its medicinal properties. The root of the ginseng plant is used as medicine. Ginseng belongs to the genus *Panax* in the family Araliaceae. Of the 17 different species assigned to this genus, *Panax ginseng*, *Panax notoginseng*, and *Panax quinquefolius* are used as medicine. *P. ginseng* is extensively practiced as medicine due to the presence of ginsenoside saponin which accounts for the pharmacological efficacy [1]. Among the 150 different types of ginsenoside saponins, Rb1, Rb2, Rc, Rd, Re, and Rg1 constitute more than 90% of the total ginsenosides in *P. ginseng*. However, Rb1, Rg1, Rg3, Rd, Re, Rh1, and Rh2 are the most frequently studied [2]. Ginsenosides have diverse pharmacological activities mainly because of their steroidal structure, which enables them to interact with cell membranes, membrane-bound ion channels, as well as extra- and intracellular receptors to produce alteration at the transcriptional level. Based on several *in vitro* and *in vivo* studies, Rb1, Rg1, and Rg3 have multiple pharmacological efficacies to neuronal, cardiovascular, and immune systems, and have neuroprotective, anticancer, and antidiabetic activities.

Ginsenoside Rb1 is abundant in roots, rhizomes, and root hairs of ginseng, compared wtih the stem and leaves. Rb1 appears to be responsible for most of the plant's pharmacological activity especially in cardiovascular, endocrine, and immune systems, and is the chief component for neuroprotection [3]. A recent investigation has suggested that Rb1 exhibits greater neuroprotection activity when administrated intranasally than orally [4]. Ginsenoside Rg1 is one of the active compounds found in ginseng. It stimulates glucose

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uptake, relieves oxidative stress, and suppresses adipocyte development and possible neuroprotective role as well [5]. It is reported to have phytoestrogen-like properties and resembles that of steroid hormone-like structure. It has been widely used to treat Alzheimer's disease (AD) in clinics [6] and believed to be the principal compound for the antiaging property of ginseng.

Ginsenoside Rg3 is a steroidal saponin that is highly enriched in Korean Red Ginseng. It exhibits a broad range of pharmacological activities notably, enhanced anticancer efficacy compared with other ginsenosides [7]. It has the best proapoptosis effect [8] and is clinically approved for use in cancer treatment [9]. Rg3 displays stereospecificity, wherein 20(*R*) isomer exhibits higher antioxidant activity [11] and more potent immune response [12] but dissolves poorly, whereas 20(*S*) shows antidiabetic and anticancer activity and readily dissolves, making it easier to use in pharmaceutics. Rb1, Rg1, and Rg3 respectively represent the protopanaxadiol (PPD), protopanaxatriol (PPT), and Korean Red Ginseng enriched fractions, which are used as parameters to evaluate the quality of ginseng and ginseng-based products. Hence, it is important to understand the molecular mechanism and signal transduction attenuated by Rb1, Rg1, and Rg3 in various pathophysiological conditions. Therefore, this review summarizes the pathways and the receptors they modulate in neuronal disorders, cardiovascular diseases (CVDs), cancer, and diabetes mellitus (DM). We strongly believe that the information shared in this review will be helpful in generating an efficient treatment strategy with ginsenoside-based medications.

1.1. Ginsenosides are triterpenoid saponins

Ginsenosides are glycosylated triterpenoid saponins with diverse chemical structures based on the sugar moieties and linkage position on the skeleton of aglycones. Triterpenoid ginsenosides are further classified into dammarane type and oleanane type (a natural triterpene found in the flowering plants), and ocotillol type [10]. Glycosylation of hydroxylated C3, C12, and C20 hydroxylated groups at the triterpenoid skeleton contributes to the diversity of PPT and PPD. Ginsenosides Rb1 and Rg3 belongs to the PPD class whereas Rg1 belongs to the PPT class (Fig. 1) [11]. Moreover, the aglycone structure and the position of sugar moieties attached to them determine the pharmacological activity of ginsenosides.

1.2. Bioavailability and safety of ginsenosides

Most of the ginsenosides are poorly absorbed in the gastrointestinal tract of humans because of their poor absorption in the intestine and distribution by blood circulation. However, they are modified/transformed by gastric juices and intestinal microflora, producing ginsenosides with increased *in vivo* physiological activity than their parent compound. Various processing methods of ginseng and biotransformation of ginsenosides yield much simpler variants with improved bioavailability molecules such as the conversion of Rb2 to Rg3 during red ginseng processing and metabolism of Rb1 to F2 and compound K by intestinal microflora [12]. These transformed ginsenosides further contribute to the chemical diversity of ginsenosides.

It has been believed from ancient days that ginseng is a reliable drug for longevity, and to fight fatigue and stress. Results from the effects of ginsenosides on cell culture practices and animal efficacy studies have provided promising effects in various disease conditions. The role of ginsenoside Rb1 as a neuroprotective agent, Rg3 as an anticancer and antidiabetic agent, and Rg1 as an antidiabetic agent has been established by many studies. It is important to note that ginsenosides were supplemented with other drugs to reduce or nullify the side effects produced by those drugs. The effect of ginseng consumption on cancer prevention was evaluated by a meta-analysis, which proved that ginseng use confers 16% lower risk of developing cancer regardless of tissue type [13].

2. Action of ginsenosides in CVD

CVDs, a group of disorders affecting the heart and blood vessels, account for 31% of all global deaths according to World Health Organization 2012 estimates. CVDs are often called as "the causes of causes" as any dysfunction of cardiovascular system might lead to the abnormalities of other bodily functions. Various signaling mechanisms have been attributed to involve the cardiovascular system at many different levels [14]. In recent years, the effects of ginsenosides on CVD has been researched extensively because of their intrinsic property of controlling reactive oxygen species (ROS), nitric oxide (NO) production, and the ability to activate various receptors in endothelial cells.

2.1. Ginsenosides target endothelial NOS, phosphatidylinositol 3-kinase/Akt pathway in endothelial cells

Nitric oxide (NO) is one of the most important compounds produced in endothelial cells by nitric oxide synthase (NOS) from L-arginine. NO has various biological activities and plays a crucial role in vascular endothelium homeostasis, prevention of injury, regulation of local cell growth, etc. [15]. Endothelial NOS (eNOS) in human aortic endothelial cells are regulated by the activation of androgen receptor and phosphotidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (PI3K/Akt) and MEK/extracellular signal-regulated kinase (ERK) pathways. Most ginsenosides have the ability to activate NO production, but Rb1 possesses proven elucidation by triggering signaling pathways mentioned above. Rb1 prevents homocysteine (Hcy)-induced oxidative damage to endothelial cells by activating the PI3K/Akt pathway and mitigating the protein kinase C (PKC) pathway to maintain the phosphorylation state of eNOS, thus enabling NO production [3]. Oxidized low-density lipoprotein is a most common risk factor for endothelial dysfunction in coronary atherosclerosis. It prevents NO production in endothelial cells by downregulating the expression of eNOS messenger RNA (mRNA) [16]. However, Rb1 could reverse this

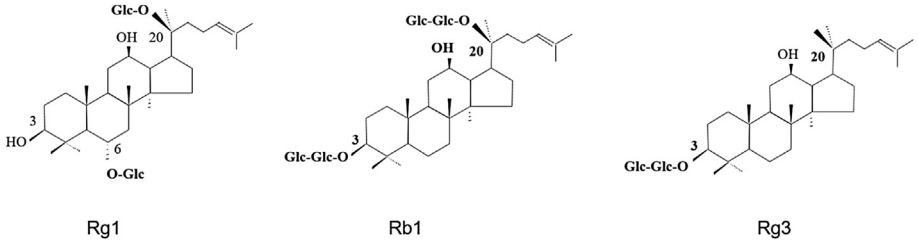

Fig. 1. Structure of ginsenosides Rg1, Rb1, and Rg3.

effect by upregulating the expression of eNOS in vascular endothelial cells, thereby sustaining the production of NO (Fig. 2). Besides Rb1, Rg3-mediated eNOS activation is dependent on glucocorticoid receptor (GR) and estrogen receptor (ER) signaling cascade involving PI3K and c-Jun N-terminal kinase [17].

2.2. Attenuating homocysteinemia

Hcy is an intermediate product in the biosynthesis of amino acids methionine and cysteine. The elevated level of Hcy is related to increased risk for CVD. In endothelial dysfunction, the effect of Hcy is linked to the generation of oxidative stress, nuclear factor kappa B (NF-κB) activation, inflammation, and inhibition of eNOS, which are collectively recognized as independent risk factors for atherosclerosis [18]. During Hcy-induced oxidative damage to endothelial cells, ginsenoside Rb1 prevents damage by inducing NO production and eNOS expression, and also by promoting the secretion of ghrelin, which suggests an important treatment strategy during homocysteinemic conditions [19].

Many patients with coronary heart disease or cerebrovascular atherosclerosis have hyperhomocysteinemia, which causes endothelial cell dysfunction and induces apoptosis. Recent studies target the secretion of ghrelin during Hcy-induced vascular endothelial dysfunction and structural damage. Rb1 excites NO production in human aortic endothelial cells 15 min after the treatment and reaches a maximum by 30 min. It was found that it induces phosphorylation of eNOS protein at Ser1177 in less than 10 min by phosphorylating Akt at Ser473 (via P13K signaling) and ERK1/2 at Thr202/Thr204 (via MEK signaling). Androgen receptor-mediated function is implicated in the phosphorylation of eNOS by Rb1 (Fig. 2) [20]. Apart from promoting eNOS phosphorylation, Rb1 could prevent the oxidative damage induced by hydrogen peroxide.

2.3. Antiangiogenesis in CVDs

Angiogenesis is the development of new blood vessels from preexisting endothelium or vasculature. It is a dynamic multistep developmental process that happens during wound healing and fetal development [21]. Furthermore, abnormal angiogenesis is contributed to the pathogenesis of diabetic retinopathy, rheumatoid arthritis, and tumor development [22]. Multiple molecular mediators and signaling mechanisms have been implicated in this process such as growth factors, intracellular signaling pathways, and cell–cell interactions. The production of NO is vital for proper vasodilatory function, as well as antiproliferative and antiapoptotic states of endothelial cells. However, a recent investigation suggests an involvement of a few microRNA (miRNAs) in promoting or suppressing angiogenesis. By using human umbilical cord endothelial cells (HUVECs), it was shown that Rg1 has a direct relationship in downregulating five miRNAs, including that of miRNA-214, which is closely associated with eNOS expression and angiogenesis [23]. MiR-214 overexpression downregulates XBP1 transcription factor, by which it suppresses angiogenesis; moreover, in chronic cardiac failure patients, miR214 is found to be upregulated [24]. Certainly, 20(*S*)-Rg3 is more active than 20(*R*)-Rg3; in addition, their mode of signaling is also different in promoting angiogenesis. 20(*S*)-Rg3 enhances eNOS production by rapidly inducing the ERK/Akt signaling pathway via the activation of peroxisome proliferator-activated receptor-γ (PPAR-γ) and induces HUVECs proliferation. However, the stereoisomer 20(*R*)-Rg3 reduces angiogenic activity in HUVECs at low concentration (μM), whereas at higher concentration (mM) it promotes angiogenesis by activating PPAR-γ. The activity of Rg3 depends on the state at which the endothelial cells are in; it promotes angiogenesis when they are activated, and it suppresses angiogenesis when they are not [22]. Rg1 induces angiogenesis by a nongenomic crosstalk with GR and fibroblast growth factor [25].

2.4. Preventive effect in cardiac injury

Cardiac hypertrophy is characterized by the adaptive increase in heart size by the enlargement of individual cardiomyocytes. Prolonged cardiac hypertrophy leads to cardiomyopathy, heart failure, or even sudden death [26]. Drugs such as angiotensin-converting enzymes and calcium channel blockers can decrease cardiac hypertrophy, and NOs have shown strong antihypertrophy effects. Among various ginsenosides, Rb1, in particular, has shown an antihypertrophic effect by NO release and reducing intracellular Ca^{2+}

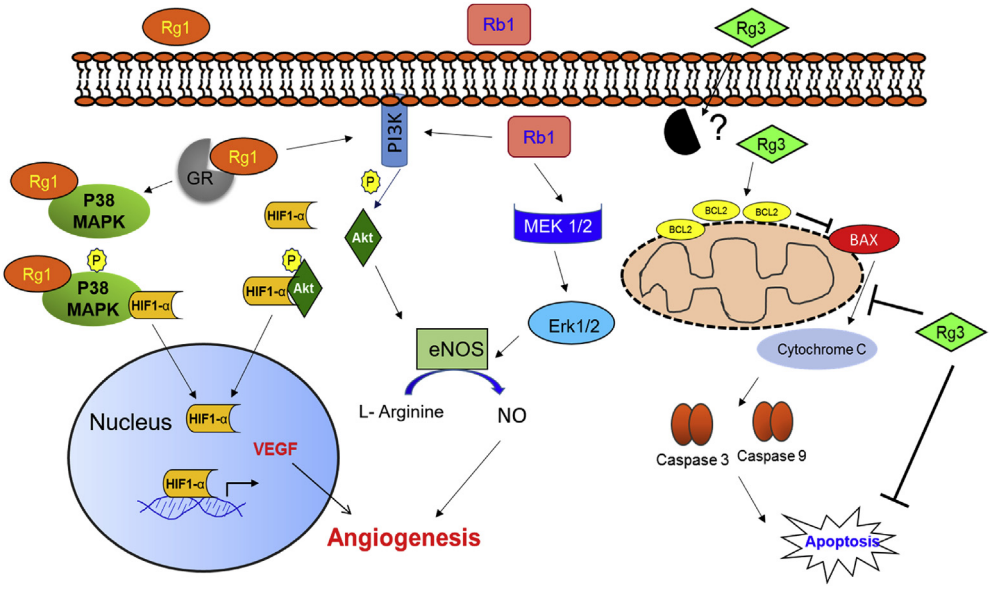

Fig. 2. Effect of ginsenosides Rb1, Rg1, and Rg3 on endothelial cells. Bax, Bcl-2-associated X protein; Bcl2, B-cell lymphoma 2; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; GR, glucocorticoid receptor; HIF1-α, hypoxia-inducible factor 1-alpha; MAPK, mitogen-activated protein kinase; PI3K/AKT, phosphatidylinositol 3-kinase/AKT; VEGF, vascular endothelial growth factor.

concentration in myocytes. Moreover, it attenuates the expression of NFAT3 and GATA4 transcription factors in cardiomyocytes to exhibit its antihypertrophic activity by reducing calcineurin signal transduction pathway [27]. Interestingly, Rg1 shows a protective effect on left ventricular hypertrophic condition by activating p38 and mitogen-activated protein kinase (MAPK) signaling, and promotes angiogenesis by increasing the expression of hypoxia-inducible factor-1 and vascular endothelial growth factor (Fig. 2) [28].

Myocardial ischemia/reperfusion (MI/R) injury is another serious cardiac arrest caused by thrombosis that results from a sudden impairment in coronary blood supply. It is partly mediated by ROS generation and exacerbated by ischemic reperfusion (IR) injury. Under hypoxic conditions, this may lead to cellular damage and acute signaling pathway, which leads to cell death. Ca^{2+} influx into vascular myocytes has been considered deleterious during IR injury. It was proven that Rg1 treatment directly suppresses the Ca^{2+} channel to inhibit Ca^{2+} overload in cardiomyocytes and improves the production of antioxidant enzymes such as T-SOD, CAT, and GSH, in cardiac myocytes during myocardial ischemia [29]. Meanwhile, Rg3 inhibits apoptosis caused by MI/R injury by inhibiting the activation of caspase-3 and caspase-9 and altering the ratio of Bcl-2/Bax while increasing the expression of p-Akt, eNOS in rat neonatal cardiomyocytes [30]. The preconditioning of ginsenosides shows a novel therapeutic potential with better results in MI/R injury. In streptozotocin-induced Mi/R injury in diabetic rats, preconditioning with Rb1 reduces cardiomyocyte apoptosis and alleviates cardiac dysfunction despite impaired PI3K/Akt signaling [31]. Rb1 preconditioning of diabetic rats at a dose of 40 mg/kg 10 min before the MI/R injury reduces the severity of MI/R and increases NOS expression and NO release [32]. Rg1 suppresses human arterial vascular smooth muscle cell proliferation by blocking tumor necrosis factor-α signaling and cell cycle arrest at G1 phase.

This inhibition is achieved by diminishing the activation of Akt, ERK1/2, and protein kinase B signaling cascades [33]. Inflammatory cells such as leukocytes adhere to intracellular adhesion molecule-1 (ICAM1) and promote inflammatory reactions. IR injury upregulates ICAM1 expression because of the generation of ROS. Rb1 treatment controls ROS production by preventing ICAM1 expression [34], thereby attenuating inflammatory reactions in IR injury. Ventricular remodeling is a phenomenon associated with lesion repair and ventricular composition during MI/R injury. This may lead to decline in left ventricular systolic function followed by heart failure and death. Apart from the lethal effects of MI/R injury, injection with ginsenoside Rb1 (2 mg/kg) for 4 wk reduces the left ventricular remodeling, presumably by the renin–angiotensin system. Likewise, continuous application of Rg1 (5 mg/kg/day) to rats with acute myocardial infarction increased the number of peripheral blood stem cells and stimulated their homing to the infarcted myocardium [32].

3. Impact of ginsenosides in ROS, cancer, and apoptosis

Cancer is rated as a dreadful disease with increasing mortality rate worldwide. Although various treatment methods are available for cancer, complementary and alternative medicines have been rapidly gaining popularity in recent years because they increased chances of patient survival with little side effects [35]. Ginsenoside saponins are considered an excellent option for their anticancer property, and their effect is directly proportional to the number of sugar moieties, the position of a hydroxyl group, and stereoselectivity [9].

3.1. *Rg3 outclass other ginsenosides in cancer prevention*

The anticancer evaluation of ginsenoside relies heavily on its parental compound. Orally administered ginsenosides are poorly absorbed, whereas some of them are broken down into simpler compounds by intestinal bacterial flora, which allows them to be absorbed readily in the body. Compared with bulkier ginsenosides, ginsenoside Rg3 is less polar—hence it has a pronounced effect in different cancer tissues. It has been broadly researched and prescribed clinically against cancer in China [36]. Radiation therapy combined with surgery or chemotherapy is applied to eradicate cancerous cells and its growth. However, cancer cells continuously evade the treatment because of the continuous activation of NF-κB transcription factor. However, Rg3 treatment in γ-ray sensitized lung cancer cells suppresses NF-κB activity significantly, leading to the inhibition of tumor progression (Fig. 3) [37]. Histone acetylation by histone acetyltransferases is characterized by increased gene transcription whereas histone deacetylation by histone deacetylase is typically correlated with gene silencing. Rg3 treatment in skin melanoma cell reduces the expression of histone deacetylase-3, which promotes the acetylation of p53 protein. The acetylated p53 protein could arrest the proliferation of cell cycle in the G0/G1 phase [38].

Matrix metalloproteinases (MMPs) are endopeptidases that specifically degrade collagens and noncollagenous proteins. The damaged liver protects itself from MMP activity by overexpressing tissue inhibitors of metalloproteinase and suppresses MMPs. The administration of Rb1 exhibits antiproliferative effect by inhibiting transforming growth factor β1 (TGF-β1), MMP-2, and tissue inhibitors of metalloproteinase-1 activation and by preventing the activation of the *α-SMA* gene [39]. In hepatocellular carcinoma, Rg3 exhibits its protective role through caspase-mediated apoptosis [40] by altering Bax and Bcl-2 expression to enable the release of pro-apoptotic factor such as cytochrome *c* [41]. Moreover, by suppressing NF-κB activation, Rg3 reduces the expression of Ki67, a nuclear protein expressed during cell cycle stages—namely, S, G1, G2, M phases, vascular endothelial growth factor, and CD34—to induce apoptosis in cancer cells. Gallbladder cancer (GBC) is another life-threatening disease with poor prognosis and treatment method. 20(*S*)-Rg3 reduces GBC progression remarkably by activating the p53-mediated apoptotic pathway [42]. In another study, when Rg3 was orally administered to mice with GBC, it induced cytotoxic effect by inducing endoplasmic stress mediated by C/EBP homology protein (CHOP) upregulation [43]. These studies reveal the potential therapeutic cure for GBC by ginsenoside Rg3.

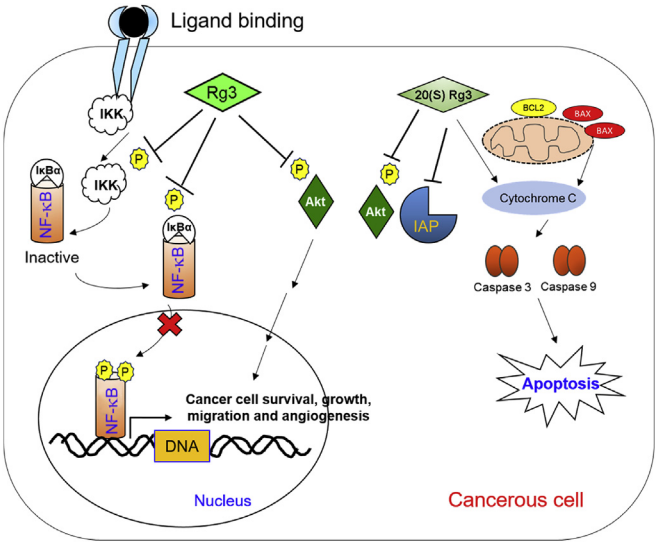

Fig. 3. Ginsenoside Rg3 and its stereoisomer 20(*S*)-Rg3 differentially attenuate cancer cell growth. IAP, inhibitors of apoptosis proteins; IKK, IκB kinase; NF-κB, nuclear factor κB.

When administered together with cisplatin, Rg3 improves the efficacy by inhibiting heme oxygenase-1 and NAD(P)H-quinone oxidoreductase-1 enzyme, and protects the kidney and liver by scavenging cisplatin-induced ROS generation [44]. The stereoisomer 20(*S*)-Rg3 prevents proliferation of ovarian cancer cells by triggering the caspase-3- and caspase-9-mediated apoptotic pathway. Rg3 downregulates PI3K/Akt and inhibitor of apoptosis family of proteins, thereby allowing caspase 3 and caspase 9 to function [45]. The role of Rg3 in colon/colorectal cancer is also well documented. It induces apoptosis and suppresses the migration of colon cancer by repressing the activation of NF-κB transcription factor [46]. The proliferation of tumor growth is inhibited by blocking nuclear translocation of β-catenin, thereby preventing Tcf transcriptional activity [47].

3.2. *Rb1 and anticancer mechanism*

Beyond its ability to suppress tumor growth and inflammation, Rb1 activates estrogen receptor subunit β (ER-β) to specifically enhance the production of pigment epithelium-derived factor (PEDF) by recruiting transcriptional activators to the promoter of PEDF. The secretion of PEDF inhibits tube formation in endothelial cells (Fig. 4) [48]. The selective binding of Rb1 to ERβ could be a potential pharmaceutical target for specific tissue types because it reduces the potential side effects. Rg1 exerts antimetastatic effect in liver cancer cells by inhibiting TGF-β1. This inhibition prevents the transition of epithelial cells to mesenchymal cells, thus preventing metastasis [49]. Chemoprevention in cancer treatment or from chemical carcinogen might involve preventing the interaction of carcinogen or endogenous free radicals to DNA, thereby suppressing disease progression [50].

Cytochrome P450 1A1 (CYP1A1) plays a significant role in the oxidation of endogenous and exogenous chemical compounds, and also most of the clinically prescribed drugs [51]. The transcriptional activation of CYP1A1 is mediated by aryl hydrocarbon receptor (AhR) binding to the promoter of CYP1A1. Ginsenosides Rg1 and Rb1 have shown a greater affinity for these receptors and enhance the activity of AhR to bind to the xenobiotic responsive element loci including CYP1A1 promoters (Fig. 4). Treatment with ginsenosides improved the expression of CYP1A1 via AhR, which might be useful for chemoprevention in chemical carcinogen [52]. Although ginsenosides are known for their proliferative effect on cells, they are an antagonist to cancer cells. It was demonstrated effectively in hepatocellular carcinoma that ginsenosides differentially activates the expression of c-Myc and hepatocyte nuclear factor-4α and drug metabolizing enzyme such as Cyp3a4 in a concentration-dependent manner. At 40 mg/L concentration, ginsenoside treatment enhances the expression of c-Myc and suppresses the proliferation of HepG2 cells, whereas at 200 mg/L it activates both c-Myc and hepatocyte nuclear factor-4 with a notable increase in Cyp3a4 [53]. These results are of high clinical significance and provide relevant information on the dosage of ginsenoside crude fractions or pure fractions used as an anticancer agent.

4. Diabetes and insulin metabolism

Diabetes mellitus (DM) is the eighth leading cause of death among both men and women worldwide and the fifth leading cause of death in women in 2012. It is acute, and chronic disease occurs owing to the impairment in insulin production by the pancreas and uptake by the body. Diabetes leads to serious complications that affect all the important organs during hyperglycemic conditions [54]. The hypoglycemic activity of ginseng and its extract has been known for a very long time. Different ginseng preparations were demonstrated to have efficacy in people with diabetes, and active research with cell lines and animal studies are exploring the possible mechanism by which the hypoglycemic action of ginsenosides could be achieved [55].

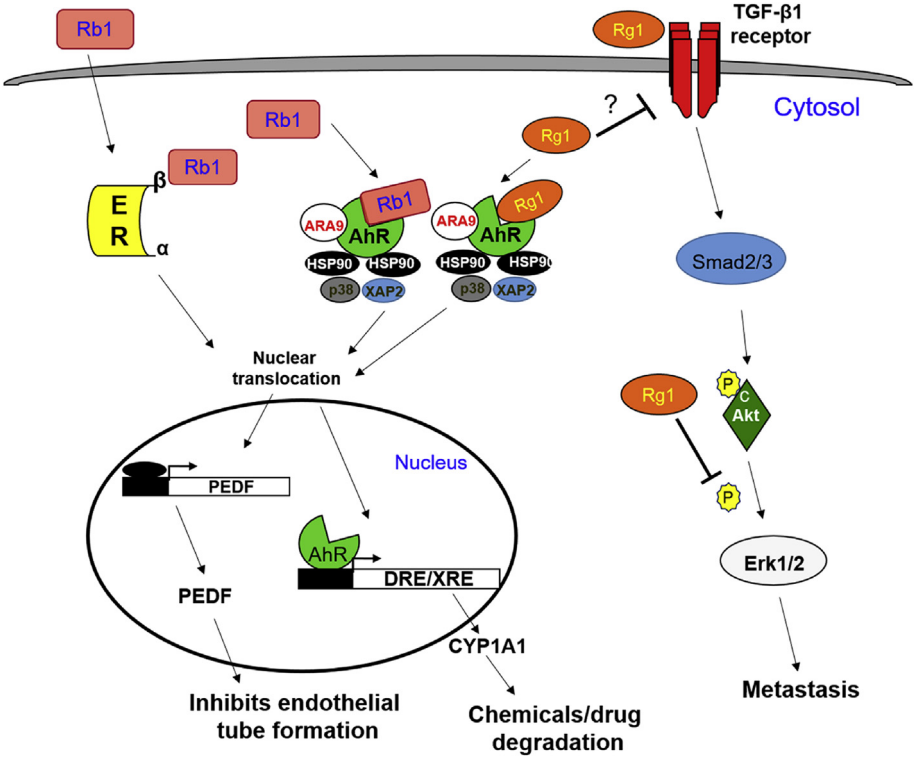

Fig. 4. Mechanism of cancer prevention by ginsenosides Rb1 and Rg1. AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PEDF, pigment epithelium derived factor; TGF-β1, transforming growth factor β1; XRE, xenobiotic responsive elements.

4.1. Enhancing glucose uptake and insulin sensitivity

Ginsenoside Rb1 has a significant antihyperglycemic effect and increases insulin sensitivity; thus, it has been used clinically to treat DM and its complications [56]. Furthermore, Rb1 has been shown to be useful in treating obesity and diabetes; notably, those effects are partially mediated by a reduction in food intake and body weight besides lowering glucose [57]. Acute intraperitoneal injection of Rb1 (10 mg/kg) significantly suppresses food intake in rats, although chronic treatment with Rb1 possesses no deleterious side effects in obese rats [58]. Glucose uptake in tissue is a critical step in maintaining glucose homeostasis, and PI3K/Akt pathway is one of the key pathways involved in the translocation of glucose transporter 4 (GLUT4) in muscles [59]. It enables GLUT4 transporter-mediated intracellular uptake of glucose to the cells. Hyperglycemic condition and enhanced unfolded protein response evoke ER stress by nicotinamide adenine dinucleotide phosphate-oxidase induced ROS generation. The stressed ER upregulates the secretion of interleukin-1β, leading to inflammation, which is a critical factor for insulin resistance. Rb1 suppresses insulin resistance due to inflammation by preventing interleukin-1β maturation and secretion in ER. It attenuates the activation of ER stress associated NLRP3 inflammasome while phosphorylating insulin receptor substrate-1 (IRS-1), thereby restoring the PI3K/Akt signaling pathway in adipose tissue [60].

Adenosine monophosphate kinase (AMPK) mediates the regulation of energy balance at the whole body level by responding to the hormones and nutrient signals. Ginsenoside Rg1 significantly enhanced the glucose uptake in myocytes by AMPK-mediated GLUT4 translocation (Fig. 5) [61]. In contrast, glucose homeostasis in hepatic cells is maintained by AMPK by inhibiting the expression of gluconeogenesis genes such as Glu-6-phosphate and phosphoenolpyruvate carboxylase. Rg1 suppresses hepatic gluconeogenesis by inducing the phosphorylation of liver kinase B1, AMPK, and forkhead box class O1 transcription factor [62]. By phosphorylating these kinases and transcription factors, Rg1 inhibits the expression of gluconeogenic genes in hepatic cells [62]. Similarly, Rb1 promotes insulin uptake in insulin-sensitive cells by GLUT1, GLUT4 translocation mediated by IRS-1 and protein kinase B [63]. Recent findings suggest that Rb1-mediated AMPK activation is amplified by the phosphorylation of downstream effector TBC1 domain family member 4, thereby enhancing glucose uptake in skeletal muscle cells. Moreover, it reduces hepatic glucose production by suppressing the expression of glucose-6-phosphate and phosphoenolpyruvate carboxykinase [57]. These actions of Rg1 and Rb1 provide a possible therapeutic target for type 2 diabetes.

The antihyperglycemic effect of ginsenoside Rg3 has been well established in recent years. Unlike Rb1 and Rg1, Rg3 stimulates the expression of IRS-1 and GLUT4 at the transcriptional level (Fig. 5). Rg3 treatment increases the phosphorylation of IRS-1 in myotubes than Akt; however, the classical AMPK pathway is unaltered. Strikingly, recent findings suggested a new role for Rg3. It improved the expression of the primary genes involved in mitochondrial biogenesis such as peroxisome proliferator-activated receptor gamma coactivator 1-α and its downstream targets. The Rg3-mediated mitochondrial biogenesis improves the energy status of skeletal muscle cells leading to enhanced insulin resistance [64]. Meanwhile, in adipocytes, Rg3 treatment stimulates glucose uptake by enhancing PI3K signaling mediated by IRS-1 signaling cascade [65]. These findings strengthen the antihyperglycemic effect of Rg3 and a possible type 2 DM drug.

4.2. Stereoselectivity and glucose metabolism

Based on the aglyone skeleton of ginsenosides, Rg3 efficiently stimulated the secretion of glucagon-like-peptide-1 in endocrine cells through the sweet taste receptor-mediated signaling cascade in type 2 DM mouse model [66]. Rg3 induces the secretion of insulin in pancreatic cell lines, and the stereoisomers of Rg3 have shown differential activities in causing insulin secretion. 20(*S*)-Rg3 is superior to 20(*R*)-Rg3 in phosphorylating AMPK and acetyl-coA carboxylase in myotubes and inducing insulin secretion in β-cells [67]. 20(*R*)-Rg3 specifically phosphorylates AMPK and Ca^{2+}/calmodulin-dependent protein kinase kinase, and facilitates GLUT4 translocation in myotubes [68]. Rg1 treatment reduces oxidative stress and attenuates myocardial apoptosis in diabetic rats. Rg1 treatment also significantly reduces CHOP, caspase-12 expression to ameliorate endoplasmic reticulum-induced apoptosis in diabetic

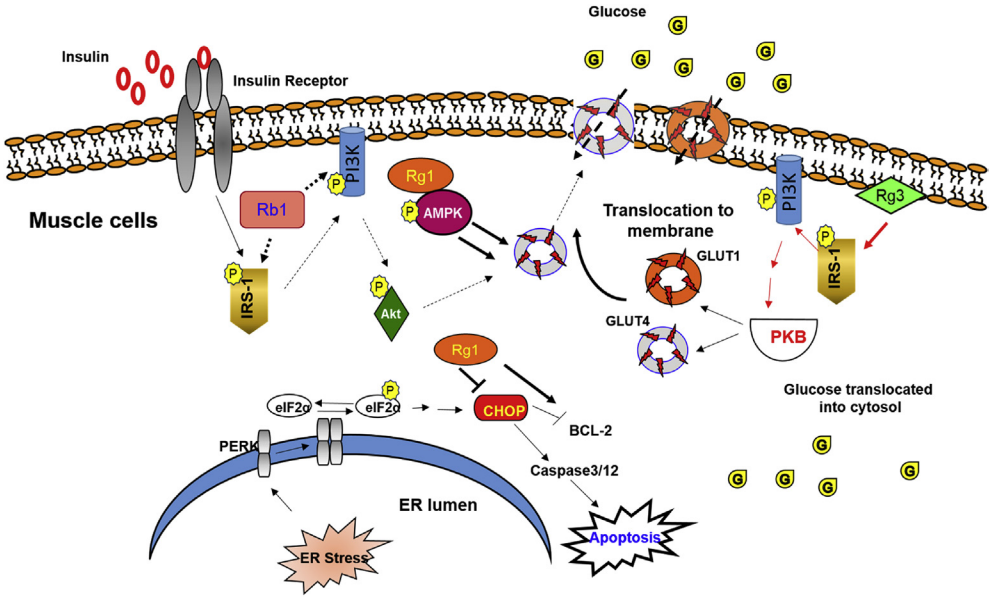

Fig. 5. Enhancing glucose uptake in muscle cells and insulin sensitive cells by ginsenosides Rb1, Rg1, and Rg3. AMPK, 5′ AMP-activated protein kinase; Bcl2, B-cell lymphoma 2; CHOP, CCAAT enhancer-binding protein homologous protein; eIF2α, eukaryotic initiation factor 2; ER, estrogen receptor; GLUT1, glucose transporter 1; GLUT4, glucose transporter 4; IRS-1, insulin receptor substrate 1; PERK, protein kinase R (PKR)-like endoplasmic reticulum kinase; PI3K, phosphatidylinositol 3 kinase; PKB, protein kinase B.

rats [69]. Besides enhancing glucose uptake and insulin metabolism, ginsenosides also protect the body cells from the aftermath of diabetics.

5. Ginsenoside signaling targets in nervous system

Numerous reports are proclaiming the neuroprotective effects of ginsenosides and ginsenoside derivatives in neurological disorders, stroke, and neuroinflammation. Ginsenosides are known to affect voltage-gated channels such as the Ca^{2+}, Na^{+}, K^{+}, ligand-gated channels 5-HT3, the α-7 nicotinic acetylcholine, and *N*-methyl-D-aspartate (NMDA) receptors [70]. The magnitude of their action depends on the receptors they bind to and the extent at which they get absorbed in the brain. Rb1 and Rg1 increase synaptosomal choline uptake and acetylcholine release in rat brain by enhancing acetylcholine mRNA expression [71], but Rb1 is more useful than Rg1 in triggering acetylcholine esterase in the hippocampus of mice. Rg3 pretreatment attenuates Hcy-induced neurotoxicity by reducing the intracellular Ca^{2+} elevation by acting on NMDA receptor (NMDAR) [70].

5.1. Attenuation of neurodegeneration

Neurodegeneration accounts for the majority of neurological disorders that result in gradual loss of neurons in the brain and spinal cord leading to ataxia or dementia. Excitotoxicity, mitochondrial disorders, and finally apoptosis have been the leading cause of neurodegeneration in AD, Parkinson's disease (PD), Huntington's disease, amyotrophic lateral sclerosis, stroke, and other dementias [72]. Neurotrophic factors support the growth, survival, and differentiation of neurons by the activation of the tropomyosin-related kinase family of receptor kinases [73]. Neurotrophic factor transcription is highly regulated; for example, brain-derived neurotrophic factor (BDNF) has eight different promoters, each of which leads to different transcripts among them. The transcription of the IVth exon is mediated by calcium and cAMP-response element through a protein signaling cascade involving MAPK-ERK, PI3K, PLC, and NMDAR activation [74]. When Schwann cells were incubated with ginsenosides Rb1 and Rg1, they induced the secretion of NGF and BDNF by an intracellular increase of cyclic AMP via protein kinase A signaling [75]. Likewise, long-term administration of Rg1 enhances the transcription of BDNF by attenuating phospho-CREB level [76] and increases BDNF signaling in hippocampal neurogenesis in mice brain. It helps to promote neuronal survival in cerebral ischemia by facilitating the production of BDNF and enhances the translation of glial-derived neurotrophic factor while downregulating the expression of caspase-3 (Fig. 6). Moreover, it reduces the expression of anti-apoptotic proteins Bcl-2 and Bax during cerebral ischemia and DEX-induced cytotoxicity [77].

Accumulation of α-synuclein plays a significant role in the pathogenesis of the genetic form of PD and other synucleinopathies. Hence, inhibition of on α-syn fibrillation is considered an effective strategy in PD. Ginsenoside Rb1 exhibits a significant inhibitory effect on α-syn fibrillation by disaggregated fibrils, and blocks α-syn polymerization. Rb1 outclasses Rg1 and Rg3 in neuroprotection and its ability to inhibit the formation of mature amyloid fibrils containing β-sheet structures [78].

5.2. Attenuation of amyloid-β formation in AD

AD is characterized by the accumulation of amyloid-β (Aβ) plaque, neurofibrillary tangles in the brain, along with hyperphosphorylated microtubule-associated protein tau [79], which promotes neurodegeneration. Cyclin-dependent kinase-5 is a crucial enzyme that phosphorylates tau, which is regulated by cyclin-related activator molecules such as p35, p39, p25, and p29. p25 is a product of calpain-mediated proteolysis of p35, which forms a stable complex with cdk5 and hyperphosphorylates tau protein. The abnormal regulation of calcium level in neuronal cells is implicated in the activation of calpain, which increases AD pathology. Rb1 pretreatment stabilizes the intracellular calcium homeostasis and microtubule integrity by suppressing the transcription of cdk5 and p25 in cortical neurons [80]. The formation of Aβ-peptide involves the proteolytic cleavage of amyloid protein precursor (APP) by α, γ, and β-secretase. α-Secretase is a metalloprotease that cleaves APP within the Aβ domain, thus generating amyloid-β peptide. Upon cleavage, APP gets released into the extracellular environment. The ER-mediated signaling pathway involving PKC and MAPK serves as a hub for the regulation of α-secretase. It has been shown previously that activation of PKC decreases Aβ production.

Rg1 has antiaging and antineurodegenerative effects; it takes two routes in attenuating Aβ aggregation: (1) by enhancing the activity of α-secretase; (2) by ER-β-dependent PI3K/Akt signaling and PPAR-γ-mediated suppression of β-secretase1 (BACE1), also in age-dependent dementia [81]. BACE1 is a membrane-bound protease that cleaves APP in the β site; its activity becomes increased in the frontal and temporal cortices of AD patients, and the transcription of BACE1 increases with the downregulation of PPAR-γ (Fig. 6) [82]. Molecular docking studies with major ginsenosides have suggested that ginsenosides Rb1, Rg1, and Rg3 showed significant inhibitory action on acetylcholine esterase, and BACE1. These results might provide a strong validation for the treatment of AD by ginsenosides [83]. It was demonstrated that treatment with Rg1 significantly upregulated the PPAR-γ protein level in AD condition, which represses the transcription of BACE1 [84]. Another study has reported that Rg1 ameliorates Aβ-induced neuronal apoptosis by ERα and GR-mediated signal transduction. This activation upregulates the phosphorylation of ERK1/2 and inhibits nuclear translocation of NF-κB. Furthermore, it facilitates the differentiation of mouse embryonic stem cells to neuronal lineage in cell culture by the activation of GR-dependent downstream phosphorylation of ERK1/2 and Akt signaling [85].

Treatment with Rb1 could increase p-Akt levels and p-ERK1/2 mediated signaling to prevent the apoptosis induced by Aβ [86]. Exposure to Aβ leads to the accumulation of ROS and lipid peroxidation, which cause caspase-3-mediated neuronal apoptosis. Rb1 treatment prevents apoptosis by modulating the Bcl2/Bax ratio and by inhibiting caspase-3 activation [56]. Rb1 enhances neural serotonin receptor (5-hydroxytryptamine) synthesis by upregulating the activities of tryptophan hydroxylases and by decreasing monoamine oxidases genes mainly by ER [87]. This action of Rb1 enables an increase in serotonin production and prevents its assimilation. Interestingly, ginsenosides have a preferential affinity to ER; Rg1 activates ER-α subunit and phosphorylates AF-1 domain in less than 5 min [88].

5.3. Cerebral ischemic injury

The increased level of extracellular glutamate concentration during cerebral ischemia amplifies the effect of stroke and traumatic injury. NMDARs are ligand-gated ion channel receptors that get activated by glutamate in nerve cells. They participate in several neurological disorders including ischemia, schizophrenia, and aging. Antagonist against NMDARs has shown to improve IR injury and other neuropathological symptoms [89]. Rg1 displays a unique expression pattern for NMDAR subunits in the brain. The intracerebroventricular infusion increases the level of NMDAR subunit NR1 expression in the temporal cortex, caudate putamen,

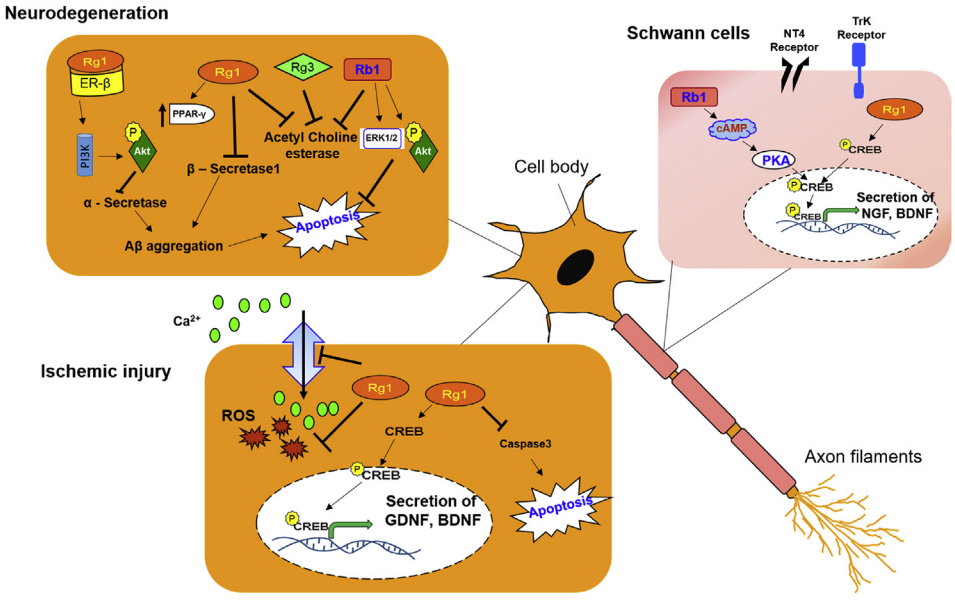

Fig. 6. Action of ginsenosides in neurodegeneration, ischemic injury, and secretion of neurotrophic factors. BDNF, brain-derived neurotrophic factor; cAMP, adenosine monophosphate; CREB, cAMP response element binding; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; GDNF, glial cell-derived neurotrophic factor; NGF, nerve growth factor; PKA, protein kinase A; PPAR-γ, peroxisome proliferator-activated receptor gamma; ROS, reactive oxygen species; Trk, tropomyosin-related kinase.

hippocampus, and granule layer of the cerebellum. NR2B mRNA is elevated in the cortex, thalamus, and caudate putamen, but NR2C mRNA is increased only in the granule layer of the cerebellum. Apart from exhibiting the differential expression pattern for NMDARs, Rg1 has also been shown to suppress their activity in pathological conditions such as hypoxic–ischemic injury by inhibiting Ca^{2+} influx through NMDARs and other voltage-dependent ion channels [90]. It promotes hippocampal neurogenesis by modulating the production of intracellular NOS and NMDARs in posttransient ischemia [91]. Long-term administration of Rg1 promotes synaptic plasticity in age-related cognitive decline by enhancing the expression of plasticity-related proteins such as NMDAR subunit 1, synaptophysin, and calcium/calmodulin-dependent protein kinase II alpha [92].

Rb1 helps to regulate homeostasis in the brain by regulating the expression of neuropeptide Y (NPY). NPY is associated with the infundibular nucleus of the hypothalamus, as part of a reciprocal circuit controlling reproduction and energy balance; it responds to hormones such as insulin, leptin, ghrelin, as well as glucose. It has a great impact on physiological functions such as food intake, energy expenditure, reproduction, and thermoregulation [93]; hence, during obesity, NPY levels become altered in the hypothalamus [94]. By triggering the classical PI3K/Akt signaling, Rb1 significantly reduces NPY gene expression in the hypothalamus of obese rats and c-Fos expression in the hindbrain. Chronic Rb1 treatment significantly reduced the NPY gene expression in rat brain and also in serum [58,95], by which Rb1 helps to maintain homeostasis in the body.

Among the other ginsenosides, only Rb1 protects neurons against oxidative injury by enhancing the Nrf2/Ho-1 pathway. The activation of Nrf2 upregulates the transcription of multiple antioxidant response element controlled genes, leading to the expression of drug metabolizing enzymes [96,97]. Interestingly, Rb1 could prevent endoplasmic reticulum stress caused by the accumulation of misfolded protein. If it is uncontrolled, it will result in apoptosis mediated by CHOP, caspase-12, and c-Jun N-terminal kinase pathways [98]. It has been reported that hyperglycemic condition evokes CHOP-mediated apoptosis in neurons. Ginsenoside Rb1 prevents neurotoxicity in high glucose-induced neuronal death by attenuating CHOP signaling mediated ER stress-mediated apoptotic signaling pathway. In neurons, glycogen synthase kinase-3β (GSK-3β) serves as an upstream activator of CHOP signaling. Rb1 treatment inhibits GSK-3β-mediated CHOP signaling, thereby preventing neuronal apoptosis due to ER stress [99]. Moreover, Rb1 treatment attenuates intracellular ROS production as well as disruption of mitochondrial dysfunction [100].

6. Conclusion and future recommendations

It is apparent that ginsenosides have many beneficial effects by interacting with various signaling pathways and effecting changes at the transcription level. The interaction of ginsenosides with signaling networks and receptors exhibits different benefits such as improving brain cognitive functions, attenuating inflammatory reactions, providing cytotoxicity to various cancerous cells, as well as improving glucose uptake and insulin metabolism in diabetic condition. Even though major ginsenosides are poorly absorbed in the intestinal tract, few attempts have been undertaken to increase their bioavailability using various methods such as conjugating them with polymers, fatty acids, and utilization of nanodelivery systems. However, these investigations are still at the preliminary level to guarantee their use for human consumption. In the future, the ginseng research must be directed channelised (1) To develop suitable drug delivery system for the ginsenosides; (2) To improve the pharmacokinetics of ginsenosides in blood circulation; (3) To assess the safety of PPD- and PPT-type ginsenosides and transformed metabolites of them in humans. For this, researchers must be encouraged to conduct improved animal testing and clinical trials to exhibit the beneficiary role of ginsenosides. Most of the available studies focused mainly on cell culture practices, but the animal studies and clinical trials are limited to describe the potential role of ginsenosides. The pharmacokinetics of crude ginseng preparations and purified ginsenosides in healthy humans must be undertaken to understand their comprehensive effect, and this has to be done sooner rather than later. As a final point, to ensure ginsenosides and ginseng-based preparations are the best alternative, medicine for various disease and disorders translational research has to be increased.

Conflicts of interest

The authors have no conflicts of interest.

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