Black Ginseng

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Black ginseng is a processed ginseng which prepared by steaming and drying of white or red ginseng for several times (usually 9). This process resulting in extensive changes in types and amounts of several secondary metabolites. Thus, primary ginsenosides (the main active inredients in ginseng) were transformed into less polar derivatives by steaming. In addition, apparent changes happened to other secondary metabolites such as the increasing of phenolic compounds, reducing sugars and acidic polysaccharides as well as the decrease in concentrations of free amino acids and total polysaccharides. Furthermore, the presence of some Maillard reaction products like maltol was also engaged. These obvious chemical changes were associated with a noticeable superiority for black ginseng over white and red ginseng in most of the comparative biological studies including anticarcinogenic, immunomodulatory, anti-Inflammatory, antidiabetic, hepatoprotective, antioxidant and tonic effects.

Keywords: Black ginseng ; Transformed ginsenosides ; Anti-cancer ; Anti-inflammatory ; Ginsenoside-Rg3 ; Ginsenoside-Rg5

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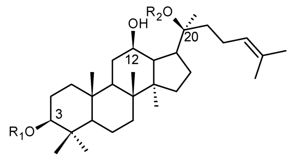
1. Introduction

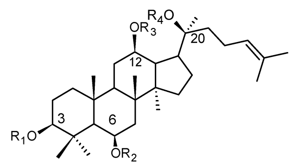
Ginseng is the roots and rhizomes of *Panax ginseng* Mey., belonging to the perennial plants of genus Panax and family Araliaceae. The most commonly used species are: *Panax ginseng* (Asian ginseng), *Panax quinquefolius* (American ginseng) and *Panax notoginseng* (Chinese notoginseng or Sanqi)[1]. Ginseng has been used in China for more than 4000 years as a tonic and restorative, promoting health, treating hemorrhage, impotence, anorexia and infections. Modern clinical studies proved various pharmacological effects for ginseng. For instance, the aphrodisiac and adaptogenic properties of ginseng were reported to result from its effects on the hypothalamic–pituitary–adrenal axis, resulting in an elevation of corticotropin and corticosteroids levels in plasma[2]. Moreover, the immune enhancing activity was correlated to its ability to regulate different types of immune cells such as dendritic cells, macrophages, natural killer cells, B cells and T cells [3]. Induction of apoptosis, inhibition of angiogenesis of cancer cells in addition to inhibition of cell proliferation and immunosurveillance are reported mechanisms for the anticancer activities of *Panax ginseng* [4]. According to *in vitro* and *in vivo* results, ginseng could treat cardiovascular diseases through antioxidation properties, reduction of platelet adhesion, vasomotor regulation, enhancing lipid profiles, and influencing several ion channels [5]. What is more, ginseng exhibited treating effects in many central nervous system disorders such as Parkinson’s disease, Alzheimer’s disease, depression, cerebral ischemia as well as several other neurodevelopmental disorders. Ginseng could exert these activities through different pathways such as neuroprotection, synaptic plasticity regulation, decreasing neuroinflammatory processes and regulation of neurotransmitter release [6].

The main bioactive secondary metabolites in ginseng are ginsenosides, which belong to dammarane-type triterpene saponins with different sugar moieties attached at C-3 and C-20. Ginsenosides are named ‘Rx’: ‘R’ refers to the root and ‘x’ describes the ascending alphabetical order of their chromatographic polarity. Accordingly, Ra is the highest polar ginsenoside, while Rb is less polar than Ra [7]. As shown in Figure 1, ginsenosides can be classified into two major classes; (1) the protopanaxadiol (PPD) type in which the sugar units are attached to the β-OH at C-3 and/or C-20 (such as Ra_1, Ra_2, Ra_3, Rb_1, Rb_2, R_c, R_d, Rg_3, Rh_2 and Rs_1) and (2) the protopanaxatriol (PPT) type in which the sugar units are attached to the α- OH at C-6 and/or the β-OH at C-20 (such as Re, Rf, Rg, Rg_1, Rg_2 and Rh_1). Moreover, some other ginsenosides, such as ocotillol group, have a five-membered epoxy ring at C-20 (for instance F11) and the oleanolic acid saponins (for example, Ro, C-IV and C-Ib) have also been isolated and identified [8]. The major ginsenosides in the white or (sun-dried) ginseng’s roots are Rg_1, Re, Rb_1, Rc, Rb_2, Rb_3 and Rd, which make up more than 70% of total ginsenosides [9]. In addition, some PPD or PPT-type ginsenosides with structure changes in side-chain were isolated and exhibited strong biological activities [10].

Figure 1. Major classes of Ginsenoside.



Protopanaxadiol (PPD) ginsenosides



Protopanaxatriol (PPT) ginsenosides

According to our previous research, the three generally-used species in *Panax genus* could be distinguished by the presence of some characteristic ginsenosides such as the notoginsenoside R_1, which is a characteristic marker for *P. notoginseng,* while the ginsenoside Rf is a marker for *P. ginseng.* Furthermore, the ocotillol-type triterpene 24(R)-pseudo-ginsenoside F_{11} presents in high amounts in *P. quinquefolius* and in very minute amounts in *P. ginseng*, and hence a high ginsenoside Rf/24(R)-pseudo-ginsenoside F_{11} ratio (>700) clearly differentiates *P. ginseng* and *P. quinquefolius* [11]. Likewise, the ratio between Rb_1 and Rg_1 is a very clear marker as the high Rb_1/Rg_1 ratio (around 10 or greater) indicates *P. quinquefolius*, while low (1–3) indicates *P. ginseng* [12]. Additionally, the ginseng plant contains some other important secondary metabolites such as ginseng oils, phytosterol, carbohydrates, amino acids, peptides, vitamins, minerals, certain enzymes and phenolic compounds (caffeic acid, syringic acid, p-coumaric acid, ferulic acid and cinnamic acid) [13].

Due to black ginseng having originated and being distributed in Asia, the term black ginseng in this article refers to black *P. ginsing*, unless otherwise stated.

2. Preparation

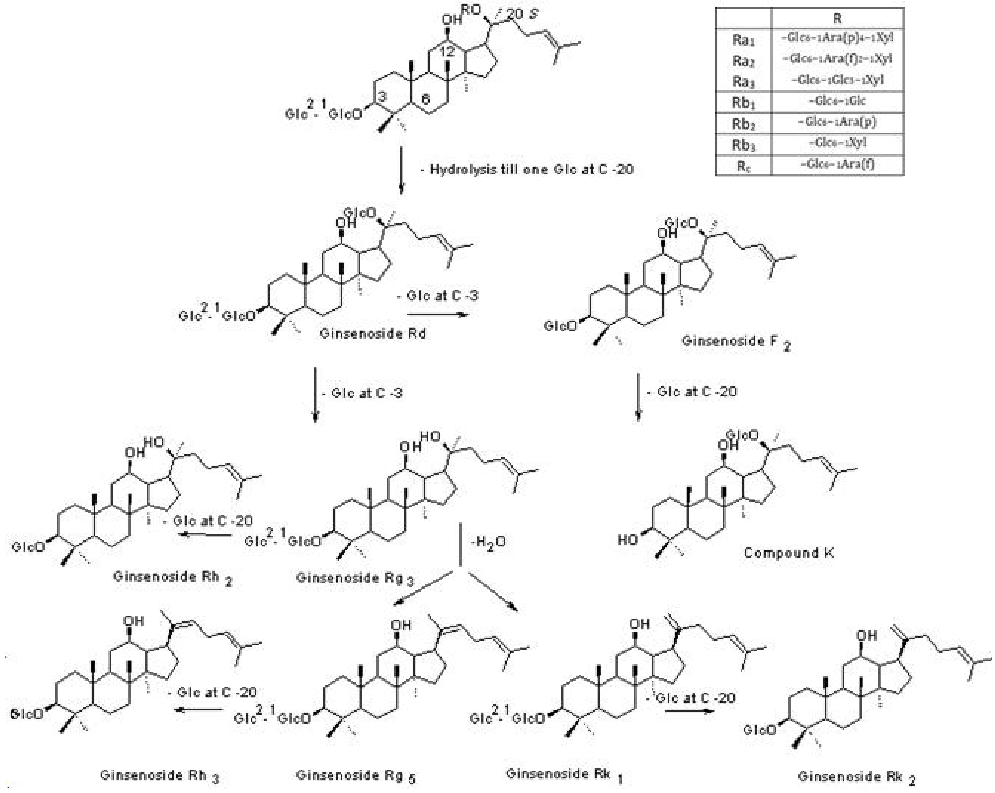
Black ginseng is usually developed from fresh or white ginseng steamed several times (usually 9 times) at 96 °C for 3 h, followed by hot air-drying at 50 °C for 24 h [14]. While the processing method of “steaming and drying 9 times” has always been used in TCM in the processing of Radix Rehmanniae [15], this method was rarely used for the processing of ginseng in the ancient TCM. Black ginseng was firstly prepared in South Korea then widely used in China and Southeast Asian countries. The optimum conditions for the preparation of black ginseng were determined to be steaming at 113.04 °C for 18 h and drying at 100 °C for 8.03 h; this method resulted in 0.75 mg/g ginsenoside Rg_3, 3.24 mg/g polyphenol, 13.72 mg/g acidic polysaccharide and 0.26 part per billion (ppb) benzopyrene [16]. Other methods have been reported in the preparation of black ginseng, such as steaming the white ginseng three times at 120 C for 30 min after soaking it in grape juice for 24 h, which was more effective in the transformation of ginsenoside Rg3 compared to the traditional method (got Rg3 approximately 18 times more than that in red ginseng) [17]. In addition, black ginseng was prepared by steaming white ginseng for 3 h, drying and steaming again for 6 h. In this procedure, the main ginsenosides were Rg_3, Rk_1 and Rg_5 and the ginsenoside Rg_3 was determined to be 11.48 mg/g of dried black ginseng [18]. The method of fermented black ginseng preparation was done by repeated steaming and drying of fresh ginseng before fermentation by incubation with Saccharomyces cerevisiae for 24 h [19]. On the other hand, flavored black ginseng was obtained by immersing white ginseng in garlic juice for 24 h, then putting it into the autoclave at 120 °C for 3 h, followed by air-drying in the oven at 60 °C. In this procedure, the primary saponins in ginseng, such as ginsenoside Re, Rg_1 and Rb_1, almost completely transformed to the rare secondary saponins and aglycones such as ginsenoside Rg_3, Rg_5 and protopanaxadiol. In addition, non-reducing polysaccharides such as starch are completely degraded into smaller reducing sugar units [20]. Sun ginseng is a new type of processed ginseng prepared by steaming of fresh ginseng at 120 °C for 2 h using an autoclave. Accordingly, ginsenosides Rg_5 and Rg_3 were found to be the most transformed ginsenosides with concentrations of 19% and 39% of all ginsenosides, respectively [21]. The optimum conditions to prepare black ginseng with safe levels of benzo(a)pyrene were reported to be: Steaming at a temperature between 80 and 120 °C and drying at a temperature less than 50 °C [22].

3. Phytochemical changes

The preparation of balck ginseng depends on the steaming and drying for several times (usually 9). The steaming process casues the transformation of several secondary metabolites in to other forms through different chemical reactions. The polar ginsenosides transform into specific less polar ginsenosides by hydrolysis, dehydration, decarboxylation and isomerization reactions. The notable structural changes are the hydrolysis of sugar moieties at C-3, C-6 or C-20 and subsequent dehydration at C-20. These reactions took place to the major primary ginsenosides (Rb_1, Rb_2, Rc, Rd, Re and Rg_1) as well as to other minor ginsenosides [23]. As shown in Figure 2, the ginsenosides Ra_1, Ra_2, Ra_3, Rb_1, Rb_2, Rb_3, Rc and Rd are converted to the ginsenosides Rg_3, F_2, compound K and Rh_2 in black ginseng through hydrolysis reactions for sugar moieties at C-3 and C-20. On the other side, the ginsenosides Rk_1 and Rg_5 are produced as a result of the dehydration reactions of Rg_3 [24] . The R-epimers of Rg_2, Rg_3 and Rh_1 were produced through isomerization of the corresponding ginsenosides and through addition reactions (selective attack of the hydroxyl group) for the dehydrated corresponding ginsenosides (Rk_1 and Rg_5 in case of 20(R)-Rg_3) [25].

Rh_1 and Rh_4 were deduced to be generated from the ginsenoside Rg_1 through hydrolysis and dehydration reactions, respectively. Interestingly, four ginsenoside (Rg_6, F_4, Rk_3, and Rh_4) were produced from the ginsenoside Rg_2 through hydrolysis and dehydration reactions [26]. The acetylated ginsenosides (20(S)-Rs_3 and 20(R)-Rs_3) were produced from the malonyl derivatives of Rb_1, Rb_2, Rc and Rd the by hydrolysis of glycosyl moiety at C-20 and decarboxylation of malonyl moiety attached to glycosyl linkage at C-3, 20(S)-Rs_3 further underwent dehydration to generate Rs_4 and Rs_5 . Generally, The steaming process causes an increase in the protopanaxadiol group to protopanaxatriol group ratio (PD/PT) from 1.9 to 8.4 in white and black ginseng, respectively [24] in addition, it also led to the production of some specific ginsenosides such as (20(S)-, 20(R)-Rg_3, Rk_3, Rh_4, Rk_1, Rg_5, …etc.) which are absent from white ginseng . As black ginseng was subjected to much more steaming, the concentration of these ginsenosides is much higher than red ginseng. Similarly, the steaming and drying processes led to some chemical changes in the other secondary metabolites. Although steaming causes a decrease in the contents of polysaccharides from 29.1% in fresh ginseng to be only 11.1% in black ginseng [27], it causes an increase in reducing sugars and acidic polysaccharides contents. The increase of reducing sugars and acidic polysaccharides contents in black ginseng with percentages of 128 % and 187.5 %, respectively, comparing the white ginseng was reported [25]. Similarly, the phenolic compounds content was increased more than three folds by steaming from 3.1 mg/g in white ginseng to be 10.6 mg/g in black ginseng [25]. In another comparative study, the phenolic contents of white ginseng and black ginseng roots of P*anax ginseng, P. notoginseng* and *P. quinquefolium* were evaluated to be 20.4±0.90 mg/g, 17.12±0.56 mg/g, 14.45±0.13 mg/g in white ginseng roots and to 34.3±0.18, 44.15±1.45, and 34.05±2.03 mg/g in the black ginseng roots, respectively. The identified phenolics included; ferulic, gentisic, cinnamic, syringic, and p-hydroxybenzoic acids combined with arginine and maltose due to Maillard reaction [28]. Another study reported the increase of salicylic acid, vanillic acid and p-coumaric acid contents from 0.121, 0.404 and 0.522 mg/100g in white ginseng to 0.394, 0.628 and 0.737 mg/100g in black ginseng, respectively, because of the steaming process [29]. In fact, the black color of black ginseng is a result of a chemical reaction named Maillard reaction, which is achemical reaction between reducing sugars and amino acids resulting in glycosylamines and or ketosamines [30]. The reported Maillard reaction products in ginseng due to steaming included: Argin-maltose [29] Arg-fru-glc, Arg-fru, maltol-3-O-β -D-glucoside in addition to maltol which increased from 2.598 mg/100g in white ginseng to 94.007 mg/100g by steaming [28]. The content of maltol was reported to be much higher in black ginseng than red and white ginseng [31]. The significant decrease of free amino acids from 17.9 mg/g in white ginseng to 2.79 mg/g after steaming has been reported [32]. Arginine's content (the most predominant amino acid in ginseng) was reduced from 10.4 to 1.38 mg/g and b-N-oxalyl-L-a,b-diaminopropionic acid (b-ODAP), a famous neurotoxin, was decreased also by 92.9%. The decrease of amino acid content in black ginseng is believed to be a result of Maillard reaction due to the detection of increased levels of Maillard reaction products [31]. These results have been authenticated by a recent study used multiple ultra-performance liquid chromatography with mass spectrometry (UPLC-MS) assay methods and proved the decrease of amino acids contents during the steaming process. The concentrations of 29 amino acids were less in red ginseng than white ginseng and were the least in black ginseng [33]. On the other hand, benzo(a)pyrene hydrocarbon was detected only in the black ginseng with a content of 0.17 μg/kg [25]. Benzo(a)pyrene is a polycyclic aromatic hydrocarbon produced as a byproduct of the incomplete burning of organic materials. It is a potent carcinogenic and listed as Group 1 carcinogens by the International Agency for Research on Cancer [33]. According to the Korea Food and Drug Administration (KFDA), the maximum acceptable level for benzo(a)pyrene on any food products is 5.0 μg/kg [25]. Accordingly, although reporting the presence of benzo(a)pyrene hydrocarbon in black ginseng, it's still in very minute amounts and much lower than the accepted limit.

Figure 2. Pathways of chemical changes of PPD- type ginsenosides during black ginseng processing .



4. Pharmacological Studies

During the past decade, there was considerable interest in investigating black ginseng's pharmacological actions using different biochemical, pharmacological and molecular biological techniques. The examined pharmacological activities included anticancer, hepatoprotective, antidiabetic, antioxidant and general tonic activities besides its effect on the central nervous and immune systems. Some of these reports were carried out to compare the biological activity of black ginseng with white and/or red ginseng. While most of these reports were designed to figure out the pharmacological potential of black ginseng in addition to its mechanism of action. Furthermore, there is extensive literature involving the biological activities of the major transformed ginsenosides, such as Rg_3, Rk_3, Rh_2, Rk_1 and Rg_5, which are present in black ginseng in much higher concentrations than red ginseng and are totally absent in white ginseng. Additionally, the amounts of polyphenols, acidic polysaccharides and Maillard reaction products increase considerably by the steaming process to be in the highest concentrations in black ginseng.

4.1 Anticarcinogenic Effects

Black ginseng exerted promising *in vitro* and *in vivo* anticarcinogenic effects against several cancer types through different mechanisms of action. Black ginseng's crude saponin fraction exerted stronger *in vitro* cytotoxic activities than red ginseng against ACFIN, HCT-15 and PC-3 cell lines with IC_{50} values ranging from 60.3–90.8 µg/mL [34]. In another comparative study, black ginseng extract exhibited cytotoxic activity against colon 26-M3.1 carcinoma cell line with an IC_{50} value of 800 µg/mL, while it was 2000 µg/mL for white ginseng [35]. In addition, black ginseng extract could inhibit basic fibroblast growth factor (bFGF)-induced endothelial cell proliferation and migration dose-dependently through its ability to inhibit the angiogenesis process [36]. Furthermore, breast cancer MCF-7 cell lines proliferation was inhibited by black ginseng due to cell cycle arrest induction in the G0/G1 phase in a dose-dependent manner [37].

Black ginseng exhibited promising anticancer activities in different *in vivo* studies such as tumor inhibition in H_{22} tumor-bearing mice dose-dependently via immune function improvement and tumor cell apoptosis induction [38]. Moreover, it performed *in vivo* anticancer activities against hepatocellular carcinoma as it reduced the size and the volume of an HepG2 cell transplanted tumor in BALB/c nude mice [39].

4.2 Immunomodulatory and Anti-Inflammatory Effects

In a comparative study, black ginseng exhibited stronger anti-inflammatory and anti-nociceptive effects than red ginseng in xylene-induced ear edema model in mice and carrageenan-induced paw edema in rats; it inhibited the pro-inflammatory mediators, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) and pro-inflammatory cytokines, IL-1β, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) [40]. In addition, the lipopolysaccharide-induced TNF-α release was significantly decreased after treatment with black ginseng extract [38]. Furthermore, the dose-dependent recovering effect of black ginseng extract against the cisplatin-induced nephrotoxicity and the reduced (pig cell LLC-PK1) cells viability, has been indicated [41]. In addition, in the same experiment, compound K could abrogate the elevated percentage of apoptotic LLC-PK1 cells significantly.

The black ginseng extract potently inhibited atopic dermatitis and asthma through suppression of the elevated IL-6 and IL-8 induced by *Dermatophagoides pteronissinus* treatment in human acute monocytic leukemia (THP-1) and human eosinophilic leukemic (EoL-1) cell lines [42]. Similarly, gamma-irradiated black ginseng extract could inhibit mast cell degranulation and suppress atopic dermatitis-like skin lesions in mice through different mechanisms, including the suppression of β-hexosaminidase and histamine in the stimulated mucosal mast cells [43].

4.3 Hepatoprotective Effect

Black ginseng exhibited a hepatoprotective effect on acetaminophen-induced mice liver injury decreasing the levels of serum alanine aminotransferase (ALT), aspartate transaminase (AST) and lipid peroxidation product malondialdehyde (MDA) significantly. Meanwhile, the antioxidant levels in liver tissues were elevated, including glutathione (GSH), cytochrome P450 E1 (CYP2E1) and 4- hydroxynonenal (4-HNE) [44]. Additionally, treatment with black ginseng ethanol extract decreased lipid accumulation in the liver and damage in the muscle of diabetic mice through the activation of AMP-activated protein kinase (AMPK) [45]. Symmetrically, black ginseng could decrease the activation of apoptotic pathways in the liver through decreasing Bax and increasing Bcl-2 protein expression levels associating a significant inhibition of APAP-induced necrosis and inflammatory infiltration [43]. Fermented black ginseng exhibited hepatoprotective activities against hydrogen peroxide (H_2O_2)-mediated oxidative stress in HepG2 human hepatocellular carcinoma cell lines. It could attenuate the increased ROS level and elevate the expression and activity of antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase. At the same time, black ginseng inhibited the phosphorylation of upstream mitogen-activated protein kinases (MAPKs) [46].

4.4 Antidiabetic Effect

In a comparative experiment, black ginseng ethanolic extract exerted stronger antidiabetic activity than red ginseng. It reduced hyperglycemia at a dose of 200 mg/kg in STZ-treated mice and improved the β-cell function by inhibition of β-cell apoptosis through suppression of the cytokine-induced nuclear factor–κB signaling pathway in the pancreas [47]. Black ginseng extract decreased the elevated blood glucose levels in streptozotocin-induced diabetic rats to a normal level [48]. Additionally, black ginseng ethanol extract decreased fasting blood sugar and glycated hemoglobin (HbA1c) via the upregulation of GLUT2 and GLUT4 expression in diabetic rats [44]. In another experiment, black ginseng ethanolic extract could significantly improve fasting blood glucose levels, glucose tolerance and decrease HbA1c in STZ-induced diabetic mice. It could increase glucose uptake in C2C12 myotubes via AMPK, Sirt1 and PI3-K pathways. In addition, a significant increase in the expression of the genes involved in glucose uptake in the muscle (glucose transporter (GLUT)1, GLUT4) and β-oxidation (acyl-CoA oxidase (ACO), carnitine palmitoyl transferase 1a (CPT1a), mitochondrial medium-chain acyl-CoA dehydrogenase (MCAD)) has been reported [49].

4.5 Anti-Obesity and Antihyperlipidemic Effects

Black ginseng ethanol extract could decrease the induced hyperlipidemia and fat accumulation in white adipose tissues and liver in the fat diet-mice through the inhibition of fat digestion [50]. Black ginseng extract efficiently reduced the total serum cholesterol levels and low-density lipoprotein (LDL) levels in the fat diet-fed mice [51] and in STZ-induced diabetic mice [48]. In addition, a significant decrease in triglyceride and non-esterified fatty acid (NEFA) levels with an increase in HDL level was noticed in male obese diabetic C57BLKS/J-db/db mice after treatment with black ginseng [52]. Water and ethanol extracts of black ginseng decreased lipid accumulation through the regulation of PPARγ, C/EBPα and AMPK phosphorylation in 3T3-L1 cells with a stronger activity for the ethanol extract . Black ginseng could regulate the expression of hepatic genes involved in gluconeogenesis (phosphoenol pyruvate carboxykinase (PEPCK), glucose6 phosphatase (G6Pase)), glycogenolysis (liver glycogen phosphorylase (LGP)) and glycogenesis (glycogen synthase (GS)) [48] and attenuate the key genes responsible for lipogenesis (acetyl-coenzyme A (CoA) acetyltransferase, 2, 3-hydroxy-3-methyl-glutaryl-CoA reductase) [50].

4.6 Effects on the Central Nervous System

Black ginseng extract could prevent the cognitive impairment induced by cholinergic dysfunction through the inhibition of acetylcholinesterase (AChE) activity after 24 h of a single administration of 200 mg/kg in the brain [53]. In a comparative experiment, both white ginseng and black ginseng of the roots of *Panax ginseng, P. quinquefolium* and *P. notoginseng* inhibited AChE and butyrylcholinesterase (BChE) dose-dependently. The efficacy of black ginseng roots was greater than that of the respective white ginseng roots of each species [54]. Black ginseng extract significantly reversed scopolamine (SCOP)-induced memory impairment in amnesic mice and also reduced escape latency by decreasing malondialdehyde (MDA) levels and restored superoxide dismutase (SOD) and catalase (CAT) activities [55]. The same effect was confirmed by the black ginseng-enriched formula (Chong–Myung–Tang) in the water maze associating the inhibition of nitric oxide production in BV2 cells and significant suppression of expression of proinflammatory cytokines such as nitric oxide synthase, cyclooxygenase-2 and interleukin-1b. Over and above, the black ginseng-enriched CMT extract diminished the protein expression of MAP kinase and NF-kB pathway factors [56].

Likewise, black ginseng protected rats against ischemia-induced neuronal and cognitive impairment and improved the escape latency with reduced loss of cholinergic immunoreactivity and nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d)-positive neurons in the hippocampus [57]. After oral administration of black ginseng extract with a dose of 200 mg/kg for 16 weeks, the cognitive deficits associated with normal aging in old-age mice was decreased, associating DNA damage protection and a significant increase in brain-derived neurotrophic factor protein expression [58].

4.7 Antioxidant Effect

There was an increase in the content of total phenolics because of steaming. This increase is thought to be a direct reason for the higher antioxidant activity of black ginseng [59]. Black ginseng roots of *Panax ginseng, P. quinquefolium* and *P. notoginseng* exhibited more total phenolic contents and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging activity than white corresponding roots. The best antioxidant activity was obtained with black ginseng roots of *P. ginseng* [53]. The same result was confirmed by another experiment through improving antioxidant activity according to the increasing number of steaming–drying cycles [60]. Black ginseng improved most of the morphological scores significantly in ethanol-treated embryos associating the significant restoration of the decreased mRNA levels of the antioxidant enzymes; cytosolic glutathione peroxidase (GPx), phospholipid hydroperoxide GPx and selenoprotein P, suggesting that black ginseng exerted this protective effect via the augmentation of antioxidative effect in the embryo [61]. Unexpectedly, in an acetaminophen-induced oxidative stress rat model, it was found that red ginseng extract exhibited more anti-oxidant activity than white ginseng and black ginseng extracts [62]. Four acidic polysaccharide fractions (BGP-60, BGP-65, BGP-70 and BGP-80) with estimated molecular weights of 28.6, 26.7, 11.4 and 3.05 kDa, respectively, were purified from black ginseng and showed strong potential antioxidant activities against DPPH, superoxide anion radicals and hydroxyl radical [63].

4.8 Tonic Effect

Black ginseng treatments at a dose of 150 mg/kg significantly increased the exercise capacity in rats. The level of blood lactic acid was decreased but the activity of citrate synthase in muscles was increased [64]. Black ginseng also increased muscle growth and could treat or prevent muscle loss related to aging through the production of myoblasts with larger multinucleated myotubes and increased diameter and thickness; the mechanism of action is thought to be the activation of Akt/mTOR/p70S6k axis [65]. The complex extract of black ginseng and fenugreek could increase cell viability, which had been attenuated because of oxidative stress through regulation of Erk kinase activation. Moreover, the oral administration of the complex could significantly increase the levels of total and bioavailable testosterone, follicle-stimulating hormone and luteinizing hormone in a hormone-deficient animal model. A dosage of 100 mg/kg of the complex extract could improve motor function and increase muscle endurance in a forced swimming test [66]. In a comparative study, the acidic polysaccharide of black ginseng exhibited a stronger anti-fatigue activity, than the neutral polysaccharide using the forced swim test through the restoring of the physiological markers for fatigue including glucose, glutathione peroxidase (GPx), creatine phosphokinase (CK), lactic dehydrogenase (LDH) and malondialdehyde (MDA) levels [67].

4.9 Topical Uses

Fermented black ginseng exhibited a significant anti-wrinkle effect at a concentration of 0.3 µg/mL through the increase of the type I procollagen expression levels in the human fibroblasts and the decrease in the MMP-1 expression level. Furthermore, at 3 µg/mL it increased the expression of TIMP-2 up to 154.55%. However, at 10 µg/mL it decreased the expression levels of MMP-2 and MMP-9 to 45.15% and 66.65%, respectively [68].

The wound healing activity of fermented black ginseng in human umbilical vein endothelial cells was mediated by angiogenesis through the MAP kinase pathway and enhanced the tube formation in HUVECs and migration in HaCaT cells was discussed [69]. Fermented black ginseng could stimulate the phosphorylation of p38 and extracellular signal-regulated kinase in HaCaT cells. Moreover, mice treated with 25 mg/mL exhibited faster wound closure in the experimental cutaneous wounds model [68].

4.10 Toxicity Studies

Single acute oral toxicity of black ginseng has been studied in rats indicating that the oral LD_{50} in the rats is higher than 15 g/kg, meaning that the black ginseng is virtually nontoxic [70]. On a cellular level, black ginseng showed no significant cytotoxicity against the normal splenocyte cells [38], while RG_3 protected normal cells against cancer by reduction of MNNG-induced DNA damage and apoptosis [71]. The eye irritation potential of fermented black ginseng was examined using the EpiOcular-EIT kit; results indicated that black ginseng considered is safe for the eyes at concentrations up to 100 μg/mL [67]. Fermented black ginseng could ameliorate the nephrotoxicity via regulating oxidative stress, inflammation and apoptosis with a DPPH radical scavenging activity stronger than that of white ginseng. Moreover, the reduced creatinine clearance levels and cell viability by cisplatin were recovered significantly after treatment [72]. Similarly, black ginseng extract and Rg_3 could recover the cisplatin-induced nephrotoxicity and the reduced (pig cell LLC-PK1) cells viability dose-dependently [39].

5. Methodology

A systematic literature search of several online databases, such as NCBI, Scopus, Science Direct, PubMed and google scholar, was conducted up to April 2019. The principal search topics were related to black ginseng: Its preparation methods, transformed ginsenosides and phytochemical changes in black ginseng in addition to the pharmacological effects of black ginseng, ginsenosides and other major secondary metabolites. The main sources of data collection included review articles and research papers published by reputed publishers such as Elsevier, Springer, Routledge and Taylor & Francis.

6. Prospects

Ginseng was first discovered and used by ancient Chinese. Traditionally, only white ginseng (or sun-dried ginseng), red ginseng and sugar ginseng were used as food and drugs, but because of the big loss of ginsenosides in the sugar ginseng, it is rarely used nowadays. Black ginseng is a newly-processed ginseng product with much stronger secondary metabolites; here are three prospects proposed to peers:

While black ginseng is a newly processed ginseng, there are several products made of it and which have been approved in the health and food markets with a large consumption in the rich areas of China and Korea. Accordingly, further deep studies are imperative and safety assessment is required. Presently, ginseng aged less than 5 years could be used as a food material in China with a dose of 3g/day at maximum. Similarly, and because of the stronger anticancer activity of black ginseng, its dose limit should be precisely issued by the China Food and Drug Administration (CFDA) in China and other countries.

Unfortunately, almost all the scientific studies of black ginseng examined it as one drug, not as a member in a formula like most of the drugs in traditional Chinese medicine. Is there another medicine that could or could not be mixed with black ginseng or should it be used as one treatment? In addition, if the black ginseng could be used in a formula, the determination of its proper dose inside the formula needs further study. Furthermore, despite there being several clinical comparative studies of the three types of ginseng (white ginseng, red ginseng and black ginseng), the anti-fatigue action associated with qi deficiency—which is a vital action of ginseng—has not been studied sufficiently.

More research should be driven toward the transformed ginsenosides of black ginseng; some of these ginsenosides have been detected in wild ginseng, such as Rg_3 and Rh_2 [73]; the engagement of these rare ginsenosides may elucidate the medicinal value of wild ginseng, which was a highly esteemed part of the old Chinese culture until now. Additionally, it has been proven that the primary saponins usually biotransform in humans’ digestive tracts into the more active transformed saponins and/or their aglycone [74]. Presently, the ginsenoside Rg_3 is developed as a cheap anticancer auxiliary medicine in China. The establishment of a simple preparation procedure of black ginseng decreased the preparation cost, which gives a good chance to develop a cheap and effective method to prepare these valuable transformed ginsenosides.

In summary, research in black ginseng revealed a massive change in the phytochemical content aligned with the priority in several pharmacological actions of white ginseng and red ginseng; consequently, black ginseng is expected to play a greater role in the health of humankind as the research goes on.

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