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Chapter

Black Garlic and Its Therapeutic Benefits

Gia-Buu Tran, Tan-Viet Pham and Ngoc-Nam Trinh

Abstract

Black garlic is a functional food produced from fresh garlic (*Allium sativum* L.) via fermentation with the whole bulbs or peeled cloves in a chamber in which temperature (60–90°C) and humidity (70–90%) are regulated for a period of time. Black garlic possesses an abundant amount of antioxidant compounds such as polyphenols, flavonoids, tetrahydro-β-carboline derivatives, and organosulfur compounds, including S-allyl-cysteine and S-allyl-mercaptocysteine, as compared with fresh garlic. Note that fermentation not only alters the nutrition components and sensory attributes but also enhances the bioactivity of black garlic. A growing body of evidences demonstrating therapeutic effects of black garlic, including anticancer, anti-obesity, immunomodulatory, hypolipidemic, antioxidant, hepatoprotective, and neuroprotective effects, have been reported in the last few decades. This book chapter provides a literature review of therapeutic effects of black garlic from fundamental to clinical studies that can be used by food and nutrition experts, researchers, and scientists to improve people’s health and wellness.

Keywords: functional food, therapeutic benefits, black garlic, nutrition, bioactivity

1. Introduction

Garlic (*Allium sativum* L.), a member of Alliaceae family, is a popular ingredient used for culinary art and traditional and modern medicine. It possesses not only a strong flavor and distinctive taste but also a variety of bioactive organosulfur compounds, comprising of allicin, allixin, diallyl disulfide, diallyl trisulfide, S-allyl-cysteine, S-allyl-mercaptocysteine, allixin, 1-propaneyl allyl thiosulfonate, (E,Z)-4,5,9-trithiendecade-1,6,11-triene 9-oxide, allyl methyl thiosulfonate, etc. Therefore, garlic has been documented in several literatures as a prominent remedy by reason of its several health benefits such as anticancer, antioxidant, antibiotic, antihyperlipidemic, anti-diabetic, antiagulant, antimicrobial, anti-constipation, antiparasitic, diuretic, and hepatoprotective effects [1–3]. In Vietnamese traditional medicinal system, garlic is prescribed to treat amebic and bacillary dysenteries, wound infection, chronic bronchitis, hypertension, whooping cough, and threadworm infection [4]. Furthermore, bioactivity and garlic components are also quite disparate among different cultivars [5, 6]. Garlic could be used directly or as food processing or brewery products including black garlic, smoked garlic, garlic oil, garlic oil macerate, extract, powder, supplement pill, garlic juice, alcoholic tincture, etc. [7].

Among the garlic processing products, black garlic is emerging as one of the most well-known functional foods in the market. As compared with the raw garlic, black garlic has a typical black color, sweet taste, and chewy texture without the
offensive odor. Moreover, several bioactivities of black garlic including anticancer, anti-obesity, immunomodulatory, hypolipidemic, antioxidant, hepatoprotective, and neuroprotective effects have been documented in literature [8]. However, a systematic review of black garlic and its therapeutic effects from fundamental to clinical studies is still lacking. This chapter of the book provides food and nutrition experts, researchers, and scientists an overview of application of black garlic in functional food for a variety of specific diseases with clinical evidences to improve people’s health and wellness.

2. Black garlic and its production

The people from Asian countries such as Thailand, South Korea, and Japan have produced and used black garlic as a traditional food for centuries, but it has been introduced into global market in recent decades. In brief, black garlic is produced by fermentation of whole bulb of fresh garlic at high humidity and temperature which in turn results in garlic to turn black via a set of nonenzymatic browning reactions, including Maillard reaction, oxidation of phenols, and caramelizing. When garlic undergoes fermentation, not only physiochemical characteristics of garlic are altered, but also the concentration of bioactive compounds is also improved [8]. Choi and collaborators [9] showed that the moisture of garlic and pH decreased along with the fermentation process, whereas the reducing sugar and total acidity were accumulated. On the other hand, color spectra and composition of amino acids of black garlic also were altered as compared with fresh garlic [9]. As the consequence, black garlic has elastic and chewy texture, as well as sweet taste without offensive flavor of garlic (Figure 1). Furthermore, black garlic possesses an abundant amount of antioxidant compounds such as polyphenols, flavonoids, tetrahydro-β-carboline derivatives, and organosulfur compounds, including S-allyl-cysteine and S-allyl-mercaptopcysteine, as compared with fresh garlic. Kim and collaborators suggested that the total polyphenol and flavonoid of black garlic increase 9.3- and 1.5-folds, respectively, after a program heat schedule as compared with fresh garlic [10]. The concentration of S-allyl-cysteine, one of the most important organosulfur bioactive compounds of garlic, also increases in black garlic from 4.3- to 6.3-folds depending of heating treatment [11].

Due to its palatability and abundant amount of bioactive compounds, black garlic has become one of the most well-known and prominent products in nutraceuticals and functional food market with a remarkable growth of consumption demand and profitability during recent years. Furthermore, black garlic has attracted not only
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3. Therapeutic effects of black garlic

3.1 Anticancer effect of black garlic

Cancer, which has been regarded as one of leading cause of death in worldwide, is a type of disease related with uncontrolled or normal growth of cells or tissues in our bodies. With the growing number of the evidences reported for anticancer effect of black garlic in recent decades, some researchers suggested that black garlic could be used as a dietary product for preventing and treating cancers from gastric cancer to leukemia. The mechanism of anticancer effects of black garlic in different types of cancer diseases is various comprising of the induction of apoptosis, stopping the cell cycle, and inhibition of tumor growth and invasion. In 2011, Wang and collaborators suggested that aqueous extract of black garlic could inhibit proliferation and triggered the apoptosis of SGC-7901 cells, a human gastric cancer cell line, with dose-dependent manner. The authors also observed the inhibitory effect of black garlic on the growth of tumor in tumor-bearing mice [16]. Moreover, black garlic has the anti-invasive effect and prevents tumor metastasis in human gastric carcinoma AGS cells through the increase of tightness of tight junction as well as the...
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downregulation of matrix metalloproteinases (MMP)-2 and metalloproteinases-9, which play a role as mediators of metastasis and invasiveness [17]. The anticancer effect of black garlic on colon cancer cell line also has been demonstrated. Moreover, treatment with alcoholic extract of black garlic could upregulate PTEN and downregulate Akt/pAkt expression, the members of phosphatidylinositol 3-kinase protein kinase B (PI3K/Akt) signal transduction pathway, leading to the modulation of p70S6K1 protein, induction of apoptosis, and arresting of the cell cycle of HT29 human colon cancer cell line [18]. Park and collaborators have reported that hexane extract of black garlic could reduce the cell viability of U937 cells, human histiocytic lymphoma. It has been found that hexane extract of black garlic exhibited induction of both intrinsic and extrinsic pathways through the alteration of the expression of apoptosis-relating proteins. They found that black garlic upregulates death receptor (DR)-4 and Fas ligand, increases Bax/Bcl-2 ratio, as well as induces the truncation of Bid protein, which involves not only endogenous mitochondrial pathway but also death receptor-mediated apoptotic pathway [19]. Some authors also proved that black garlic could inhibit cell growth and enhance sensitization of Lewis cells, lung cancer cell line, against ionizing radiation [20].

3.2 Benefit effect on dyslipidemia and anti-obesity

Obesity is a threatening problem to public health in Western and developed countries which causes several metabolic syndromes and chronic diseases. Obesity can be prevented by a combination of physical activity and healthy diet balance between energy intake and expenditure. Note that several functional foods such as γ-oryzanol, butyric acid, legumes, bromelain, peas, lentil, fabas beans, conjugated linoleic acid, diacylglycerols, purified black raspberries, apples, bilberries, sea buckthorn, etc. have been indicated as preventing and/or treating obesity agents via several mechanisms including the induction of satiation, decreasing of appetite, regulation of lipid metabolism, and thermogenesis [21]. Among them, black garlic is known as a prominent lipid and weight-lowering ingredient. In 2015, Ha and collaborators showed that the diet supplemented with 1.5% black garlic extract could only reduce weight but also decrease kidney and epididymal fat in high-fat model [22]. Moreover, black garlic extract attenuates dyslipidemia induced by high-fat diet. In Ha's work, the authors observed the lowering effect of black garlic on the plasma level of total lipid, total cholesterol, and triglyceride. On the contrast, high-density lipoprotein cholesterol (HDL) of black garlic group was higher than high-fat diet group. Of note, treatment with black garlic reduces both glucose and insulin in plasma levels in high-fat diet. One of the explanations for the anti-obesity effect of black garlic is that black garlic could ameliorate diet-induced obesity through downregulation of transcription factors and enzymes related with fat and cholesterol syntheses such as sterol regulatory element-binding protein-1c (SREBP-1c), acetyl-coA carboxylase (ACC), fatty acid synthase (FAS), glucose-6-phosphate dehydrogenase (G6PDH), hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, and acyl-CoA cholesterol acyltransferase (ACAT) or fatty acid oxidation rate via increase of the expression of carnitine palmitoyltransferase-1 (CPT-1), a key enzyme related with lipolysis and fatty acid breakdown [22]. Furthermore, Chen and collaborators also recommended that the anti-obesity effect of methanol extract of black garlic could be related with regulation of lipogenesis, adipokine biosynthesis, fatty acid oxidation, fatty acid and glucose transport, and lipolysis in both the adipose tissue and liver [23]. Furthermore, Seo and collaborators found that black garlic extract could augment the anti-obesity and cholesterol-lowering effect of exercise in animal models [24]. Combination of black garlic and exercise showed a greater effect on decreasing visceral fat, epididymal fat, and liver weight.
as compared to exercise-alone group and exhibited lowering triglyceride effects in high-fat diet-induced rats. On the other hand, consumption of black garlic (6 g/day) for a long term (12 weeks) also has the cardioprotective effect in patients from a double-blind, randomized placebo-controlled trial by diminishment of atherosclerosis markers and improvement of dyslipidemia. In Jung study, black garlic supplement group exhibited a significant increase of high-density lipoprotein cholesterol levels and low-density lipoprotein cholesterol/apolipoprotein B along with a decline of apolipoprotein B as compared to placebo group [25]. Recently, some researchers have developed fermented products from garlic extract that also manifested an anti-obesity effect similar with black garlic extract. Jung and collaborators demonstrated that fermented black garlic extract, a product created by fermented Saccharomyces cerevisiae (KTCT7910) in medium supplemented with 5%, also exhibited the hypolipidemic and anti-obesity effects but not hypoglycemic effect [26]. In 2016, Lee and collaborators fermented black garlic extract with Lactobacillus plantarum BL2 to produce functional food that could reduce body weight and epididymal, retroperitoneal, and mesenteric adipose tissue mass as well as plasma level of triglyceride and total cholesterol in diet-induced obese mice. The fermented product inhibited adipose tissue hypertrophy via the downregulation of a set of proteins related with adipogenesis and lipogenesis including peroxisome proliferator-activated receptor γ (PPARγ), CCAAT-enhancer-binding protein α (C/EBPα), stearoyl-CoA desaturase (SCD-1), SREBP-1c, and FAS [27].

3.3 Black garlic and its antioxidant properties

Black garlic contains abundant antioxidant compounds including polyphenols, alkaloids, flavonoids, S-allyl-cysteine, and antioxidant intermediate products derived from Maillard reaction [8, 9]. Several studies suggest that black garlic not only scavenges the free radicals in vitro but also activates the antioxidant enzymes in vivo. Wang and Sun reported that black garlic ethanol extract has an identical DPPH radical inhibitory effect with vitamin C in concentrations 200 and 250 μg/ml and comparable OH radical scavenging effect with vitamin C in concentrations 400 and 500 μg/ml. Black garlic extract could reduce malondialdehyde (MDA) concentration in serum, an end product of lipid peroxidation, and enhance serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in oxidative damage murine model induced by benzene bromide [28]. These results were similar with Lee and collaborators work. A diet supplemented with 5% black garlic aqueous extract could decrease oxidative stress and diabetes complications. Black garlic exerts a strong antioxidant capacity through the strong scavenging 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) or ABTS radical activity, which is four times higher than those of raw garlic, and suppression of oxidative stress marker, such as thiobarbituric acid reactive substances (TBARS) content in the liver, and activation of antioxidant defense system, including SOD, GSH-Px, and catalase (CAT) in db/db mice, a genetically modified model for diabetes mellitus [29]. Additionally, Ha and collaborators suggested that black garlic could specifically upregulate the mRNA expression of nuclear factor erythroid 2-like factor (Nrf2)-related antioxidant proteins, such as heme oxygenase-1 (HO-1), glutathione S-transferase alpha 2 (GSTA2), and NAD(P)H quinone oxidoreductase-1 (NQO1) to prevent accumulation of reactive oxidative species in the liver [30]. According to a Hungarian research, black garlic also improves superior postsischemic cardiac function, infarct size reduction, and HO1 and inducible NOS (iNOS) levels after ischemia/reperfusion, which in turn exerts a cardioprotective effect [31]. Moreover, antioxidant effect of black garlic has also been proved in clinical study. In previous
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study, Wang and collaborators performed a double-blind, parallel design study with a population of untrained males with similar age and body mass index during 14 days to compare the effect of black garlic and placebo on exercise-induced oxidative stress and recovery of muscle function [32]. They found that black garlic improved the recovery rate of circumference of biceps brachii after eccentric exercise and diminished reactive oxygen metabolites (dROMs), lipid peroxide, and 8-iso prostaglandin F2α concentrations, a new indicator of oxidative stress and related with atherosclerosis [32, 33]. Recently, Liu and collaborators have demonstrated the beneficial effect of black garlic on coronary heart disease patient. Black garlic exhibits an augmentation on chronic heart failure by increasing the left ventricular ejection fraction value and the scores of the quality of life and circulating antioxidant levels along with a decline of brain natriuretic peptide (BNP) precursor N-terminal, a biomarker for severity of heart failure [34]. Note that some research implies that the processing method has a remarkable effect on antioxidant of black garlic [8, 15, 35]. The temperature and moisture are the most important parameters that determine the quality and antioxidant capacity of black garlic. In Sun and Wang’s work, scavenging DPPH free radical activity of black garlic produced at 85°C in 85% humidity was higher than ones generated at 75 and 60°C in the same humidity after fermentation. The authors observed that at the same temperature, the indicated humidity (75%) would produce black garlic with highest Trolox equivalent antioxidant capacity followed by 85, 80, and 70% humidity [35]. The separation of garlic cloves also has positive correlation with antioxidant capacity. Angeles and collaborators proposed that peeled black garlic cloves exhibited a higher antioxidant capacity than whole black garlic bulbs fermented at same condition in the end of production [15].

3.4 Black garlic and its anti-inflammatory effect

Inflammation is a process by which our immune system responds to injury, infection, and toxin. Inflammation plays a vital role not only in wound healing and repairing process but also in protecting our body from foreign invaders, including viruses and bacteria. However, chronic inflammation may have a negative impact on our health which has been manifested in a variety of chronic diseases from heart disease to rheumatoid arthritis and lupus. Consumption of anti-inflammatory ingredients or foods, such as ω-3 polyunsaturated fatty acid, monounsaturated fatty acids, β-carotene, quercetin, kaempferol, malvidin, peonidin, daidzein, genistein, extra virgin olive oil, tomato juice, walnut, red wine, flaxseed flour, and cherry, may help combat certain diseases related to chronic inflammation [36]. Recently, some reports have suggested black garlic as a prominent agent for treatment of inflammation and septicemia-related diseases. Aqueous extract of black garlic hinders the production of nitric oxide (NO) and proinflammatory cytokines, including tumor necrosis factor α (TNFα) and prostaglandin (PG)-E2, and suppresses NO synthase and TNFα and cyclooxygenase-2 expression through a mechanism-related mitogen-activated protein kinase and nuclear factor-KB in liposaccharide (LPS)-stimulated murine macrophages. Furthermore, black garlic extract supplement impedes serum TNFα, interleukin-6 (IL6), and interleukin-1 β (IL1β) production and prevents mice from LPS-induced death [37, 38]. These findings are identical with the results from Zhang and Jilg experiments, in which six different black garlic extracts, including hot aqueous extract, ethanol supernatant extract, ethanol precipitate extract, deproteinized ethanol supernatant extract, and deproteinized ethanol precipitate extract, not only ameliorate regulatory effect of LPS on macrophages growth inhibition but also abate TNFα, IL-6, and IL1β generation in LPS-treated macrophages [39]. Moreover, the chloroform extract of black
garlic inhibits TNFα-induced reactive oxygen species (ROS) formation, mRNA and protein expression of vascular cell adhesion molecule-1 (VCAM1), and activation of NFKB pathway and reduces adhesiveness of THP-1 monocytes to human umbilical vein endothelial cells (HUVECs) [40]. In previous research, hexane extract of black garlic also regulates human endometrial stromal cell proliferation and cell progression via suppression of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK). Moreover, Kim and collaborators demonstrate that hexane extract of black garlic has potential to render the NFKB and activator protein 1 (AP1) activation, which in turn decreases VCAM1 and ICAM1 expression [41]. Fermented black garlic, a product of fermentation of Lactobacillus rhamnosus, also hinders production of inflammatory mediators, such as TNF α, IL-6, IL1β, iNOS, and COX2, and retards an inflammatory signal transduction pathway, the NFKB pathway [42].

3.5 Neuroprotective effect of black garlic

A growing body of literature indicates that black garlic has beneficial effects to memory and nerve system through antiamnesic effect, improvement of cognitive impairment, and prevention from neuroinflammation and neurotoxicity. In previous study, Nurmasitoh and collaborators demonstrated that ethanol extract of black garlic has manifested a strong protection of murine medial prefrontal cortex from monosodium glutamate-induced oxidative stress via improvement of the working memory performance and prevention of the pyramidal neurons from modification of neuronal architecture [43]. Furthermore, a variety of doses of black garlic extracts (from 2.5 to 10 mg/200 g body weight) also inhibit deleterious effect of monosodium glutamate on spatial memory and total number of pyramidal neurons in CA1 region of the hippocampus [44]. These findings are identical with the results from Indonesian report, in which black garlic improves motor coordination function and the number of Purkinje cells in the cerebellar cortex of the rat brain [45]. The protection effect of black garlic on nerve system from neuroinflammation, a pathological evidence of Alzheimer’s disease, and cognitive impairment has been well documented [46]. B amyloid (Aβ) deposition leads to inflammation of the neuron, which triggers a host defense response to neuronal damage and eventually neuronal degeneration. Nillert and collaborators had established a neuronal degenerative model by treatment with 1 μl of aggregated Aβ(1–42) in the lateral ventricles; eventually they observed that ethanol extract-aged black garlic, a variant form of black garlic produced by aging the fresh garlic in room temperature, could ameliorate short-term recognition memory and inhibit activation of microglia as well as production of IL1β, a proinflammatory cytokine [46]. Additionally, ethyl acetate fraction of aged garlic extract protects PC12 neuron-like cells and ICR mice from neurotoxicity and amnesia induced by Aβ(25–35) [47]. Note that both aged and black garlcs are rich of S-allyl-cysteine content, a stable bioactive organosulfur compound which also exerts anti-inflammatory, neuroprotective, and antioxidant effects [48].

3.6 Hepatoprotective effect of black garlic

The liver is a vital organ which exerts detoxicity, protein biosynthesis, and digestive biochemical production. However, the liver is vulnerable with medications, chemicals, alcohol, solvents, infection, and nutritional supplement. Black garlic has proved to protect the liver from side effects including hepatotoxicity and apoptosis of cyclophosphamide, an anticancer medicine [49]. Ahmed indicated that black garlic supplement at the dose 200 mg/kg body weight recovers the histological
change, DNA damage, and blood biochemical parameter alteration (bilirubin, alanine transaminase (ALT), aspartate transaminase (AST)) as well as increases the hepatic antioxidant enzyme levels (CAD, SOD, GSH-Px) as compared with cyclophosphamide-treated group. According to Lee's research, black garlic could prevent rat clone-9 hepatocytes from hepatic damage induced by tert-butyl hydroperoxide in vitro [50]. One of explanations for hepatoprotective effect of black garlic is that black garlic could improve cell death and reduce lipid peroxidation, oxidative stress, and inflammation through regulation of JNK signaling cascade. Note that black garlic exerts the hepatoprotective effect not only in acute toxicity but also in chronic conditions. One study from Korea reports that black garlic decreases the fat accumulation, epididymal, total fat pad, and liver weight alteration and reduces elevation of hepatic enzymes (ALT, AST, alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)) and blood lymphocyte DNA damage in chronic alcohol-induced hepatic damage model [51]. They also observed a decrease of TBARS content in the liver, heart, and plasma and reduction of cytochrome P450 2E1 activity companion with a rise of hepatic GSH level and antioxidant enzyme activities such as GSH-Px, CAT, and glutathione reductase in black garlic-treated group. From these evidences, the authors imply that the strong antioxidant effect of black garlic is related to the mechanism of protection against liver damage induced by chronic alcohol exposure. Moreover, Shin and collaborators suggested that black garlic supplement not only protects the liver from acute toxicity induced by carbon tetrachloride or D-galactosamine but also improves lipid profile and liver injury in hepatic steatosis model [52]. A Vietnamese report also proved the hepatoprotective effect of single-clove black garlic on liver injury in sub-chronic toxicity model (Figure 3) [14].

Figure 3.
Liver sections from carbon tetrachloride (CCL₄)-intoxicated mice and CCL₄ treated with single-clove black garlic extract mice. Carbon tetrachloride treatment (1 ml of mixture of CCL₄ in 50% olive oil/kg body weight, twice per week, for 28 days) not only alters the gross appearance of the liver (swelling liver, hard texture, pale brown with coarse surface) but also results in a severe hepatic inflammation and necrosis in microscopic level (panels A and B, respectively). Supplement with single-clove black garlic extract (200 mg/kg body weight) could improve liver morphology (semihard texture, redness, slight coarse surface) and histological structure of the liver along with reduction of inflammation (panels C and D, respectively).
4. Conclusions

Black garlic is a well-known garlic preparation which is fermented in regulated high humidity and temperature not only to remove strong unpleasant flavor of fresh garlic but also to improve its nutrient composition, bioactives, and taste values. After being introduced in the market in last few decades, black garlic has become an emerging functional food on account of its wide-range biological functions, including antioxidant, anti-inflammatory, anticancer, lowering hyperlipidemia, anti-obesity, hepatoprotective, and neuroprotective effects. Its bioactivities and therapeutic benefits have been the subjects to a numerous extensive researches in both in vitro and in vivo levels. In recently, there are only a few clinical studies which prove the health benefits of black garlic on cardiovascular diseases. Therefore, further researches focused on other medical application and safety aspect of black garlic are required to provide a comprehensive overview about therapeutic effects of black garlic.

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Conflict of interest

The authors declare there are no conflicts of interest regarding the publication of this work.

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