Application of Anticancer Agents from Fermentation of Soy Using Bifidobacterium and Lactobacillus Sp., A Review

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Abstract: Cancer drug development presently focuses on rational drugs that have the ability to cross the cell membrane and bind to a specific enzyme or receptor. Current research in cancer treatment aimed at finding new prognostic targets for therapy, the goal of research is to find natural substances that modulate the regulatory signals such as cell cycle, growth factor and cell death. Soybean (Glycine max. L.) a common part of diet possess a high content of proteins and important source of phytoestrogen. Recent study states that phytoestrogen posses pharmacological, toxicological and mechanical properties for various chronic diseases such as cancer, osteoporosis and atherosclerosis. The isoflavones exerts as glycoside linkage which limits the absorption, therefore hydrolysis of soy milk with probiotic bacteria cleave the glycosidic linkage by β-galactosidase thereby increasing the concentration and activity of bioactive molecules. The major isoflavonoids found in soybean are genistein and daidzein, that resembles structure of estrogen and they can interact with the estrogen receptor. Thus fermenting soy increases the concentration of isoflavones (genistein and daidzien) which exhibits its anticancer activity.

Key words: Soy · Lactobacillus Sp. · Bifidobacterium Sp. · Isoflavones · Anticancer Agent · Drug Targets

INTRODUCTION

Soybeans have been reported to contain high concentration of isoflavones with potential health-enhancing properties in preventing breast cancer, prostate cancer, heart disease and osteoporosis. Due to its chemical structure, similarity with estrogen which is produced by the human body, most of these studies have shown that isoflavones possess some health benefits as summarized in the following text. Cancer arises from one single cell, the transformation from a normal cell into a tumor cell is a multistage process, typically a progression from a pre-cancerous lesion to malignant tumors (WHO 2013). Cancer is one of the leading causes of death in the world, particularly in developing countries affecting the young, old, rich, poor, men, women and children which represents a tremendous burden on patients, families and societies. The World Breast Cancer Report 2012 says that the global burden of breast cancer increases, requiring an urgent action against it. The diagnosed cases of breast cancer have increased 3.1% annually during the past 30 years. An overview of 1.6 million cases is expected to be diagnosed in 2012. (Published by the International Prevention Research Institute headquartered in Lyon, France). A death rate of 39,970 breast cancer patients (39,520 women and 450 men) occurred in 2011. Breast cancer ranks second as a cause of cancer death in women (after lung cancer). Breast cancer is still an important public health problem and the second most lethal cancer in women worldwide. Prostate cancer (PCa) is the second most common cause of cancer and the sixth leading cause of cancer death among men worldwide.

Soy milk contains several nutritional advantages over dairy milk including reduced levels of cholesterol and saturated fat as well as the absence of lactose. Soy a natural source of phytoestrogen contains isoflavone with potential anticarcinogenic activity [1]. Isoflavones are phytochemicals present in leguminous plants, especially in soybeans. The health promoting effects of soybean consumption have more recently been linked to the biological activities of a specific group of phenolic
compounds found uniquely within soybean known as isoflavonoids [2]. Soy isoflavones have been implicated in health benefits, including the potential to reduce the risk of age-related and hormone-related diseases including cancer, menopausal symptoms, cardiovascular disease and osteoporosis [3, 4]. Many studies demonstrated an association of soy and its reduced risk of cancers such as prostate and breast cancers. The consumption of soy has been linked to many biological properties because of its phenolic compounds known as isoflavonoids. Isoflavone present in soy foods are conjugated with sugar as β-galactoside linkage forms which are not easily absorbed and require hydrolysis for bioavailability and subsequent metabolism. Glucosidase is considered to be key enzyme for the conversion of isoflavone form in fermented soybean foods and are effective in converting isoflavone glycoside to aglycones [5, 6]. *Bifidobacterium* and *Lactobacillus* are predominant members of intestinal microflora however these two strains are widely studied for the production of β-glucosidase [7-10]. Probiotic microorganisms possess glucosidase, α-galactosidase and β-galactosidase, which plays an important role in the hydrolysis of isoflavone glycosides to the bioavailable aglycones forms [8]. Microorganisms having β-galactosidase activity, mainly lactic acid bacteria *Lactobacillus* sp. and *Bifidobacterium* sp., are used in fermenting soy milk, this fermentation process will lead to the transformation of isoflavone to bioactive molecules having a wide variety of therapeutic properties. In addition to being natural, nutritious and safe, the traditional fermented soybean products exhibit potent anticarcinogenic effects [11, 12]. Fermented soy milk provides nutritional and higher protein products at economical base comparable to regular fermented milk products [13].

**Isoflavones and Estrogens:** The phytoestrogens consumed by humans naturally exist in soybean and are called as isoflavones [14]. The soy isoflavones are part of diagonal compound which is structurally and functionally similar to human estrogen, the most potent mammalian estrogen, structural similarity is depicted in the Figure 1. The major isoflavones, namely, genistein and daidzein, have several features in common with estradiol-17β, including an aromatic A ring with hydroxyl group in the same plane at a distance similar to that in estradiol. Because of the structural similarity of these isoflavones to biological estrogens, genistein and daidzein both bind to estrogen receptors (ERs), suggesting that they might either induce cell proliferation (estrogen agonist) or prevent hormone-dependent growth of cancer cells by virtue of their potential estrogen-antagonistic activity [15]. This similarity of isoflavone opened the view to use this as preventive drug for many kinds of hormone dependent disease [16]. Phytoestrogens not only inhibit the growth of Estrogen-Receptor (ER)-dependent cancer cells, but also inhibits the growth of ER-independent cancer cells. The mechanisms of their anticancer effects include induction of apoptosis and alterations of cell cycle distribution [17, 18].

**Fermentation of Soymilk with Probiotic Bacteria:** Due to its extraordinary sources such as valuable proteins, unsaturated fatty acids, soluble and insoluble dietary and health characteristic, soy milk has become very interesting food. Typical soy foods are divided into two categories as non fermented and fermented soy products. Traditional non-fermented soy foods include fresh green soy bean, whole dry soy beans, soy nuts, soy sprouts and whole fat soy flour whereas traditional fermented soy foods are

![Fig 1: Structures of genistein, daidzein and estrogen (estradiol)](image-url)
considered to have more health promoting benefits when super processes. It is being suggested that the fermentation process increases the availability of isoflavones in soy (Soy and cancer survivors dietary supplement).

Since 1900 the concept of probiotics progressed when Elie Mechnikoff hypothesized that the long and healthy lives of Bulgarian peasants were the outcome of their consumption of fermented milk and milk products. Main probiotics such as members of genera \textit{Lactobacillus}, \textit{Bifidobacterium} and \textit{Streptococcus} are commonly used [20]. The cultured or probiotic forms of soy milk enhanced with genistein and daidzein have their unique carcinogenic activity by strongly inhibiting the formation of cellular mutation. A Japanese study found that the level of genistein in the fermented soybean products was higher than in normal soybeans /non-fermented soy milk. In general, isoflavones in soybeans subsist mainly as glucoside forms and rarely as aglycone. During fermentation the glucoside conjugates of isoflavones are converted into aglycones by the effect of \(\beta\)-glucosidase. The Figure 2 reveals the \(\beta\)-galactosidase bond of phytoestrogen (glucosides) is cleaved to produce aglycone (genistein and daidzein) by microbes during fermentation. Depending on the various growth conditions, a fermentation process may dramatically modify the content and the composition of these bioactive compounds into their active anticancer isoflavones [11]. Soy bean particularly rich in isoflavone aglycones (genistein and daidzein), are becoming one of the hotspots of research which is thought to act against cancer in several ways interfering with cancer promoting enzymes, by blocking the activity of hormones in the body and by interfering the process by which tumors receive nutrients and oxygens [21].

**Daidzein and Genistein:** Daidzein (4',7-dihydroxyisoflavone) belongs to the isoflavone subclass of flavonoids found in fruits, nuts, soy beans and soy based products [22, 23]. Daidzein has been garnered interest as a nontoxic compound capable of inducing tumor cell death in a variety of cancer types [24]. Daidzein act as an antioxidant and prooxidant, this capacities such as the pro-apoptotic effect of daidzein, could contribute to cancer chemotherapy and the inhibition of tumor growth. Genistein (4',5,7-trihydroxyisoflavone) is a major isoflavone of plants such as soy beans and is commonly found in a variety of human foods [25]. It has been reported that administration of dietary genistein has enhanced the activities of antioxidant enzymes in various organs, such as the small intestine, liver and kidney [26]. Genistein has been shown to inhibit the growth of various cancer cells through the modulation of genes that are intimately related to the regulation of cell cycle and programmed cell death. Genistein inhibits the growth of several cancer cells including leukemia, lymphoma, ovarian, cervical, leiomyoma, melanoma, neuroblastoma, gastric, pancreatic, breast and prostate cancer cells [27].

**Roles of Isoflavones**

**Antioxidant Properties:** The structural similarity of isoflavones to 17\(\beta\)-estradiol hormone have increased the activity of phenolics to save as antioxidants [28, 29]. Free radicals reacting with some essential molecules such as DNA, membranes and proteins may cause life-threatening damages. Isoflavone with potent antioxidant activity may be a useful therapy to lower LDL cholesterol oxidation. Previous studies has shown that genistein is the most potent antioxidant among the soy isoflavones, followed by daidzein [30, 31]. It also inhibits LDL oxidation, by scavenging reactive oxygen species (ROS) or blocking generation of ROS involved in numerous pathological events [32]. In addition Genistein and daidzein inhibit the production of hydrogen peroxide and superoxide anion generation in cells, through an indirect regulation of antioxidant enzymes. As reported perviously genistein and daidzein has an antioxidant effect by scavenging free radical that protects cell by inhibiting the expression of stress response related gene and reduce the activation of carcinogenesis [33].
Application of Isoflavones as Drug Targets

Inducer of Apoptosis: Genistein could induce apoptosis in a variety of human cancer cells through caspase-3 activation and down-regulation of Bcl-2, an antiapoptotic factor, thereby increasing the proapoptotic Bax protein. Studies state that daidzein increases caspase 9 activity there by decreases the expression of cyclin D, in addition genistein also inhibits Akt and Nf-κB to induce apoptosis [34, 35]. The Figure 3 shows the ability of genistein to depolymerize the microtubules in a dose-dependent manner, prevent mitosis in cancer cells, in cascade way leads to cell cycle arrest and, eventually, apoptosis (Fig.4). Daidzein has been reported to activate the catalase promoter to stimulate caspase-3 and apoptosis and to down-regulate the activities of Bcl-2 and Bcl-xL [36, 37].

Protein tyrosine kinase is the important component for cell growth and differentiation. Increased or aberrant expression of tyrosine kinase is regarded as one of the important factors influencing tumor development and progression. It plays a crucial role in signal transduction from an external cellular environment to interior of the cell. Permanent increased level of tyrosine kinase results in cascade signaling transduction leads to malignant transformation and uncontrolled tumor growth. Receptor tyrosine kinase gets activated by EGF (Epidermal Growth Factor), PDGF (Platelet Derived Growth Factor) and NGF (Nerve Growth Factor) which undergo confirmation changes and catalyzes phosphorylation for normal function of cells. The contrary stage of this mechanism results in the development of cancers. Genistein may inhibit cell tyrosine the important growth factor in signaling pathway. A study conducted in 1987 stated that genistein was proved to be a good inhibitor of EGF receptor [40]. Genistein attenuate the growth of cancer cells by inhibiting protein tyrosine kinase signaling pathway, cell inhibition is also reported to occur due to increase in apoptosis [41, 42]. Anticancer properties of genistein indicates lower risk of cancer development and cancer-related death especially in case of breast and prostate cancer. The possible anticancer applications of genistein concerned the transfer of γ-phosphate groups from ATP to tyrosine residues of proteins, an important phosphorylation for controlling signal transduction pathways involved in proliferation, differentiation, cell migration and many other cellular activities [43].

Fig 3: Effect of genistein on cellular microtubules [38].

Fig 4: Genistein induced apoptosis in prostrate cancer cell [39].
Isoflavones Inhibitors of Protein Tyrosine Kinase (PTK) and Topoisomerase II Enzymes: The growth inhibition of cancer cells could be due to cell cycle arrest, which ultimately results in cessation of cell proliferation. Increasing evidence suggests that high concentrations of genistein induce apoptosis or necrosis in various cells, such as breast cancer cells, colon cancer cells [54-56]. Genistein is a known inhibitor of Protein-Tyrosine Kinase (PTK), which may attenuate the growth of cancer cells by inhibiting PTK-mediated signaling mechanisms [47]. Genistein also acts by inhibiting topoisomerase II enzyme. Topoisomerase II is a nuclear enzyme that actively participate in DNA replication transcription. This enzyme directs the molecular process of DNA topology, chromatin condensation / decondensation, chromosome separation. It modulates the topological structure of DNA by generating transient double stranded breaks in the backbone of the double helices [48]. Increased activity of these enzymes results in rapid proliferation of cells especially in S and G₂ phase of cell cycle. Studies indicates that genistein, a nonintercalator, inhibits topoisomerase II activity by stabilizing the DNA-topoisomerase II complex, called the cleavable complex [2, 49]. Stabilization of this complex in tumor cells leads to double and single strand breaks in cellular DNA, leading to growth inhibition or cell death. Antiproliferative activity in cancer cells involves the induction of apoptosis associated with cell cycle arrest at the G₀/G₁ or G₂ phase, depending on the cancer cell type [24, 50, 51] where as daidzein causes cell cycle arrest at the G₁ and G₂/M phases in human breast cancer MCF-7 and MDA-MB-453 cells [41].

Angiogenesis Inhibitor: Angiogenesis, the generation of new blood vessels from pre-existing blood vessels, is required for the growth as well as expansion of solid tumors [52, 53]. Chemotherapeutic agent do not specifically target tumor cells rather interfere with cell division of normal cells and causes damage. Considering the side effects of chemotherapies, interest is taken in drugs targeting tumor vasculature, which can act as antiangiogenic agent that could disrupt angiogenesis. TGF-β (transforming growth factor beta) is a known major factor that regulates cell proliferation and TGF-β signaling is an important feature in the regulation of angiogenesis. Hence, therapeutic agents are being devised either to interrupt the pathogenic steps of tumor angiogenesis or to directly destroy the tumor vasculature. A number of epidemiological studies show that the soy products may have a protective effect against human cancers of the breast, colon, or prostate [54-56]. Genistein has been known to inhibit TGF-β signaling and vessel endothelial cell proliferation, therefore genistein inhibits angiogenesis.

This discovery of cytotoxic and antiproliferative activity of isoflavones, via inhibition of tyrosine kinases, topoisomerase II, G₂/M block of the cell cycle, angiogenesis has suggested possible application of genistein and daidzein in anticancer therapy. The growth inhibitory function of the two isoflavones on cancer cells vary with concentration, cell type and exposure time. Purified genistein and daidzein alone or in combination with chemotherapy, radiation therapy and /or immunotherapy have been proved to inhibit the growth of various cancer cells in vitro and in vivo by inducing apoptosis through cell cycle progression, decrease of surviving expression or down-regulation of human telomerase catalytic subunit mRNA [57].

CONCLUSION

Collectively the outcome of this review suggest that isoflavones from the fermented soy represents an adjunctive therapeutic property as good anticancer agent targeting the cell proliferation, induction of apoptosis and exert inhibitory effects on carcinogenesis. Therefore it results that isoflavones could be administered as conventional therapeutics for the prevention of tumor progression or treatment of most malignancies. These facts provide an excellent opportunity for primary prevention of the most common cancers worldwide. Furthermore, isoflavone sensitizes the effect of radiotherapy and cytotoxic chemotherapeutic drugs used in variety of cancers, thus opening avenues for devising novel therapeutic options.

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REFERENCES


