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Research

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Phytoestrogens as Pharma Foods

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ABSTRACT

Phytoestrogens are a diverse group of plant-derived compounds that structurally or functionally mimic mammalian estrogens and show potential benefits for human health. They can serve as potential alternatives to the synthetic selective estrogen receptor modulators which are currently being used in hormone replacement therapy. Estrogens play many important physiological roles in men and women. In women, life is severely affected by a variety of estrogen-related conditions such as osteoporosis, cognitive and cardiovascular disease, increased risk of breast cancer and other symptoms that decrease the overall quality of life. Phytoestrogens are effective in maintaining bone mineral density, prevent bone loss, and help in the prevention and/or treatment of such health related problems. They can be classified as flavonoids, isoflavonoids, coumestans, stilbenes, lignans and terpenoids. The main isoflavones, genistein and daidzein found in soybean, can exist as glucosides or as aglycones, and are readily hydrolyzed in the gut to their aglycones. The aglycones are easily transported across intestinal epithelial cells. Terpenoids (ferutinine, tschimgine, and tschimganidine) found in the Umbelliferae family have estrogenic activities. The main dietary source of phytoestrogenic stilbenes is trans-resveratrol from red wine and peanuts. Plant-derived foods may be an adequate source for a variety of phytoestrogens capable of producing a range of pharmacological effects and protection from various life threatening diseases. This article provides the comprehensive information about the main groups of phytoestrogens, their food as well as herbal or botanical sources, potential health benefits and probable health hazards.

KEYWORDS: Phytoestrogens; Pharma foods; Nutraceuticals; Estrogen antagonists; Flavonoids; Isoflavonoids.

ABBREVIATIONS: DES: Diethylstilbestrol; SECO: Secoisolariciresinol; ER: Estrogen receptors; SHBG: Soy-based infant formulas; PPARs: Peroxisome proliferator-activated receptors; CV: Corn oil vehicle; G: Genistein; SBIFs: Soy-based infant formulas; BD: Beta-defensin-2; S1P: Sphingosine-1-phosphate; CAMP: Cathelicidin antimicrobial peptide; VDR: Vitamin D receptor; SERM: Selective Estrogen Receptor Modulator; CVD: Cardiovascular disease; LDL: Low Density Lipoprotein; HRT: Hormone Replacement Therapy; BMD: Bone Mineral Density; AD: Alzheimer's Disease.

INTRODUCTION

It was observed that Asian populations have lower rates of cardiovascular disease, menopausal symptoms, breast cancer (and other hormone dependent cancers), diabetes and obesity than Western populations.[1] The diet of Asian populations revealed that soy is the major part of food in an Asian diet. This observation has fueled the widely held belief that consumption of soy foods reduces the risk of disease. Phytoestrogens were first observed in 1926,[2] but it was unknown if they could have any effect in human or animal metabolism. In the 1940s, it was noticed for the first time that red clover (a phytoestrogens-rich plant) pastures had effects on the fecundity of grazing sheep.[2,3]

Phytoestrogens as the name suggests are the estrogens (xenoestrogens) that are derived from the plants and not generated within the endocrine system. They can be consumed by

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eating phytoestrogenic plants and so are also known as "dietary estrogens". A phytoestrogen is a plant nutrient that is somewhat similar to the female hormone estrogen. Due to this similarity, lignans may have estrogenic and/or anti-estrogenic effects in the body.

They are a diverse group of naturally occurring non-steroidal plant compounds that because of their structural similarity with estradiol (17-β-estradiol), have the ability to cause estrogenic or/and anti-estrogenic effects,[2] by sitting in and blocking receptor sites against estrogen. Research has shown that phytoestrogens have many health benefits such as reduction in incidence of cardiovascular diseases, prostate cancer and breast cancer. They also provide protection against post menopausal diseases including osteoporosis. Besides, both phytoestrogens such as flavonoids and lignan also possess antioxidant activity.

The major groups of phytoestrogens include flavones, isoflavones, coumestans and lignans. The former three chemically are flavonoids. Phytoestrogens in particular isoflavones are found in high amounts in soybean and their products like tofu whereas lignans are mainly found in flax seed.

Dietary estrogen (phytoestrogen) are found in wide variety of food products (including herbs), even though the level varies depending on the source. The food products with the highest total phytoestrogen content are nuts and oil seeds followed by soy products (Tables 1 and 2). The total phytoestrogen content presented is the sum of isoflavones (genistein, daidzein, glycitein, formononetin), lignans (secoisolariciresinol, matairesinol, pinoresinol, lariciresinol), and coumestan (coumestrol).

Food Sources of Phytoestrogens

The main food sources rich in phytoestrogens are nuts and oilseeds, followed by soy products, cereals and breads, legumes, meat products and other processed foods that may contain soy, vegetables, fruits, alcoholic and nonalcoholic beverages. Flax seed and other oilseeds contained the highest total phytoestrogen content, followed by soybeans and tofu.[4] The highest concentrations of isoflavones are found in soybeans and soybean products followed by legumes, whereas lignans are the primary source of phytoestrogens found in nuts and oilseeds (e.g. flax) and also found in cereals, legumes, fruits and vegetables.

Phytoestrogen (PE) content varies in different foods, and may vary significantly within the same group of foods (e.g. soy beverages, tofu) depending on processing mechanisms and type of soybean used.[5] Legumes (in particular soybeans), whole grain cereals, and some seeds are high in phytoestrogens. Some other examples of foods that contain phytoestrogens are linseed (flax), Sesame seeds, Wheat berries, Fenugreek, Oats, Barley,

Phytoestrogen food sources	Phytoestrogen content (µg/100g)
Flax seed	379380
Soy beans	103920
Tofu	27151
Soy yogurt	10275
Sesame seed	8008
Flax bread	7540
Multigrain bread	4799
Soy milk	2958
Hummus	993
Garlic	604
Mung bean sprouts	495
Dried apricots	445
Alfalfa sprouts	442
Dried dates	329
Sunflower seed	216
Chestnuts	210
Olive oil	181
Almonds	131
Green bean	106
Peanuts	34.5
Onion	32
Blueberry	17.5
Corn	9
Coffee regular	6.3
Water melon	2.9
Milk (cow)	1.2

Table 1: Foods high in phytoestrogen content.

Food items	Total phytoestrogens (µg/100g)
Vegetables	
Soy bean sprouts	790
Garlic	604
Winter squash	115
Green beans	106
Broccoli	94
Cabbage	80
Fruits	
Dried prunes	184
Peaches	65
Strawberry	52
Raspberry	48
Watermelon	2.9
Nuts and other legume seeds	
Pistachios	383
Chestnuts	210
Walnuts	140
Cashews	122
Hazel nuts	108
Lentils	37
Beverages	
Red wine	54
Green tea	13
White wine	12.7
Black tea	8.9
Coffee	5.5
Beer	2.7

Table 2: Total phytoestrogen content in vegetables, fruits, nuts and drinks.

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Beans, Lentils, Yams, Alfalfa, Mung beans, Apples, Carrots, Pomegranates, Wheat germ, Rice bran, Lupin, Kudzu, Coffee, Licorice root, Mint, Ginseng, Hops, Bourbon, Beer, Fennel and Anise, Red clover (sometimes a constituent of green manure).

Due to the molecular similarities with estrogens, phytoestrogens mildly mimic and sometimes act as antagonists of estrogen. Studies have proved that phytoestrogens play an important role in the regulation of cholesterol and the maintenance of proper bone density post-menopause. Evidence is accruing that phytoestrogens may have protective action against diverse health disorders, such as prostate, breast, bowel and other cancers, cardiovascular disease, brain function disorders and osteoporosis.[2,3,6] However, phytoestrogens cannot be considered as nutrients, since the lack of these in the diet does not produce any characteristic deficiency syndrome nor do they participate in any essential biological function.[2]

Phytoestrogens Structure

Chemically phytoestrogens belong to a large group of substituted natural phenolics compounds: the coumestans, prenyl-flavanoids and Isoflavones. These are the three most active estrogenic compounds in this class. Isoflavones are the most researched phytoestrogens and is commonly found in soy and red clover. Apart from this, lignans, stilbenes and terpenoids have also been identified as phytoestrogens but they are not flavonoids.[2] Another term 'mycoestrogens' refers to the mold metabolites of fungus Fusarium that is frequently found in pastures as well as in alfalfa and clover.[7,8] The major phytoestrogens along with their food sources are given in Table 3.

The Major Classes of Phytoestrogens are Discussed below:

Isoflavones

Isoflavones are found exclusively in the family Fabaceae (Leguminosae) and soybeans are a very rich source of them. The isoflavonoids encompass several structurally and biosynthetically related classes such as flavonols, anthocyanins, flavanones, coumestans, and chalcones. Isoflavonoids differ structurally from other classes of flavonoids in having the phenyl ring attached at the 3- rather than at 2-position of the heterocyclic ring. They have similar structure to estrogen and have the capacity to exert both estrogenic and anti-estrogenic effects, they may block the effects of estrogen in some tissues e.g. the breast and womb lining but act like an estrogen in providing possible protection against bone loss and heart diseases. In this subclass, the most thoroughly investigated and interesting compounds with regard to estrogenicity are genistein, daidzein, biochanin A and formononetin. The estrogen effect of isoflavones is much less powerful than the estrogen hormones. This is why isoflavones and phyto-estrogens exercise a balancing effect when the level of estrogens is low, such as during the menopause, and cause less menopause symptoms. A closely related compound to the isoflavonoids is 8-prenyl-naringenin, an isoflavanone, found in hops (*Humulus lupulus*), an ingredient used in beer. Populations in China, Japan, Taiwan and Korea are estimated to consume high quantities of isoflavones and women of these countries complain fewer incidences of osteoporosis and related health problems, especially hot flushes, cardiovascular diseases, lower incidence of hormone dependent breast and uterine cancer.[9]

Flavones

The flavones are a group of naturally occurring chemical compounds widely distributed in the plants. Natural flavones include apigenin, chrysin, quercetogetin, luteolin, and tricetin. Their major food sources are parsley, celery, citrus peels, capsicum, and pepper. Apigenin (4,5,7-trihydroxyflavone) commonly present in fruits and vegetables with proven anti-inflammatory and anticarcinogenic effects in various animal tumor model systems. It has been shown to suppress angiogenesis in melanoma and carcinoma of the breast, skin and colon.[10,11] Apigenin has shown potential to inhibit growth in several human cancer cells, including breast, colon, skin, thyroid, leukemia, and prostate.[9]

Stilbenes

Stilbenes belong to the family of phenylpropanoids and share most of their biosynthesis pathway with chalcones.[12] An example of stilbene is resveratrol found in grapes and has several health benefits. It exists in 2 structural isomeric forms, *cis* and *trans*, with the trans form being more common and possessing greater biological activity. One of the richest sources of this is

Class	Phytoestrogens	Food sources
Isoflavones	Genistein, biochanin A, diadzein (with its metabolites: O-DMA and equol), formononetin, glycetin	Soy, peanut, clover, sunflower seed, walnut
Flavones	Apigenin, chrysin, quercetogetin, luteolin, tricetin	Parsley, celery, citrus peels, capsicum, pepper
Stilbenes	Resveratrol	Grape, peanuts
Lignans	Secoisolariciresinol, matairesional, enterodiol, enterolactone	Soybean, peanut, broccoli, cashew nut, kiwi, pomegranate, triticale straw, flaxseeds, cereals
Coumestans	Coumestrol	Mung beans or soy sprouts, alfalfa sprouts, clover

Table 3: Phytoestrogens of human interest and their food sources.[9]

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Polygonum cuspidatum, a weed that is used in traditional Chinese and Japanese medicines. The primary dietary sources in the human diet are peanuts, grapes and wine. It has exhibited antioxidant, cardio-protective, chemo-preventative, anti-inflammatory, and estrogenic properties, as well as interaction with signal transduction pathways. It has shown to inhibit oxidative-induced apoptosis in a variety of cell lines and reduced oxidative stress in RPE cells. The antioxidant activity of resveratrol may also be associated with protection against the progression of atherosclerosis. The structural similarity of resveratrol to the synthetic estrogen diethylstilbestrol (DES) suggests that it may have estrogenic activity, cardio-protection and prevention of estrogen-dependent cancers. The estrogenic activity of resveratrol may also help prevent bone loss in post-menopausal women.[9,11]

Lignans

The lignan family is a large group of naturally abundant molecules that can be found in a plethora of superior plants where flaxseed is a particularly rich source. Lignans, along with isoflavones and coumestans, comprise the three major classes of phytoestrogens. When plant lignans are consumed, intestinal bacteria convert some into two mammalian lignans, enterolactone and enterodiol. These compounds are absorbed from the digestive tract, circulate and are excreted in the urine.[13-16]

Among lignans, secoisolariciresinol (SECO) and matairesinol are of particular interest. Secoisolariciresinol and matairesinol are two lignan dimers which are not estrogenic by themselves, but readily convert to the mammalian lignans, enterodiol and enterolactone, respectively, which are estrogenic. These are of great interest because of their estrogenic, anti-estrogenic, anti-carcinogenic, antiviral, antifungal and antioxidant activities. Particularly abundant in flaxseed, these molecules can also be found, for example, in soybean, peanut, broccoli, cashew nut, kiwi[17] and pomegranate,[14] triticale straw,[15] greater burdock[18] or *Forsythia intermedia*, asparagus, whole grains and tea.[9] Due to the structural similarity of enterolignans with mammalian oestrogens, these compounds are potentially interesting for combating some hormone-dependent cancers.[19-22] Some epidemiologic investigations have shown that the risk of breast, prostate and colon cancers is lower in countries or regions in which the diet is particularly rich in lignans.

Coumestans

Coumestans are another important group of plant (family Fabaceae) phenols that show estrogenic activity. The main coumestans with phytoestrogenic effects are coumestrol and 4′-methoxycoumestrol. Coumestrol was first isolated from ladino clover (*Trifolium repens* L.), strawberry clover (*Trifolium fragiferum* L.) and alfalfa (*Medicago sativa* L.). Coumestrol and genistein have higher binding affinities to ER-β than the other phytoestrogen compounds. Under *in vitro* conditions, coumestrol has been reported to inhibit bone resorption and to stimulate bone mineralization. Coumestans are less common in the human diet than isoflavones yet similar to isoflavones, in that they are also found in legumes, particularly sprouts of alfalfa and mung bean (*Vigna radiata*) and they are especially high in clover however, low levels have been reported in brussel sprouts and spinach.[9]

Terpenoids

Ikeda et al[23] surveyed estrogenic and antiestrogenic activities of terpenoids phytochemicals found in the Umbelliferae family and revealed that three compounds tschimgine, tschimganidine and ferutinine have agonistic and/or antagonistic activities for ER-α and ER-β. Ferutinine and tschimganidine are sesquiterpenoids and tschimgine is a monoterpenoid. Ferutinine isolated from *Ferula jaeschkeana* was reported to increase uterine weight and prevent pregnancy when administered orally in rats. It may modulate estrogen signaling similar to phytoestrogens specifically, estrogen receptor subtype selective PE and may be useful as natural SERMs.[9,24,25]

MODE OF ACTION

Phytoestrogens bind to the specific receptor sites known as estrogen receptors (ER). These receptor sites are of two types, alpha (ER-α) and beta (ER-β). Generally phytoestrogens display higher affinity for ER-β compared to ER-α.[26] The high affinity of phytoestrogens to estrogen receptors is due to their unique structural configuration that enables them to display estradiol-like effects.[2]

1. Phytoestrogens possess a phenolic ring for binding to estrogen receptor.
2. They have a low molecular weight similar to estrogens (mol. wt. 272).
3. Phytoestrogens possess a ring of isoflavones that mimics the ring of estrogens at the receptors binding site.
4. The distance between two hydroxyl groups at the isoflavones nucleus is similar to that occurring in estradiol.
5. There is an optimal hydroxylation pattern that favours binding with estrogen receptors.

Phytoestrogens also modulate the concentration of endogenous estrogens by binding or inactivating some enzymes and also affect the bioavailability of sex hormones by depressing or stimulating the synthesis of sex hormone binding globulin (SHBG).[3] Research has shown that phytoestrogens bind and transactivate peroxisome proliferator-activated receptors (PPARs). Both ERs and PPARs influence each other and therefore, induce differential effects in a dose-dependent way. The final biological effects of genistein are determined by the balance among these pleiotrophic actions.[27]

Ecology of Phytoestrogens

Phytoestrogens are naturally occurring substances since ancient times and are involved in plant defense systems particularly against fungi. They function as dietary phytochemicals and are considered co-evolutive with mammals.[9] besides phytoestrogens,

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there are some man-made novel exogenous estrogens known as xenoestrogens. They are used as food additives and in ingredients like cosmetics, plastics and insecticides. Xenoestrogens have environmentally similar effect as phytoestrogens as proved in a study of populations.[28]

Phytoestrogens and Birds

Studies have shown that consumption of plants containing unusual content of phytoestrogens under drought conditions decreases the fertility in quail. It has been found that parrot food available in nature possess weak estrogenic activity. Studies are being conducted on screening methods for environmental estrogens present in manufactured supplementary food, with the purpose to enable reproduction of endangered species.[29]

In another, it was found that developmentally inappropriate exposures to estrogenic compounds are known to alter morphology and function of the reproductive tract in various species. Chickens are continually exposed to the relatively potent estrogenic soy isoflavones through the diet. Previous experiments have demonstrated that the primary soy isoflavone genistein induces proliferation of the chick oviduct. However, information is lacking as to specific reproductive tract developmental effects of genistein exposure in chicks. Experiments were done to compare specific oviduct morphological and functional responses to genistein exposure with responses elicited by a classical estrogen, diethylstilbestrol (DES). To avoid the effects of dietary soy isoflavones, the experimental diets were formulated with dried egg white, rather than the usual soybean meal, as a protein source. 100 one day-old female chicks were assigned evenly to 10 treatments: egg white based diet with daily oral gavages of corn oil vehicle (CV); 1 mg DES; 2.0 mg genistein (G2); 20 mg genistein (G20); or 40 mg genistein (G40). At 15 days of age, half the birds from each treatment received a single injection of 2 mg progesterone in a corn oil vehicle to induce ovalbumin synthesis in the oviduct. The classical oviduct responses to estrogen, induction of progesterone receptor and initiation of ovalbumin synthesis, were examined by immune-histochemistry. At 16 days of age, DES treatment increased oviduct weight and percentage of final body weight as compared to all other treatments ($p<0.05$). Immunohistochemistry of formalin fixed oviduct samples revealed that the DES, G20, and G40 treatments significantly increased specific staining for progesterone receptor and ovalbumin in the chick oviduct as compared to CV and G2 treatments. It was concluded that genistein can function as a classical estrogen in the chick oviduct and that dietary exposures to genistein may alter oviduct development.[30]

Effect of Phytoestrogens on Humans

Phytoestrogens are readily absorbed and circulated in plasma and the unabsorbed portion is finally excreted in the urine. The metabolic pathway of phytoestrogens is completely different in humans as compared in grazing animals. This is due to their difference in digestive systems.[9]

Clinical trials on males have shown no observable changes in testicular or ejaculate volume when their diet was supplemented with isoflavone. A meta-analysis of 15 placebo-controlled studies has also shown that the incorporation of soy foods does not alter the bioavailability of testosterone concentrations in men.[31]

Contrary to this, some epidemiological studies have shown the protective effects of phytoestrogens in females against breast cancer. It has also been found that females with history of breast cancer should consume the soya products with caution since soybean can stimulate the growth of estrogen receptor-positive cells *in vitro*. However, the potential for tumour growth is related only with small concentration of genistein and the protective effect was found to be related with larger concentrations of same phytoestrogens.[5] Although not much information is available on the mechanism of action of isoflavones to inhibit tumour growth, but the *in vitro* studies justify the need to evaluate the impact of isoflavones on breast tissue in females.[32] Epidemiologic studies suggest that consumption of soy estrogens is safe for patients with breast cancer and that it may in fact decrease mortality and recurrence rates.[33] It has been reported that phytoestrogens such as genistein may help to prevent photo aging in human skin and promote formation of hyaluronic acid.[34]

Effect of Phytoestrogens on Infants

It has been found that there are no adverse effects of phytoestrogens on infants.[35] Research has shown that there are no adverse effects on human growth, development, or reproduction due to the consumption of soy-based infant formula as compared to conventional cow-milk formula.[36-37] In a clinical studies of infants fed SBIFs [soy-based infant formulas] have resolved questions and raised no clinical concerns with respect to nutritional adequacy, sexual development, neurobehavioral development, immune development, or thyroid disease. SBIFs provide complete nutrition that adequately supports normal infant growth and development. FDA has accepted SBIFs as safe for use as the sole source of nutrition. Although clinical guidelines published by American Academy of Pediatrics stated that isolated soy protein-based formulas may be used to provide nutrition for normal growth and development, but there are few indications for their use in place of cow milk-based formula. These indications are especially for infants with galactosemia and hereditary lactase deficiency (rare) and in situations where a vegetarian diet is preferred.[38]

Ethnopharmacology of Phytoestrogenic Plants

Phytoestrogenic plants are used in the treatment of menstrual, menopausal and including fertility problems. Phytoestrogen containing plants are *Pueraria mirifica* and its close relative, kudzu, Angelica, fennel and anise. In an another study, phytoestrogens rich plant red clover has been shown to be safe, but ineffective in relieving menopausal symptoms, black cohosh is also used for menopausal symptoms, but does not

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contain phytoestrogens. Panax Ginseng contains phytoestrogens and has been used for menopausal symptoms.[39]

Biological Activities of Phytoestrogens

Antimicrobial activity and Phytoestrogens

In a study, synergistic antimicrobial activities of phytoestrogens were observed in crude extracts of two sesame species against some common pathogenic microorganisms. Methanolic and ethanolic extracts exhibited broad spectrum antimicrobial effect against all the tested pathogenic micro-organisms except *Streptococcus pneumoniae* and *Staphylococcus aureus* respectively, while the aqueous extract exhibited inhibitory activity on *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Candida albicans*. The result confirmed the folkloric claims of the antimicrobial effectiveness of locally consumed sesame leaves extracts especially against bacterial and common skin infection in many areas of Nigeria.[40]

In another study, it was found that soybean phytoestrogen enhances the antimicrobial peptides beta-defensin-2 (BD) synthesis in endometrial epithelial cells with lipopolysaccharides and polyinosinic-polycytidylic acid stimulation. This study provided the first evidence and a role of BD in mucosal defense against pathogen in glandular endometrial epithelium. The differential modulation of the expression and secretion of BD by soybean phytoestrogen could be applied for protection of female reproductive tract from pathogen invasions.[41]

In an another study, it was discovered that a signaling lipid, sphingosine-1-phosphate (S1P), generated by sphingosine kinase 1, regulates a major epidermal antimicrobial peptide's cathelicidin antimicrobial peptide (CAMP)] expression *via* an NF-κB→C/EBPα-dependent pathway, independent of vitamin D receptor (VDR) in epithelial cells. Activation of estrogen receptors (ERs) by either estrogens or phytoestrogens also is known to stimulate S1P production, but it is unknown whether ER activation increases CAMP production. The researchers investigated whether a phytoestrogen, genistein, simulates CAMP expression in keratinocytes, a model of epithelial cells, by either a S1P-dependent mechanism(s) or the alternate VDR-regulated pathway. Exogenous genistein, as well as an ER-β ligand, WAY-200070, increased CAMP mRNA and protein expression in cultured human keratinocytes, while ER-β antagonist, ICI182780, attenuated the expected genistein and WAY-200070-induced increase in CAMP mRNA/protein expression. Genistein treatment increased acidic and alkaline ceramidase expression and cellular S1P levels in parallel with increased S1P lyase inhibition, accounting for increased CAMP production. In contrast, *si*RNA against VDR did not alter genistein-mediated up-regulation of CAMP. Taken together, genistein induces CAMP production *via* an ER-β→S1P→NF-κB→C/EBPα- rather than a VDR-dependent mechanism, illuminating a new role for estrogens in the regulation of epithelial innate immunity and pointing to potential additional benefits of dietary genistein in enhancing cutaneous antimicrobial defense.[34]

Phytoestrogens for Cancer Prevention

Phytoestrogens display an array of pharmacologic properties and investigation of their potential as anticancer agents has increased dramatically. Phytoestrogens have been investigated as natural alternatives to hormone replacement therapy and their potential as chemopreventive agents. Scientists have investigated the effects of equol, genistein and coumestrol on cell growth in fully estrogenized MCF-7 cells, simulating the peri-menopausal state and long term estrogen deprived MCF7:5C cells which simulate the postmenopausal state of a woman after years of estrogen deprivation and compared the effects to that of steroidal estrogens: 17 β-estradiol (E2) and equilin present in conjugated equine estrogen. Steroidal and phytoestrogens induce proliferation of MCF-7 cells at physiologic concentrations but inhibit the growth and induce apoptosis of MCF7:5C cells. Although steroidal and phytoestrogens induce estrogen responsive genes, their anti-proliferative and apoptotic effects are mediated through the estrogen receptor. Knockdown of ER-α using siRNA blocks all estrogen induced apoptosis and growth inhibition. Phytoestrogens induce endoplasmic reticulum stress and inflammatory response stress related genes in a comparable manner as the steroidal estrogens. Inhibition of inflammation using dexamethasone blocked both steroidal and phytoestrogen induced apoptosis and growth inhibition as well as their ability to induce apoptotic genes. Together, this suggests that phytoestrogens can potentially be used as chemopreventive agents in older postmenopausal women but caution should be exercised when used in conjunction with steroidal anti-inflammatory agents due to their anti-apoptotic effects.[42]

In yet another study, it was proved that some phytoestrogenic compounds are associated with reduced risk of endometrial cancer. The development of endometrial cancer is largely related to prolonged exposure to estrogens without cyclic exposure to progesterone. Unopposed estrogens increase mitotic activity in endometrial cells, whereas progesterone reduces this activity. The identification of factors that lower endogenous estrogen levels is therefore, important in efforts to prevent this disease.[43]

Estrogens found in plant foods (phytoestrogens), such as isoflavones found in soybeans and lignans found in whole grains, seeds, and dried fruit, have been shown to lower endogenous estrogen levels. They also stimulate the production of sex hormone–binding globulin (SHBG) by the liver. Higher SHBG levels result in more bound and thus less free estradiol, reducing the amount of estrogens available for binding with estrogen receptors. Phytoestrogens also bind competitively to estrogen receptors, thereby blocking binding by estradiol and other estrogens. Because of their weak estrogenic potential (0.1% that of estradiol), phytoestrogens do not elicit a strong estrogenic response and thus have an antiestrogenic effect that inhibits the growth and proliferation of estrogen-dependent

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cancer cells.[43]

Phytoestrogens for Breast Cancer Treatment

Flaxseed is the richest dietary source of lignans, a type of phytoestrogen. They are found in a variety of foods, including soy, flaxseeds, other nuts and seeds, whole grains, and some vegetables and fruit. Most of the research regarding flaxseed and breast cancer focuses on the lignans found in flaxseeds, and their potential for weak estrogenic or anti-estrogenic effects in a woman's body.[44] Lignans, can also change estrogen metabolism. In postmenopausal women, lignans can cause the body to produce less active forms of estrogen. This is believed to potentially reduce breast cancer risk. There are evidences that adding ground flaxseeds into the diet decreases cell growth in breast tissue as well. This would be the type of change that would be expected to decrease breast cancer risk.[8] It is well known that all cells have the ability to go through a process called apoptosis, or programmed cell death. It is believed that through this process, the body can prevent damaged cells from reproducing, and eventually developing into cancer. Researchers have shown that flaxseed sprouts can increase apoptosis. Some cell and animal studies have shown that two specific phytoestrogens found in lignans, named enterolactone and enterodiol, may help suppress breast tumor growth. Animal studies have shown that both flaxseed oil and lignans can reduce breast tumor growth and spread, even for ER- cancer cells. This result suggests that flaxseeds may have anti-cancer benefits that are unrelated to any type of effect on estrogen or estrogen metabolism.[45]

Tamoxifen is a medication known as a Selective Estrogen Receptor Modulator (SERM). It binds with estrogen receptors, without activating growth in breast cancer cells. In this way, tamoxifen prevents a women's own estrogen from binding with these cells. As a result, breast cancer cell growth is blocked.[46] One study in mice concluded that flaxseed inhibited the growth of human estrogen-dependent breast cancer, and strengthened the tumor-inhibitory effect of tamoxifen. Multiple other studies with mice have shown that dietary flaxseed works with tamoxifen to inhibit breast tumor growth.[47] Researchers are not confirmed about the results will apply to women with breast cancer, but this approach of adding flaxseeds to the diet looks promising. And several studies in women have shown that higher intake of lignans, the key phytoestrogen in flaxseeds, is associated with reduced risk of breast cancer.[48] Further, lignans in the diet are associated with less aggressive tumor characteristics in women who have been diagnosed with breast cancer. In other words, women who have already been eating lignans at the time of diagnosis seem to have tumors that are less aggressive.[49]

Phytoestrogens for Preventing Post Menopausal Osteoporosis

Aging causes the progressive loss of bone-mineral density, a process that accelerates during pre-menopause and increases fracture risk. Postmenopausal osteoporosis has become a social problem and it requires appropriate management strategies. Replacement therapy is effective for both prevention and therapy, but recent findings have shown that its long term administration is not as safe as was previously thought, so alternative treatments are urgently needed. Dietary phytoestrogens are emerging as a valid alternative to estrogens in the treatment of menopause-related diseases, such as the climacteric syndrome, cardiovascular diseases, osteoporosis, and dementia. Research has shown that dietary changes in Western habits favoring an increased intake of phytoestrogens-rich foods, could contribute to prevent and to reduce the incidence of postmenopausal osteoporosis in this population.[9]

Phytoestrogens promote estrogenic actions in mammals. They not only act estrogenically as estrogen agonists, but also anti-estrogenically as antagonists by blocking or altering ERs, thus they more closely resemble natural Selective Estrogen Receptor Modulators (SERMS). In short, they perform a complex function as agonists or antagonists depending on the tissue, ER type and quantity and the endogenous hormonal milieu.[5]

In vitro studies have shown that phytoestrogens can be the ideal candidates for treatment of osteoporosis because they are able to stimulate osteoblastic activity and inhibit osteoclast formation. This double positive action is obtained at a range of concentrations (10^{-5} to10^{-7} M) consistent with human ingestion of genistein.[50] The discovery of ERα and ERβ receptors in the bone, the positive effect of selective SERMs such as raloxifene in animals and in humans, and the fact that by virtue of their similarity to raloxifene in forming bonds with the estrogen receptors, phytoestrogens such as genistein, have selective effects on the bone. This protective effect of the phytoestrogens on the bone is produced through the binding of these substances to the estrogen receptors and particularly ER-β ER-β expression is increased during bone mineralization and the high affinity of genistein towards ER-β could make its action efficient at physiological levels.[9] Animal studies have shown that numerous phytoestrogens including coumestrol, genistein, daidzein and others have bone sparing effects in the rat.[51]

Evidence for measurable effects in humans is equally mixed. At least one study has found that post-menopausal women consuming high quantities of soy foods have better femoral and/or lumbar spine density compared to women who consume less soy.[52] A 2009 meta-analysis of randomized clinical trials conducted in humans, however, found only a weak association between increased consumption of soy isoflavones and improved bone-mineral density, leading the authors to conclude that soy isoflavones were unlikely to meaningfully reduce the risk of osteoporosis.[53] Thus adding soya to diet can help stave off bone loss in mid-life especially for women.

Phytoestrogens for Prostate Cancer

Worldwide disparities exist between geographic regions with regard to prostate cancer incidence and mortality. Countries in East Asia have lower rates of prostate cancer compared with Western countries such as Canada and the US. Some suggest

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that dietary differences between the two geographic regions, particularly the higher amount of phytoestrogens consumed in East Asia, is responsible for the difference in prostate cancer incidence. Phytoestrogens are hormonally active compounds present in plant foods that are being studied extensively for their potential roles in hormonally-sensitive neoplasms such as prostate cancer. The mechanism of action of the soy isoflavones is incompletely understood, but in regards to prostate carcinogenesis likely involves estrogenic effects, cell cycle inhibition, anti-angiogenesis and induction of apoptosis. Recent clinical studies have provided mixed results with regard to a clear association between prostate cancer and soy consumption.[54]

The evidence for a protective role of phytoestrogens is not conclusive enough for a general recommendation for their use as dietary supplements, but phytoestrogens can be considered for therapeutic use in prostate cancer patients under certain circumstances. A literature review was performed to study the evidence regarding the chemo-preventive role of phytoestrogens in healthy men, the protective role in early prostate cancer, and a possible therapeutic role in advanced prostate cancer patients. Dietary supplementation with phytoestrogens for chemoprevention of prostate cancer is still a debatable subject. Numerous pre-clinical *in vitro* studies have been promising, and novel molecular mechanisms of action for phytoestrogens continue to be identified. However, human clinical trials including studies done on prostate biomarkers and on the effects of phytoestrogens on steroid hormones are complicated by the possibility of local paracrine effects in prostatic tissue by phytoestrogens that are steroid-like in structure. Their interaction with multiple enzymes represents a paradigm for the complexity of phytoestrogen effects and a window into a potential reason that study results are inconsistent or difficult to explain. A final outcome of the phytoestrogen effect in the intact human may be difficult to discern because these agents can inhibit or induce enzymes, destroy cancer cells, yet will have intrinsic estrogenic effects themselves. Larger multi-center, multi-national, randomized controlled trials are needed before definitive recommendations can be made on the usefulness of phytoestrogens for chemoprevention and therapy for prostate cancer. However, combinations of phytoestrogens with radiation therapy and other antioxidants in advanced or metastatic prostate cancer can be considered because there are limited effective therapy options for this group of patients.[55]

In a study, a large meta-analysis suggests that both fermented and non-fermented soy is protective against cancer.[56] While tofu was the only individual food showing a protective effect, the phytoestrogens genistein and daidzein were also associated with a lower risk of prostate cancer. Further evidence of the protective effect of genistein can be concluded from studies using rodent models and human cell lines.

Mentor-Marcel et al[57] investigated the effects of genistein on the progression of prostate cancer in the TRAMP mouse model. When dietary genistein was used to elevate mouse serum genistein to levels comparable to that of Asian men, the rate of poorly differentiated adenocarcinoma decreased in a dose-dependent manner, while survival improved as a function of decreased tumor burden.[57]

In yet another study, using a rat hormonal carcinogenesis model has shown that a soy isoflavone mixture that includes genistein and diazein is able to protect against carcinogenesis in the dorsolateral and anterior prostate lobes.[58] *In vitro* studies revealed that genistein inhibited growth of two prostate cancer cell lines alone or in combination with selenium.[59] The treatment also induced apoptosis through caspase-dependent pathways and reduced expression of matrix metalloproteinase 2, which has been associated with active invasion and metastases.

Phytoestrogens for Cardiovascular Diseases

Cardiovascular disease (CVD) is the number one cause of morbidity and mortality in men and women worldwide. According to the WHO, by 2015, almost 20 million people will die from CVD each year.[60] In menopause the risk of CVD greatly increases due to the loss of estrogen. Lipid profiles, vascular reactivity, cellular proliferation and thrombosis are factors that affect CVD and on which phytoestogens have shown beneficial effects.[61] Mechanisms suggested explaining the prevention of CVD and the reduction of atherosclerosis are: improvement of plasma lipid concentrations, reduction of thrombus formation such as inhibition of platelet action, improvement of systemic arterial compliance and antioxidant activity. Studies suggest that isoflavones as antioxidants may affect atherogenesis by reducing the oxidation of LDL. Phytoestrogens are a subcategory of compounds called flavonoids, a group composed of hundreds or more types of molecules. The 2 classes of phytoestrogens are isoflavones, notably found in soy products and lignans, present in nuts, fruits, cereal grains, tea, and coffee.[62] Hwang et al[63] reported that extracts of soy, alfalfa and acerola cherry (*Malpighia glabra* L., Malpighiaceae) may synergistically interact to prevent LDL oxidation. Because of their assumed health benefit, isoflavone content is advertised in many foods that contain soybeans, and isoflavones are sold as nutritional supplements.

Phytoestrogens for Relief from Menopausal Symptoms

This is the most widely attributed health benefit of phytoestrogen consumption. Research has shown that intake of phytoestrogens provides relief from menopausal symptoms including hot flushes and night sweats. Studies have shown that there is a slight reduction in hot flushes and night sweats with phytoestrogen-based treatment. Extracts containing high levels of genistein appeared to reduce the number of daily hot flushes but it needs to be investigated further. Overall no indication suggested that other types of phytoestrogens work any better than no treatment. Moreover, no evidence was found of harmful effects on the lining of the womb, stimulation of the vagina or other adverse effects with short-term use.[64]

The association between phytoestrogen intake and breast

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cancer risk in a large prospective study in a Dutch population with a habitually low phytoestrogen intake was investigated and it was concluded that in Western populations, a high intake of isoflavones or mammalian lignans is not significantly related to breast cancer risk. Despite this uncertainty, dietary supplements continue to be popular, particularly among women seeking a "natural" alternative to hormone replacement therapy.[65] In yet another review it was concluded that no evidence shows a benefit of phytoestrogens enriched or-derived products for menopausal vasomotor symptoms with the exception of products containing a minimum of 30 mg per day of genistein which have been evaluated for upto two years in four studies.[66]

Phytoestrogens for Bone Health and Osteoporosis

Estrogen deficiency is a major risk factor for osteoporosis in postmenopausal women. Although hormone replacement therapy (HRT) has been rampantly used to recompense for the bone loss, but the procedure is coupled with severe adverse effects. Hence, there is a boost in the production of newer synthetic products to ward off the effects of menopause-related osteoporosis. As of today, there are several prescription products available for the treatment of postmenopause osteoporosis; most of these are estrogenic agents and combination products. Plant-derived natural products, mostly phytoestrogens (isoflavones, lignans, coumestanes, stilbenes, and flavonoids) are used to prevent menopause-related depletion in bone mineral density (BMD). Although, a number of papers are published on menopause-related general symptoms, sexual dysfunction, cardiovascular diseases, Alzheimer's disease, diabetes, colon and breast cancers, there is paucity of literature on the accompanying osteoporosis and its treatment.[67] In a recent study, the effect of soy protein with and without phytoestrogens on bone turnover was determined in post menopausal women i.e. within two years of the onset of menopause when the bone loss is at its greatest. It was found that there was a significant decrease in bone turn over markers of resorption and formation after supplementation with 15 g soy protein and phytoestrogens for 6 months. An initial effect on osteoclast followed by decreased osteoblast function may have beneficial effects on bone health. There was no significant change in bone turn over makers with 30 g soy protein alone for 6 months.[68]

Phytoestrogens and Cognition

Cognition and memory functioning have been reported to decrease around menopause and therefore, studies have investigated the association of ERT and cognition, as well as phytoestrogens and cognition. However, limited studies are available on the effects of phytoestrogens on cognitive functioning. The mechanisms are not understood, but it has been suggested that phytoestrogens act as estrogen agonists and may increase spine density and synapse formation in the hippocampus of adults. In addition, phytoestrogens may interact with the transcription of neurotrophin genes.[69]

Neuroprotective effects of phytoestrogen compounds found in soy have been demonstrated in animal research and cell culture studies. In particular, phytoestrogens have been shown to reduce Alzheimer's Disease (AD) related pathology, potentially alleviating risk of AD progression. In addition to their antioxidant properties, soy products also have the ability to affect cognition *via* interaction with estrogen receptors. However, observational studies and randomized controlled trials in humans have resulted in inconclusive findings within this domain. There are several possible reasons for these discrepant data. Studies which report no effect of phytoestrogens on cognition have mainly been carried out in European cohorts, with an average low dietary consumption. In contrast, investigation of Asian populations, with a higher general intake of tofu (a non-fermented soy product) has shown negative associations with cognitive function in those over the age of 65. Consideration of type of soy product is important, as in the latter sample, protective effects of tempeh (fermented soy) were also observed. Limited data provide evidence that effects of phytoestrogens on cognition may be modified by dosage, duration of consumption and cognitive test used. Additionally, characteristics of the study population including age, gender, ethnicity and menopausal status appear to be mediating variables. Phytoestrogen treatment interventions have also shown time-limited positive effects on cognition. These findings are consistent with estrogen treatment studies, where initial positive short-term cognitive effects may occur, which reverse with long-term continuous use in elderly women. Well controlled, large scale studies are needed to assess the effects of phytoestrogens on the aging brain and provide further understanding of this association.[70]

Phytoestrogens: Side Effects

As it is already known, phytoestrogens are structurally similar to endogenous estrogens such as 17 β-estradiol and are produced by plants. The most well-understood phytoestrogen action on animal physiology, due to ingestion or exposure to contaminated water, involves competitive binding to estrogen receptors. Because of this ability, some phytoestrogens have documented medicinal potential,[71] but in uncontrolled conditions they may adversely affect reproduction.[72] Furthermore, phytoestrogens may also interfere directly with steroid biosynthesis, intracellular signaling, cell proliferation, and gene expression,[73] which has raised concerns in the medical community about their safety.[8]

Consequences of exposure to these compounds are still unclear, as phytoestrogens have been reported to have estrogenic as well as anti-estrogenic effects on vertebrates.[74] Generally, phytoestrogens are considered safe for humans at common exposure levels, such as those found in soy products, but the large-scale anthropogenic production of phytoestrogens in runoff from agricultural areas, wood pulp mill discharge, and sewage treatment plant effluent may still pose a threat to aquatic ecosystems.[75]

In another study, a meta-analysis of side effects was performed comparing phytoestrogen treatment with placebo or no treatment in randomized controlled trials and it was found

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that phytoestrogen supplements have a safe side-effect profile with moderately elevated rates of gastrointestinal side effects. It was also found in the investigation studies that rates of vaginal bleeding, endometrial hyperplasia, endometrial cancer and breast cancer were not significantly increased among phytoestrogen users.[76]

CONCLUSION

Thus from the foregoing it is quite evident that diets rich in plant-derived products may supply a variety of phytoestrogens capable of producing a range of pharmacological effects in the human body. As people live longer, women are spending more of their lives in menopause, affected by a variety of estrogen-related conditions such as osteoporosis, cognitive and cardiovascular disease, increased risk of breast cancer and other symptoms that decrease the overall quality of life. Epidemiological evidence and experimental data from animal studies are highly suggestive of the beneficial effects of phytoestrogens on human health and their potential to be used as pharma foods, but the clinical data supportive of such effects are either not available, or are awaiting design and execution of appropriate prospective large-scale clinical studies. Due to the functional and structural differences of phytoestrogens, their biological activities are also highly variable and there may be other effects that have not yet been studied. Future research should focus on specific soy components, variability in phytoestrogen metabolism and effects of phytoestrogens on specific target tissues.

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ETHICAL ISSUES

There is none to be declared

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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