Soy Isoflavones in Integrative Oncology: Increased Efficacy and Decreased Toxicity of Cancer Therapy

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Abstract
Soy consumption in human diet has been linked to decreased incidence of a variety of cancers, suggesting a potential role of soy products in cancer prevention and control. Furthermore, a substantial body of evidence in the literature suggests that soy supplementation may improve the efficacy and prevent the adverse effects of cancer chemotherapy and radiation therapy. Isoflavones constitute the predominant anticancer bioactive compounds in soy. Genistein, which is the most abundant and active isoflavone in soy, has a multitude of effects on cancer cells, including inhibition of NF-κB activation and DNA methylation, enhancement of histone acetylation, inhibition of cell growth and metastasis, and antiangiogenic, anti-inflammatory, and anti-oxidant effects. Isoflavones are orally bioavailable, easily metabolized, and usually considered safe. In this article, we review in vitro and in vivo evidence as well as the results of clinical and epidemiological studies on the effects of soy isoﬂavones, with a focus on sensitization of cancer cells to chemotherapy and radiation while at the same time protecting normal cells from the harmful effects of these treatments.

Keywords
prostate cancer, genistein, chemotherapy, radiotherapy

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Introduction
Many studies have been conducted to investigate the interaction of dietary constituents and antineoplastic therapies in both animal models and clinical trials.¹ In particular, soy isoﬂavones have been suggested to have cancer preventive effects.² Epidemiological studies have shown a signiﬁcant difference in cancer incidence among different ethnic groups, which could be attributed in part to dietary habits. For example, the incidence of breast cancer and prostate cancer (PCa) are much higher in the United States and Europe where dietary soy intake is low as compared with countries such as China and Japan, where dietary consumption of soy products is much higher. Genistein, a predominant isoflavone in soy, has been shown to inhibit cancer development, growth, and metastasis in animal models. It may act by modulating the genes related to cell cycle control and apoptosis.³

Chemotherapy is a common form of treatment for many types of cancer. Chemotherapeutic drugs are designed to interfere with rapidly dividing cells such as cancer cells. This means that they may also harm normal cells that divide normally—that is, the cells in the bone marrow, mouth, gastrointestinal tract, nose, nails, vagina, and hair cells, which undergo constant division making them vulnerable to the toxic effects of chemotherapy as well as radiotherapy. Therefore, major research efforts have been aimed at discovering ways of protecting normal proliferating cells from cancer therapy. Many chemotherapeutic agents work by producing free radicals or reactive oxygen species (ROS) which, if not quenched, can damage the cell and its various constituents. However, these species also affect the normal cells. Detoxification of free radicals and ROS is, therefore,
Soy Isoflavones: Structure and Metabolism

 Isoflavones are polyphenolic plant-derived compounds that have both estrogenic (estrogen agonist) and antiestrogenic (estrogen antagonist) activity. They are the major flavonoids in legumes, particularly soybean, where they are present bound to sugars (glycosides). Soy isoﬂavones include genistein, daidzein, and glycitein. These compounds are structurally characterized by their 3-phenylchro-men-4-one backbone, which consists of 2 benzene rings linked by a heterocyclic pyran ring. In addition to the heterocyclic core, genistein and its related isoﬂavones are polyphenols (contain several hydroxyl groups attached to core phenyl rings). Genistein, or 4′,5,7-trihydroxyisoflavone and daidzein are the most prominent isoﬂavones in soy products. The structure of genistein is shown in Figure 1. Soybeans and soy products constitute the richest source of isoﬂavones in human diet.

Genistein can also be synthesized, but humans obtain it from the diet. In soy and soy products, the primary chemical form of genistein exists in the conjugated form known as genistin. Therefore, people are exposed to genistin far more than genistein when consuming soy and soy products in their diet. Genistein undergoes first-pass metabolism in the liver as well as enterohepatic circulation after absorption from the intestine. The byproducts of isoﬂavone metabolism may produce different effects on the body. Studies showed that 90% of genistein in the blood undergoes extensive liver metabolism and exists in either the glucuronidated or sulfated form, whereas only 10% exists in the nonconjugated or free form.

Soy Isoflavones in Cancer Therapy: Effects and Mechanisms

Soy, soybean, or soya bean, is a native East Asian legume, which is widely grown for its edible beans. The beans and its products have been reported to have beneficial effects in cancer, which is largely attributed to the presence of isoﬂavones. In a recent meta-analysis of 30 articles, a clear statistically significant association between soy consumption and decreased PCa risk was reported. Populations in countries like China and Japan, which consume soy foods as part of the regular diet, showed low incidence of PCa. In the analysis of the potential impacts of soy food and isoﬂavone intake and that of the circulating isoﬂavones, in both primary and advanced PCa, total soy food (P < .001), genistein (P = .008), daidzein (P = .018), and unfermented soy food (P < .001) intakes were found to be significantly associated with decreased risk of PCa. Neither soy food intake, nor circulating isoﬂavones were associated with advanced PCa risk. The meta-analysis provided a comprehensive updated analysis of previously published data demonstrating that soy foods/isoﬂavones were associated with reduced PCa risk.

Genistein is one of the most predominant and active flavonoids in soybeans. It can alter a variety of biological processes in estrogen-related malignancies, which include PCa, and has been found to act mainly by altering apoptosis, the cell cycle, and angiogenesis and inhibiting metastasis. The molecular mechanisms of the anticancer and therapeutic effects of genistein has been suggested to involve caspases, B cell lymphoma 2 (Bcl2)-associated X protein, Bcl-2, kinesin-like protein 20A, extracellular signal-regulated kinase 1/2, nuclear transcription factor κB (NF-κB), mitogen-activated protein kinase, inhibitor of NF-κB, Wingless and integration 1 β-catenin, and phosphoinositide 3 kinase/Akt signaling pathways. Its inhibitory effect on NF-κB is particularly important because the inhibition suppresses inflammation. The NF-κB and the serine/threonine-specific protein kinase Akt both maintain a homeostatic balance between cell survival and apoptosis. Interestingly, genistein has also been reported to be a potent angiogenesis and metastasis inhibitor, which is promising in cancer prevention, control, and treatment. Genistein also shows synergistic behavior with anticancer drugs, such as doxorubicin, docetaxel, and tamoxifen, suggesting a potential role in combination therapy.

In colon cancer in humans, the soy isoﬂavones, especially genistein, inhibit cell growth and facilitate apoptosis and cell cycle arrest in the G2/M phase. The cell cycle arrest in the G2/M phase is accompanied by the activation of ATM/ p53, p21waf1/cip1 and GADD45α as well as downregulation of cdc2 and cdc25A, as demonstrated by quantitative polymerase chain reaction and immunoblotting. Interestingly, genistein induced G2/M cell cycle arrest in a p53-dependent manner and suggested the crucial role of the
orally for a maximum of 6 months. There was no decline in
ing 40 mg of a soy isoflavone mixture (n = 33) twice daily
lycopene alone (n = 38) or together with a capsule contain-
to receive a tomato extract capsule containing 15 mg of
out hormone therapy. Participants were randomly assigned
radical prostatectomy and/or radiation therapy with or with-
enrolled 71 patients who had rising PSA levels after failing
isoflavone intake in PCa patients.10 Vaishampayan et al
PCa cell lines. PSA stabilization has been observed with soy
in apoptosis and inhibition of cell growth in androgen-sensi-
vitro studies with lycopene and genistein showed an increase

Dietary intake of lycopene and soy lowers the PCa risk. In
prevention and control of cancers, which makes it a promis-
progression.37 In various studies, of all the isoflavones
of normal cells by reducing lipid peroxidation or halting

Soy isoflavones, mainly genistein, have been reported to
have a number of biological effects other than their effects
in cancer. These have been summarized in Table 1.

Isoflavones as Potential Candidates in
the Prevention and Control of PCa

Epidemiological studies have shown the beneficial effects
of soy isoflavones in breast, prostate, and colon cancers in
countries such as China and Japan, where the dietary con-
sumption of soy isoflavones is high.34 A recent randomized,
double-blind phase 2 clinical study that aimed to investigate
the effect of synthetic genistein concentrations and its safety
in patients with localized PCa showed that genistein at a
dose that can be easily obtained from a diet rich in soy
reduced the level of serum prostate-specific antigen (PSA)
with no adverse effects of clinical significance.35 A compre-
prehensive meta-analysis on the extent of the possible associa-
tion between soy-based food consumption and the risk of
PCa indicated an inverse relationship between dietary soy
isoflavone (genistein and daidzein) consumption and
PCa incidence and mortality.36 Nutritionally relevant levels
of genistein may modulate the expression of prostate
tissue biomarkers associated with cancer prediction and
progression.37 In various studies, of all the isoflavones
investigated, genistein appears to be the most potent in the
prevention and control of cancers, which makes it a promis-
molecule in PCa.

The use of soy isoflavones has also been considered in
combination with other natural products, such as lycopene.38
Dietary intake of lycopene and soy lowers the PCa risk. In
vitro studies with lycopene and genistein showed an increase
in apoptosis and inhibition of cell growth in androgen-sensitive
(LNCaP) and androgen-independent (PC3 and VeCaP)
PCa cell lines. PSA stabilization has been observed with soy
isoflavone intake in PCa patients.10 Vaishampayan et al
enrolled 71 patients who had rising PSA levels after failing
radical prostatectomy and/or radiation therapy with or with-
out hormone therapy. Participants were randomly assigned
to receive a tomato extract capsule containing 15 mg of
lycopene alone (n = 38) or together with a capsule contain-
ing 40 mg of a soy isoflavone mixture (n = 33) twice daily
orally for a maximum of 6 months. There was no decline in
serum PSA in either group qualifying for a partial or com-
plete response. However, 35 of 37 (95%) evaluable patients
in the lycopene group and 22 of 33 (67%) evaluable patients
in the lycopene plus soy isoflavone group achieved stable
disease, described as stabilization in serum PSA level. Data
suggested that lycopene and soy isoflavones had activity in
PCa patients and may delay progression of both hormone-
refractory and hormone-sensitive PCa. However, the effect
was not additive. Importantly, a recent phase 2 dose-escalating
study in PCa patients examined plasma, prostate, and urine
biomarkers of carotenoid and isoflavone exposure and
described foundation for tomato-soy juice in future human
clinical trials.39 Investigating the efficacy of more com-
ounds of natural origin might be a fruitful area of research
in PCa control and treatment. Clinical trials with a mecha-
nistic approach could elucidate potential clinical uses of
natural compounds.

Soy Isoflavones in Chemotherapy
Toxicity

Chemotherapy often results in the generation of ROS in
excess, and thus the oxidative stress, which is evidenced
by increased lipid peroxidation and decrease in the levels
of total radical-trapping capacity of the tissue and body
fluids.40-42 The anthracyclines generate by far the highest
levels of oxidative stress. The cytochrome P450 monooxy-
genase system is the primary site of the generation of ROS
during cancer chemotherapy.43 It is important to highlight
that the drugs that do not depend on the generation of ROS
in their mechanism of action can only mediate their anticanc-
er effects on cancer cells that exhibit unrestricted progression
through the cell cycle and have intact apoptotic pathways.44
However, oxidative stress can interfere with chemotherapy-
induced apoptosis and with cell cycle progression by inhib-
itating the transition of cells from the G0 to G1 phase, slowing
the progression through the S phase by inhibition of DNA
synthesis, inhibiting cell cycle progression of the G1 to S
phase, and checkpoint arrest. By reducing oxidative stress,
antioxidants may counteract the effects of chemotherapy-
induced oxidative stress on cell cycle and apoptosis. Thus,
antioxidants may enhance the cytotoxicity of antineoplastic
drugs.5 In contrast, antioxidants might also protect cancer
cells against the oxidative damage induced by chemother-
apy, which suggests a harmful effect as a result of their use.1
Antioxidants may decrease chemotherapy-induced damage
of normal cells by reducing lipid peroxidation or halting
cancer cell proliferation.6 In their study, Block et al
systematically reviewed randomized controlled clinical trials
evaluating the effects of concurrent use of antioxidants in
chemotherapy on toxic side effects. The review suggested
that antioxidant supplementation may reduce the toxic
effects of ROS-generating chemotherapies. Additionally,
analysis suggested that concurrent use of supplements and
Chemotherapeutic drugs are designed to interfere with rapidly dividing cells and kill cancer cells as well as normal cells, such as healthy intestinal mucosal cells, because of their rapid rate of division. Methotrexate (MTX) is one of the common antineoplastic agents that destroys mucosal cells and may result in mucositis, stomatitis, diarrhea, decreased nutrient absorption, translocation of gastrointestinal bacteria, and anorexia. Various soy products have been shown to provide dramatic protection against MTX toxicity in animal models. Funk and Baker investigated isolated soybean components in a semipurified diet and showed that they alter MTX toxicity and that soybean meal and soybean concentrate offered the greatest protection, completely alleviating MTX-induced anorexia and diarrhea when included as sole protein source and fed 14 days prior to and 7 days following intraperitoneal MTX injection at 20 mg/kg body weight. In addition, although the rats fed casein-based semipurified diet had necrotic intestine, those fed the soybean-containing complex diet or semipurified diet containing soybean concentrate or soybean isolate showed no signs of necrosis in any part of the

| Table 1. Miscellaneous Effects of Soy Isoflavones. |
|--------------------------------------------------|
| **Property** | **Effect** | **Reference** |
| Free radical scavenging effect | Protects cells in central nervous system | Wei et al61 |
| | Protection against oxidative modification of LDL | Tikkanen et al62 |
| | Protection of human cortical neuronal HCN1-A and HCN2 cells | Ho et al63 |
| | Protection of primary cortical neurons from iron-induced free radical reaction and lipid peroxidation | Sonee et al64 |
| | Protection of dopaminergic neurons from lipopolysaccharide-induced injury by inhibiting microglia activation | Wang et al65 |
| | Rescue human amniotic fluid mesenchymal stem cells and Schwann cells from apoptosis by suppressing the macrophage deposits, associated inflammatory cytokines, and fibrin deposits | Pan et al66 |
| | Prevention of endoplasmic reticulum stress-mediated neurotoxicity by inhibiting tau hyperphosphorylation in SH-SYSY cells | Park et al67 |
| | Alleviation of the endoplasmic reticulum stress-mediated and DNA damage-mediated neurodegeneration caused by homocysteine | Park et al68 |
| Anti-inflammatory | Suppression of lipopolysaccharide-induced inflammation in rat liver | Zhao et al69 |
| Effect on immune system | Immunomodulation | Sakai and Kogiso70 |
| Antiviral | Antiviral properties in vitro and in vivo against a wide range of viruses | Andres et al71 |
| Tyrosine kinase inhibitor | Tyrosine kinase inhibition | Akiyama et al72 |
| Effect on vascular system | Prevention of atherosclerosis and related vascular events | Holzer et al73 |
| Antihypertensive | Attenuation of hypertension, targeting the kidney to increase renal blood flow, sodium excretion | Martin et al74 |
| Osteopenia | Positive effect in cyclosporin A–induced osteopenia only in sites with high turnover and improvement of the osteoprotective effect of L-arginine | Clementi et al75 |
| Epidermal hyperplasia | Reduction in epidermal hyperplasia caused by topical retinoid treatment | Rittie et al76 |
| Hepatoprotective effect | Effect via suppression of necrosis of hepatocytes and the cellular infiltration in liver parenchyma and prevention of the development of fatty and protein dystrophy in the liver and normalization of the activity of aminotransferases | Saratikov et al77 |
| Drug toxicity | Protection of post–neural tube closure defects of rodents induced by cyclophosphamide | Zhao et al78 |
| | Protection of normal and cancer cells against genotoxic potential of tamoxifen | Wozniak et al79 |
| Radiation injury | Protection against acute radiation injury | Landauer et al80 |

Abbreviation: LDL, low-density lipoprotein.
small intestine. In a different study,\textsuperscript{51} the same group aimed to determine whether MTX toxicity associated with a casein-based semipurified diet could be ameliorated or prevented by adding fiber or by replacing casein with another protein source. Results of the study showed that addition of amorphous cellulose to semipurified casein-based diet slightly reduced toxicity symptoms. Addition of crystalline cellulose, hemicellulose, and pectin did not lessen toxicity symptoms, whereas replacing casein with soybean concentrate totally alleviated the toxicity symptoms. These findings suggested that it may be possible to develop soybean-based enteral products that would minimize gastrointestinal toxicity experienced by cancer patients undergoing MTX chemotherapy.

Chevreau and Funk-Archuleta,\textsuperscript{50} indicated that soy concentrate was superior in alleviating MTX toxicity compared with commercial enteral products. Rats fed soy concentrate maintained food intake above 90% of preinjection levels, which was greater than all other groups at day 3 and those receiving hydrolyzed or intact casein without fiber on day 4 (P < .05). The soy concentrate group also gained more weight when compared with other groups fed hydrolyzed or intact casein without fiber (P < .05) and had no diarrhea. The same study showed that crypt necrosis occurred in all groups except those consuming the soy concentrate diet or enteral product containing soy fiber. Funk (52) investigated the ability of a soy-derived antiapoptotic fraction that could inhibit apoptosis in an in vitro assay to inhibit MTX-induced gastrointestinal toxicity in rats. Rats fed high doses of the soy-derived antiapoptotic fraction–supplemented diets experienced significantly less weight loss and diarrhea and better food intake (P < .05). They also performed mouse embryonic C3H10T1/2 cell apoptosis assay and demonstrated that soy-derived antiapoptotic fraction was a potent inhibitor of apoptosis. It is well known that many chemotherapeutic agents, including MTX, increase the incidence of apoptosis, particularly in the gastrointestinal tract.\textsuperscript{53} Thus, the studies suggested that the mechanism behind the protection of undesirable gastrointestinal MTX toxicity by soy-derived antiapoptotic fraction was likely to be a result of reduction of apoptotic cell death.

Similarly, despite the beneficial chemotherapeutic effects of bleomycin on cancer cells, cytotoxicity and genotoxicity of bleomycin on normal cells persists as a major problem in chemotherapy. Lee et al\textsuperscript{12} demonstrated a number of effects of genistein pretreatment on the toxicity of bleomycin in normal lymphocytes and human leukemia (HL-60) cells. It increased the frequencies of micronuclei in HL-60 cells, decreased the frequencies of micronuclei in human lymphocytes during G0 and G2, increased DNA damage in HL-60 cells, and reduced DNA damage in blood lymphocytes. As a result, dual antagonistic effects of genistein were observed from this study—genistein enhanced the bleomycin-induced cytotoxicity in HL-60 cells, whereas it protected normal blood lymphocytes. In another study, cisplatin was tested in vitro in human lymphocytes for its toxicity. This study showed that cisplatin when combined with genistein considerably reduced the genotoxicity, possibly because of the free radical scavenging activity of genistein.\textsuperscript{54}

The results of the in vitro and in vivo studies supported a novel chemotherapy strategy for treating cancer patients by concurrent administration of chemotherapy with soy isoflavones. Results of a clinical trial conducted by Tacyildiz et al\textsuperscript{55} demonstrated that genistein reduced the adverse effects of chemotherapy in pediatric cancer patients. In this study, 9 cycles of chemotherapy were administered without genistein supplementation, whereas 57 cycles were administered with genistein supplementation. Patients served as their own controls, and the clinical-laboratory data from the first cycle were compared with the data from subsequent cycles. Chemotherapy doses and schedules between first and subsequent cycles remained the same. Genistein levels were 2 to 6 times higher (range = 0.215-0.411 mg/L; median = 0.258 mg/L) during genistein supplementation compared with the no supplementation period (range = 0.058-0.111 mg/L; median = 0.061 mg/L). The results of the study showed that there was less myelosuppression, oral mucositis, infections, and requirement of blood products during the cycles given with genistein supplementation. In addition, 3 children receiving genistein during abdominal radiotherapy experienced less pain and no diarrhea, which is a common side effect of abdominal radiation. Studies with soy products and chemotherapy toxicities are summarized in Table 2.

Bioavailability of Isoflavonoids

Pharmacokinetic studies in healthy adults compared plasma kinetics of pure daidzein, genistein, and their β-glycosides administered as a single-bolus dose to 19 healthy women.\textsuperscript{56} Results demonstrated differences in the pharmacokinetics of isoflavone glycosides compared with their respective β-glycosides. Even though all isoflavones were efficiently absorbed from the intestine, there were striking differences in the fate of aglycones and β-glycosides. Mean times to attain peak plasma concentrations, tmax for the aglycones genistein and daidzein were 5.2 and 6.6 hours, respectively, whereas for the corresponding glycosides, it was delayed to 9.3 and 9.0 hours, respectively, which was consistent with the residence time needed for hydrolytic cleavage of the glycoside moiety. The apparent volume of distribution of isoflavones confirmed extensive tissue distribution. In this study, the plasma genistein concentration was consistently higher than that of daidzein when equal amounts of the 2 isoflavones were administered, and this was accounted for by more extensive distribution of daidzein (236 L) compared with genistein (161 L). The systemic bioavailability of genistein (mean area under
| Agents | Toxicities                            | Treatment                                                                 | Results                                                                 | Reference       |
|--------|--------------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------|-----------------|
| Methotrexate | Gastrointestinal toxicity           | 34 SD rats; groups; control group (casein), casein + soy fraction (0.164% of diet) group, casein + soy fraction (0.493% of diet), casein + soy fraction (1.643% of diet) | Improved food intake $P < .05$, weight gain $P < .05$, decreasing incidence of diarrhea $P < .05$ | Funk-Archuleta et al^48 |
| Bleomycin | Cytotoxicity and genotoxicity       | Human blood lymphocytes obtained from healthy women and human leukemia cell line HL-60 cells (KCLB 10240) pretreated with genistein followed by bleomycin | Enhanced bleomycin-induced cytotoxicity in human leukemia (HL-60) while protecting normal blood lymphocytes | Lee et al'^12 |
| Cisplatin | Genotoxicity                        | Human lymphocyte culture of 2 healthy donors treated with cisplatin only and in combination with genistein and gingerol separately in the presence of a metabolic activation system | Reduced genotoxicity because of the free radical scavenging activity of genistein | Beg et al'^54 |
| Various combination chemotherapy regimens + radiotherapy | Various toxicities             | 8 Pediatric patients with cancer (served as their own controls); 9 cycles of chemotherapy without soy isoflavone, 57 cycles of chemotherapy with soy isoflavones; chemotherapy doses and schedules same | Genistein levels were 2 to 6 times higher (range = 0.215-0.411 mg/L; median = 0.258 mg/L) during genistein supplementation compared with the no supplementation period (range = 0.058-0.111 mg/L; median = 0.061 mg/L); genistein supplementation: less myelosuppression (shorter duration of neutropenia), oral mucositis, infections (shorter duration of antibiotic use), blood product requirements; no diarrhea during abdominal radiotherapy | Tacyildiz et al'^55 |
| Etoposide | Alopecia                            | 10 SD rats; etoposide injected daily in 11-day-old SD rats at 1.2 mg/kg ip for 3 days, 5 days before the first injection of etoposide, soymetide-4 orally for 8 days concomitantly with indomethacin, AH23848B, pyrilamine, cimetidine, and PDTC | Oral administration soymetide-4: suppression of alopecia induced by etoposide in neonatal rat models | Tsuruki et al'^81 |
| Methotrexate | Gastrointestinal toxicities        | Rats; 5 enteral products containing casein or soy isolate in various forms to 10 rats for 7 days before injection and 7 days after injection of MTX (20 mg/kg) | Soy concentrate diet consumption; maintained food intake above 90% of preinjection levels, which was greater than all other groups at day 3 and those receiving hydrolyzed or intact casein without fiber on day 4 ($P < .05$), no diarrhea, weight gain when compared with other groups fed hydrolyzed or intact casein without fiber ($P < .05$), crypt necrosis (in intestine) occurred in all groups except those consuming the soy concentrate diet | Chevreau and Funk-Archiuleta'^50 |

(continued)
Table 2. (continued)

| Agents      | Toxicities         | Treatment                                                                 | Results                                                                                     | Reference  |
|-------------|--------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|------------|
| Methotrexate| Gastrointestinal   | Male SD rats; 5 different experiments; products tested: soybean meal, soybean concentrate, soybean isolate and soybean fiber; 14 days prior to and 7 days following intraperitoneal MTX injection | Soybean meal and soybean concentrate offered the greatest protection, completely alleviating MTX-induced anorexia and diarrhea; soybean concentrate and soybean isolate prevented the necrosis (in the small intestine of MTX-injected animals) observed in animals fed the casein-based semipurified diet | Funk and Baker⁵¹  |
| Methotrexate| Gastrointestinal   | Male SD rats; 6 different experiments; products tested: (1) fiber sources, including crystalline cellulose, amorphous cellulose, hemicellulose, and pectin; (2) protein sources, including casein, soybean concentrate, whey isolate, egg albumen, com gluten meal, and hamburger; in experiments 1 to 4, diets for 14 days before MTX injection, experiments 5 and 6 to evaluate time periods prior to or after MTX dosing on toxicity development | Toxicity was lower when 25% of the protein normally supplied by casein was replaced with soybean concentrate, and no toxicity symptoms were present when 50% or more of the protein was provided by soybean concentrate | Funk and Baker⁵²  |
| Cisplatin   | Nephrotoxicity     | Mice; control (n = 10), genistein (10 mg/kg; n = 10), cisplatin (20 mg/kg; n = 10), and cisplatin plus genistein (n = 10) | Genistein; significantly reduced cisplatin-induced renal injury, ameliorated the cisplatin-induced upregulation of ICAM-1 and MCP-1 expression, resulting in decreased infiltration of macrophages into the kidney, significantly reduced cisplatin-induced generation of ROS and NF-κB activation in HK-2 cells, reduced cisplatin-induced apoptosis in kidney through downregulation of p53 induction | Sung et al⁸²  |

Abbreviations: AH23848B, an antagonist of the PGE2 receptor EP4; HK-2, human kidney; HL-60, human leukemia cells; ICAM-1, immunostaining for intercellular adhesion molecule-1; MCP-1, immunostaining for monocyte chemoattractant protein-1; MTX, methotrexate; NF-κB, nuclear factor-κB; PDTC, pyrrolidine dithiocarbamate ammonium; ROS, reactive oxygen species; SD, Sprague-Dawley.

The curve [AUC] = 4.54 µg/[mL h]) was much higher than that of daidzein (mean AUC = 2.94 µg/[mL h]). Bioavailability was greater when ingested as β-glycosides rather than as aglycones. The pharmacokinetics of methoxylated isoflavones showed distinct differences depending on the position of the methoxyl group in the molecule. Glycitin, found in 2 phytoestrogen supplements, underwent hydrolysis of the β-glycoside moiety and little further biotransformation, leading to high plasma glycitein concentrations. Biochanin A and formononetin, 2 isoflavones found in 1 phytoestrogen supplement, were rapidly and efficiently demethylated, resulting in high plasma genistein and daidzein concentrations typically observed after the ingestion of soy-containing foods. These differences in pharmacokinetics and metabolism have implications in clinical studies because it cannot be assumed that all isoflavones were comparable in their pharmacokinetics and bioavailability. An analysis of 33 phytoestrogen supplements and extracts revealed considerable differences in the isoflavone content from that claimed by the manufacturers. Plasma concentrations of isoflavones show marked qualitative and quantitative differences depending on the type of supplement ingested.⁵⁶ Various studies, including in vivo studies, have shown that genistein from soy extracts, its free form, and its glycoside genistin are readily bioavailable.⁵⁵ Extensive metabolism of
genistein in the intestine, and postabsorption, has been documented both in humans and experimental animals. Among the several metabolites identified in the blood and excreta are dihydrogenistein, dihydrodaidzein, 6'-hydroxy-O-desmethylangolensin, 4-ethylphenol, glucononide and sulfate conjugates of genistein and its metabolites, and 4-hydroxyphenyl-2-propionic acid. The gut microflora cleaves the C-ring of the isoflavonoid skeleton to give 4-hydroxyphenyl-2-propionic acid and dihydrogenistein. The metabolism in the gut wall and liver is also known to yield gluconuronide and sulfated products. Few reports suggest that conjugation plays a role in rapid elimination by biliary and urinary excretion.

**Adverse Effects of Soy Isoflavones**

In an analysis of data from PubMed and EMBASE from 1975 to 2015 (articles selected with the search terms isoflavone, phytoestrogen, soy, genistin, and PCa), isoflavones are reported not to play an important role in reducing PSA in PCa patients or healthy men, but the intake of various types of phytoestrogens with lower concentrations in the daily diet was reported to produce synergistic effects against PCa. The analysis suggests that the prostate tissue may concentrate isoflavones to potentially anticarcinogenic levels but cautioned that the isoflavones may also act as an agonist in PCa.

Phytoestrogens are structurally and functionally analogous to estrogens. Phytoestrogens and their active metabolites such as equol can remain in food/meat and influence the hormonal balance of the consumers. In animals, intake of phytoestrogens may affect fertility, sexual development, and behavior.

Consumption of soy as dietary supplement may cause mild stomach and intestinal side effects such as constipation, bloating, and nausea and may also cause allergic reactions involving rash, itching, and anaphylaxis in some people.

**Conclusion**

Chemotherapeutic agents and radiation induce oxidative stress and inflammation, producing side effects in cancer patients. Soy isoflavones, owing to their multiple mechanisms of effects, including the antioxidant and anti-inflammatory effects, may be used as dietary supplements to ameliorate the adverse reactions to anticancer drugs and radiation. At the same time, they may increase the efficacy of cancer chemotherapy and radiation, especially in PCa. The effect of soy isoflavones, particularly genistein, in the prevention and control of PCa has been supported by preclinical studies, meta-analyses, and clinical trials, but larger placebo-controlled clinical trials are needed to investigate the potential use of genistein for amelioration of the adverse effects of anticancer drugs and radiation therapy.

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**References**

1. D’Incalci M, Steward WP, Gescher AJ. Modulation of response to cancer chemotherapeutic agents by diet constituents: is the available evidence sufficiently robust for rational advice for patients? Cancer Treat Rev. 2007;33:223-229.
2. Sarkar FH, Li Y. Soy isoflavones and cancer prevention. Cancer Invest. 2003;21:744-757.
3. Zhang Z, Wang CZ, Du GJ, et al. Genistein induces G2/M cell cycle arrest and apoptosis via ATM/p53-dependent pathway in human colon cancer cells. Int J Oncol. 2013;43:289-296.
4. Tabassum A, Bristow RG, Venkateswaran V. Ingestion of selenium and other antioxidants during prostate cancer radiotherapy: a good thing? Cancer Treat Rev. 2010;36:230-234.
5. Conklin KA. Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. Integr Cancer Ther. 2004;3:294-300.
6. Lawenda BD, Kelly KM, Ladas EJ, Sagar SM, Vickers A, Blumberg JB. Should supplemental antioxidant administration be avoided during chemotherapy and radiation therapy? J Natl Cancer Inst. 2008;100:773-783.
7. Fukutake M, Takahashi M, Ishida K, Kawamura H, Sugimura T, Wakabayashi K. Quantification of genistein and genistin in soybeans and soybean products. Food Chem Toxicol. 1996;34:457-461.
8. Sfakianos J, Coward L, Kirk M, Barnes S. Intestinal uptake and biliary excretion of the isoflavone genistein in rats. J Nutr. 1997;127:1260-1268.
9. Adlercreutz H, Markkanen H, Watanabe S. Plasma concentrations of phyto-oestrogens in Japanese men. Lancet. 1993;342:1209-1210.
10. Hussain M, Banerjee M, Sarkar FH, et al. Soy isoflavones in the treatment of prostate cancer. Nutr Cancer. 2003;47:111-117.
11. Sarkar FH, Li Y. The role of isoflavones in cancer chemoprevention. Front Biosci. 2004;9:2714-2724.
12. Lee R, Kim YJ, Lee YJ, Chung HW. The selective effect of genistein on the toxicity of bleomycin in normal lymphocytes and HL-60 cells. Toxicology. 2004;195:87-95.
13. Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, Clinton SK. Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. J Nutr. 1999;129:1628-1635.
14. Applegate CC, Rowles JL, Rand AM, Jeon S, Erdman JW. Soy consumption and the risk of prostate cancer: an updated systematic review and meta-analysis. Nutrients. 2018;10:E40.
15. Spagnuolo C, Russo GL, Orhan IE, et al. Genistein and cancer: current status, challenges, and future directions. Adv Nutr. 2015;6:408-419.
16. Ali S, Mann DA. Signal transduction via the NF-kappaB pathway: a targeted treatment modality for infection, inflammation and repair. Cell Biochem Funct. 2004;22:67-79.
17. Phillip CJ, Giardina CK, Bilir B, et al. Genistein cooperates with the histone deacetylase inhibitor vorinostat to induce cell death in prostate cancer cells. BMC Cancer. 2012;12:145.
18. Li Y, Ahmed F, Ali S, Philip PA, Kucuk O, Sarkar FH. Potentiation of the radiation effect with genistein in cervical cancer cells. Clin Cancer Res. 2007;12:2491-2498.
19. Khoshyomn S, Manske GC, Lew SM, Wald SL, Penar PL. Synergistic action of genistein and cisplatin on growth inhibition and cytotoxicity of human medulloblastoma cells. Pediatr Neurosurg. 2000;33:123-131.
20. Sahin K, Tuzcu M, Basak N, et al. Sensitization of cervical cancer cells to cisplatin by genistein: the role of NFKappaB and Akt/mTOR signaling pathways. J Oncol. 2012;2012:461562.
21. Hillman GG, Forman JD, Kucuk O, et al. Genistein potentiates the radiation effect on prostate carcinoma cells. Clin Cancer Res. 2001;7:382-390.
22. Hillman GG, Wang Y, Kucuk O, et al. Genistein potentiates inhibition of tumor growth by radiation in a prostate cancer orthotopic model. Mol Cancer Ther. 2004;3:1271-1279.
23. Raffoul JJ, Banerjee S, Che M, et al. Soy isoflavones enhance radiotherapy in a metastatic prostate cancer model. Int J Cancer. 2007;120:2491-2498.
24. Yashar CM, Spanos WJ, Taylor DD, Gercel-Taylor C. Potentiation of the radiation effect with genistein in cervical cancer cells. Gynecol Oncol. 2005;99:199-205.
25. Tang Q, Ma J, Sun J, et al. Genistein and AG1024 synergistically increase the radiosensitivity of prostate cancer cells. Oncol Rep. 2018;40:579-588.
26. Djuric Z, Chen G, Doerge DR, Heilbrun LK, Kucuk O. Effect of soy isoflavone supplementation on markers of oxidative stress in men and women. Cancer Lett. 2001;172:1-6.
27. Kaptiots S, Hermann M, Held I, Seelen C, Ehrlinger H, Gmeiner BM. Genistein, the dietary-derived angiogenesis inhibitor, prevents LDL oxidation and protects endothelial cells from damage by atherogenic LDL. Arterioscler Thromb Vasc Biol. 1997;17:2868-2874.
28. Ruiz-Larrea MB, Mohan AR, Paganga G, Miller NJ, Bolwell GP, Rice-Evans CA. Antioxidant activity of phytoestrogenic isoflavones. Free Radic Res. 1997;26:63-70.
29. Blay M, Espinel AE, Delgado MA, et al. Isoflavone effect on gene expression profile and biomarkers of inflammation. J Pharm Biomed Anal. 2010;51:382-390.
30. Duan W, Kuo IC, Selvarajan S, Chuay KY, Bay BH, Wong WS. Antiinflammatory effects of genistein, a tyrosine kinase inhibitor, on a guinea pig model of asthma. Am J Respir Crit Care Med. 2003;167:185-192.
31. Gong L, Li Y, Nedeljkovic-Kurepa A, Sarkar FH. Inactivation of NF-kappaB by genistein is mediated via Akt signaling pathway in breast cancer cells. Oncogene. 2003;22:4702-4709.
32. Hall WL, Vafeiadou K, Hallund J, et al. Soy-isoflavone-enriched foods and inflammatory biomarkers of cardiovascular disease risk in postmenopausal women: interactions with genotype and equol production. Am J Clin Nutr. 2005;82:1260-1268.
33. Kang JS, Yoon YD, Han MH, et al. Estrogen receptor-independent inhibition of tumor necrosis factor-alpha gene expression by phytoestrogen equol is mediated by blocking nuclear factor-kappaB activation in mouse macrophages. Biochem Pharmacol. 2005;71:136-143.
34. Gescher A, Pastorino U, Plummer SM, Manson MM. Suppression of tumour development by substances derived from the diet—mechanisms and clinical implications. Br J Clin Pharmacol. 1998;45:1-12.
35. Lazarevic B, Boezelijn G, Diep LM, et al. Efficacy and safety of short-term genistein intervention in patients with localized prostate cancer prior to radical prostatectomy: a randomized, placebo-controlled, double-blind phase 2 clinical trial. Nutr Cancer. 2011;63:889-898.
36. Hwang YW, Kim SY, Jee SH, Kim YN, Nam CM. Soy food consumption and risk of prostate cancer: a meta-analysis of observational studies. Nutr Cancer. 2009;61:598-606.
37. Lazarevic B, Hammarstrom C, Yang J, et al. The effects of short-term genistein intervention on prostate biomarker expression in patients with localised prostate cancer before radical prostatectomy. Br J Nutr. 2012;108:2138-2147.
38. Vaishampayan U, Hussain M, Banerjee M, et al. Lycopene and soy isoflavones in the treatment of prostate cancer. Nutr Cancer. 2007;59:1-7.
39. Grainger EM, Moran NE, Francis DM, et al. A novel tomato-soy juice induces a dose-response increase in urinary and plasma phytochemical biomarkers in men with prostate cancer. J Nutr. 2019;149:23-35.
40. Faber M, Coudray C, Hida H, Mousseau M, Favier A. Lipid peroxidation products, and vitamin and trace element status in patients with cancer before and after chemotherapy, including adriamycin: a preliminary study. Biol Trace Elem Res. 1995;47:117-123.
41. Sangeetha P, Das UN, Koratkar R, Suryaprabha P. Increase of short-term genistein intervention on prostate biomarker expression in patients with localised prostate cancer before radical prostatectomy. Br J Nutr. 2012;108:2138-2147.
42. Faber M, Coudray C, Hida H, Mousseau M, Favier A. Lipid peroxidation products, and vitamin and trace element status in patients with cancer before and after chemotherapy, including adriamycin: a preliminary study. Biol Trace Elem Res. 1995;47:117-123.
43. Conklin KA. Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. Nutr Cancer. 2000;37:1-18.
44. Nicolson GL. Lipid replacement therapy: a nutraceutical approach for reducing cancer-associated fatigue and the
adverse effects of cancer therapy while restoring mitochondrial function. Cancer Metastasis Rev. 2010;29:543-552.

45. Block KI, Koch AC, Mead MN, Tothy PK, Newman RA, Gyllenhaal C. Impact of antioxidant supplementation on chemotherapeutic efficacy: a systematic review of the evidence from randomized controlled trials. Cancer Treat Rev. 2007;33:407-418.

46. Block KI, Koch AC, Mead MN, Tothy PK, Newman RA, Gyllenhaal C. Impact of antioxidant supplementation on chemotherapeutic efficacy: a systematic review of the evidence from randomized controlled trials. Int J Cancer. 2008;123:1227-1239.

47. Cunningham D, Morgan RJ, Mills PR, et al. Functional and structural changes of the human proximal small intestine after cytotoxic therapy. J Clin Pathol. 1985;38:265-270.

48. Funk-Archuleta MA, Foehr MW, Tomei LD, Hennebold KL, Bathurst IC. A soy-derived antiapoptotic fraction decreases methotrexate toxicity in the gastrointestinal tract of the rat. Nutr Cancer. 1997;29:217-221.

49. Pinkerton CR, Milla PJ. Methotrexate enterotoxicity: influence of drug dose and timing in the rat. Br J Cancer. 1984;49:97-101.

50. Chevreau N, Funk-Archuleta M. Effect of enteral formulas on methotrexate toxicity. Nutr Cancer. 1995;23:185-204.

51. Funk MA, Baker DH. Effect of soy products on methotrexate toxicity in rats. J Nutr. 1991;121:1684-1692.

52. Funk MA, Baker DH. Effect of fiber, protein source and time of feeding on methotrexate toxicity in rats. J Nutr. 1991;121:1673-1683.

53. Barry MA, Behnke CA, Eastman A. Activation of programmed cell death (apoptosis) by cisplatin, other antitumor drugs, toxins and hyperthermia. Biochem Pharmacol. 1990;40:2353-2362.

54. Beg T, Siddique Y, Afzal M. Protective action of flavonoids and genistein on cisplatin toxicity in vitro. J Young Pharm. 2012;4:124-125.

55. Tacyildiz N, Ozyoruk D, Yavuz G, et al. Soy isoflavones ameliorate the adverse effects of chemotherapy in children. Nutr Cancer. 2010;62:1001-1005.

56. Setchell KD, Brown NM, Desai P, et al. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. J Nutr. 2001;131(4, suppl):1362S-1375S.

57. Tamura M, Hori S, Nakagawa H. Dihydrodaidzein-producing Clostridium-like intestinal bacterium, strain TM-40, affects in vitro metabolism of daidzein by fecal microbiota of human male equal producer and non-producers. Biosci Microflora. 2011;30:65-71.

58. Shelnut SR, Cimino CO, Wiggins PA, Ronis MJ, Badger TM. Pharmacokinetics of the glucuronide and sulfate conjugates of genistein and daidzein in men and women after consumption of a soy beverage. Am J Clin Nutr. 2002;76:588-594.

59. Zhang HY, Cui J, Zhang Y, Wang ZL, Chong T, Wang ZM. Isoflavones and prostate cancer: a review of some critical issues. Chin Med J (Engl). 2016;129:341-347.

60. Jargin SV. Soy and phytoestrogens: possible side effects. Ger Med Sci. 2014;12:Doc18.

61. Wei H, Wei L, Frenkel K, Bowen R, Barnes S. Inhibition of tumor promoter-induced hydrogen peroxide formation in vitro and in vivo by genistein. Nutr Cancer. 1993;20:1-12.

62. Tikkanen MJ, Wålhåk K, Ojala S, Vihera V, Adlercreutz H. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc Natl Acad Sci U S A. 1998;95:3106-3110.

63. Ho KP, Li L, Zhao L, Qian ZM. Genistein protects primary cortical neurons from iron-induced lipid peroxidation. Mol Cell Biochem. 2003;247:219-222.

64. Sonee M, Sum T, Wang C, Mukherjee SK. The soy isoflavone, genistein, protects human cortical neuronal cells from oxidative stress. Neurototoxicology. 2004;25:885-891.

65. Wang X, Chen S, Ma G, Ye M, Lu G. Genistein protects dopaminergic neurons by inhibiting microglial activation. Neuroreport. 2005;16:267-270.

66. Pan HC, Yang DY, Ho SP, et al. Escalated regeneration in sciatic nerve crush injury by the combined therapy of human amniotic fluid mesenchymal stem cells and fermented soybean extracts. Natto. J Biomed Sci. 2009;16:75.

67. Park YJ, Jang YM, Kwon YH. Isoflavones prevent endoplasmic reticulum stress-mediated neuronal degeneration by inhibiting tau hyperphosphorylation in SH-SY5Y cells. J Med Food. 2009;12:528-535.

68. Park YJ, Jang Y, Kwon YH. Protective effect of isoﬂavones against homocysteine-mediated neuronal degeneration in SH-SY5Y cells. Amino Acids. 2010;39:785-794.

69. Zhao JH, Arao Y, Sun SJ, Kikuchi A, Kayama F. Oral administration of soy-derived genistin suppresses lipopolysaccharide-induced acute liver inflammation but does not induce thymic atrophy in the rat. Life Sci. 2006;78:812-819.

70. Sakai T, Kogiso M. Soy isoflavones and immunity. J Med Invest. 2008;55:167-173.

71. Andres A, Donovan SM, Kuhlenschmidt MS. Soy isoflavones and virus infections. J Nutr Biochem. 2009;20:563-569.

72. Akiyama T, Ishida J, Nakagawa S, et al. Genistin, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem. 1987;262:5592-5595.

73. Holzer G, Esterbauer H, Kronke G, et al. The dietary soy flavonoid genistin abrogates tissue factor induction in endothelial cells induced by the atherogenic oxidized phospholipid oxPAPC. Thromb Res. 2007;120:71-79.

74. Martin D, Song J, Mark C, Eyster K. Understanding the cardiovascular actions of soy isoflavones: potential novel targets for antihypertensive drug development. Cardiovasc Hematol Drug Targets. 2008;8:297-312.

75. Clementi G, Fiore CE, Mangano NG, et al. Role of soy diet and L-arginine in cyclosporin-A-induced osteopenia in rats. Pharmacol Toxicol. 2001;88:16-19.

76. Rittle L, Varani J, Kang S, Voorhees JJ, Fisher GJ. Retinoid-induced epidermal hyperplasia is mediated by epidermal growth factor receptor activation via specific induction of its ligands heparin-binding EGF and amphiregulin in human skin in vivo. J Invest Dermatol. 2006;126:732-739.

77. Saratikov AS, Chuchalin VS, Rat’kin AV, Rat’kin EV, Fedoreev SA, Bulgakov VP. Hepatoprotector properties of soybean isoflavone combined supplementation protects the post-neural tube closure defects of rodents induced by cyclophosphamide in vivo and in vitro. Neurotoxicology. 2010;31:180-187.
79. Wozniak K, Kolacinska A, Blasinska-Morawiec M, et al. The DNA-damaging potential of tamoxifen in breast cancer and normal cells. *Arch Toxicol.* 2007;81:519-527.

80. Landauer MR, Srinivasan V, Seed TM. Genistein treatment protects mice from ionizing radiation injury. *J Appl Toxicol.* 2003;23:379-385.

81. Tsuruki T, Takahata K, Yoshikawa M. A soy-derived immunostimulating peptide inhibits etoposide-induced alopecia in neonatal rats. *J Invest Dermatol.* 2004;122:848-850.

82. Sung MJ, Kim DH, Jung YJ, et al. Genistein protects the kidney from cisplatin-induced injury. *Kidney Int.* 2008;74:1538-1547.