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REVIEW

# Anti-carcinogenic and anti-metastatic effects of flax seed lignan secolariciresinol diglucoside (SDG)

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## ABSTRACT

Flaxseeds contain phenolic compounds called lignans and secolariciresinol diglucoside (SDG) is a major lignan with putative health benefits such as antioxidant and anticancer effects. The role of SDG and its metabolites such as enterolignans is gaining attention due to their mitigating effects against cancers especially prostate and breast cancer. Several epidemiological, in vitro and animal studies add evidence to this anti-cancer benefit of SDG. However, more research activities, especially clinical and pharmacokinetic studies in humans are required to corroborate this evidence. This review attempts to focus on the roles of SDG and its metabolites in preventing breast tumors, including an evaluation of potential mechanisms of action.

**Keywords:** *Flaxseed lignans, secoloarciresinol diglucoside, enterolignans, antitumor, breast cancer, estrogen receptor signaling*

## Introduction

Plant lignans are biphenolic compounds found in roots, stem, cereals, oilseeds, nuts, legumes and fruits.[1] The major lignans are secoisolariciresinol (SECO) and matairesinol with traces of pinoresinol, lariciresinol and isolariciresinol.[1] Their reported health benefits such as antitumorogenic, antioxidant, and cardio protective effects make them ideal nutraceutical candidates.[2] Plant lignans undergo bioconversion by intestinal bacteria into mammalian lignans namely enterodiol (ED) and enterolactone (EL), also referred to as enterolignans.[3] The richest dietary source of lignans is flaxseed, also called as linseed (Linum usitatissimum L.), where SECO is found in a diglucoside form as secolariciresinol diglucoside (SDG).[2] Compared to other sources, SDG occurs predominantly at a very high concentration in flaxseeds (% yield being 0.37%).[4] In flaxseeds, however, SDG is further esterified with 3-hydroxy-3-methylglutaric acid (HMGA) and other phenolic compounds such as p-coumaric acid and ferulic acid glycosides to form SDG oligomers.[5] Following ingestion, the plant lignan SDG is converted to enterolignans by bacteria in the human colon.6 SDG first undergoes hydrolysis to yield the aglycone plant lignan secoisolariciresinol (SECO). SECO is then converted to enterodiol and demethylated to yield ED, which is then oxidized to form EL.[6] Anaerobic microbes such as *Peptostreptococcus*, *Eubacterium* and *Eggerthella* were found to catalyze the demethylation and dehydroxylation of

SDG and pinoresinol.[2, 7] ED and EL can also undergo further phase I and phase II biotransformations with extensive formation of glucuronide and sulfate conjugates.[8] EL and ED bind to the estrogen receptors and wield weak estrogenic or anti-estrogenic effects due to their structural similarity with estradiol (E2), the most active form of estrogen.[9] This underscores the physiological role of SDG and the derived enterolignans in modulating estrogen receptor dependent biological pathways. In this review, the role of flaxseed lignans in cancer prevention especially breast cancers will be discussed in detail.

## Plant lignans and breast cancer

Elevated levels of serum EL are linked to lower mortality rates in postmenopausal breast cancer patients.[10, 11] A prospective study and meta-analysis conducted in 2,182 breast cancer patients, found that post-diagnostic EL concentrations were significantly associated with diminished all-cause and breast cancer-specific mortality.[12] The authors also postulated a 6% reduction in mortality risk per 10 nmol/L increment in EL levels.[12] ED and EL either alone or in combination with tamoxifen (TAM), were found to modulate breast cancer cell adhesion, invasion and migration in two ER−human breast cancer cell lines (MDA-MB-435 and MDA-MB-231).[13, 14]

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## SDG-a potent nutraceutical for breast cancer prevention

SDG is shown to be protective against certain types of cancers (i.e. breast, lung and colon) because of their antioxidant, antiproliferative, anti-estrogenic or anti-angiogenic properties or possibly due to their ability to inhibit certain enzymes (Figure 1).[19] However, in this present review, only the anti-carcinogenic effects of SDG and its metabolites with a special focus on breast tumors is discussed extensively. Table 1 presents a summary of various *in vitro* and *in vivo* studies that have been used to evaluate the potential therapeutic efficacy of SDG and related metabolites. A number of animal studies have demonstrated the ability of SDG in preventing breast cancers. These studies showed that a 1.5 mg/d SDG dosage in rats resulted in a reduction of mammary gland terminal end buds and alveolar buds, which may reduce mammary cancer risk.[20] In another study, a 20.1 mg/100 g diet dosage during suckling reduced the chemically induced mammary tumor incidence in adulthood.[21]

A study by Saggar et al.[22] determined the effects of SDG and flaxseed oil (FO), alone or in combination, on the growth of established human estrogen receptor positive (ER+) breast tumors in ovariectomized (OVX) athymic mice. The study also evaluated the potential anticancer mechanism(s) of action of the components. In the study, OVX mice with established ER+ human breast tumors (MCF-7) were treated for 8-wk with basal diet (BD, control) or BD supplemented with SDG (1 g/kg), FO (38.5 g/kg), or SDG + FO.[22]

## Effect of SDG on palpable tumor growth, cell proliferation and apoptosis

Both SDG and FO increased the regression rate (slope) of palpable tumor during an 8-wk treatment; however, SDG was predominantly effective in reducing the palpable tumor area. Although a significant interaction existed between SDG and FO on cell proliferation (Ki-67 LI) of MCF-7 tumors, SDG alone reduced the cell proliferation by 25.9% when compared to the control.[22]

## Effect of SDG on biomarkers of estrogen receptor signaling pathway

The effects of SDG on the expression of selected estrogen receptor (ER) signaling pathway genes (PGR, ERβ, ERα and PS2) and growth factor related genes such as epidermal growth factor receptor (EGFR), insulin-like growth factor 1 receptor (IGF-1R), B-cell lymphoma 2 (BCL2) and proteins like phosphorylated mitogen-activated protein kinase (PMAPK), and phosphorylated AKT (PAKT) were measured.[22] SDG treatment resulted in a significant reduction of mRNA expression of PS2, IGF-1R and BCL2, whereas FO had a significant main effect on the reduction of PAKT protein expression. SDG alone inhibited the mRNA expression of ERα, ERβ and the protein PMAPK. However, no significant effects on the expressions of the ER signaling pathway gene CD1, the angiogenesis gene marker vascular endothelial growth factor (VEGF), the human epidermal growth factor receptor type 2 gene HER2, and protein AIB1 were observed.[22]

## Mechanisms of action and structure–function properties of SDG

Under normal conditions, E2 binds to ER, and the activated complex then binds to DNA upstream of estrogen responsive genes. This induces the recruitment of ER coactivators resulting in increased transcription of the estrogen responsive genes PS2, PGR, and CD1 and finally the increased proliferation of cancer cells.[23] In the above study, the predominant effect of SDG was revealed to significantly reduce PS2 mRNA expression. Also, its interaction with FO enhanced the expression of other estrogen responsive genes such as PGR, ERα, and ERβ.[22] These indicate that the effect of SDG may partly involve the binding of ED and EL to ER and the ER-mediated signaling pathway. It is now known that EL binds to ER, while binding preferentially to ERα rather than ERβ.[24] The fact that EL has a predilection for binding to ERα[23] unlike estradiol, which binds with equal affinities to both ERα and ERβ,[25] implies that the mechanism of action of lignans may vary from that of estrogen. Even though the precise roles of ERα and ERβ are yet to be determined, the ratios of ERα to ERβ are significantly different in tumors when compared to normal tissues.[26] It is possible that a change in ERα and ERβ expressions in the SDG and FO groups seen in this study may have contributed to the mechanism through which the lignans are working. Rickard et al.[27] found that rats administered with either FS or SDG lignans had lower plasma levels of IGF-1R than the control. IGF-1R is evinced as a key mediator of breast

tumor growth, since its expression in mammary tumors is much higher than in normal mucosa.[28]

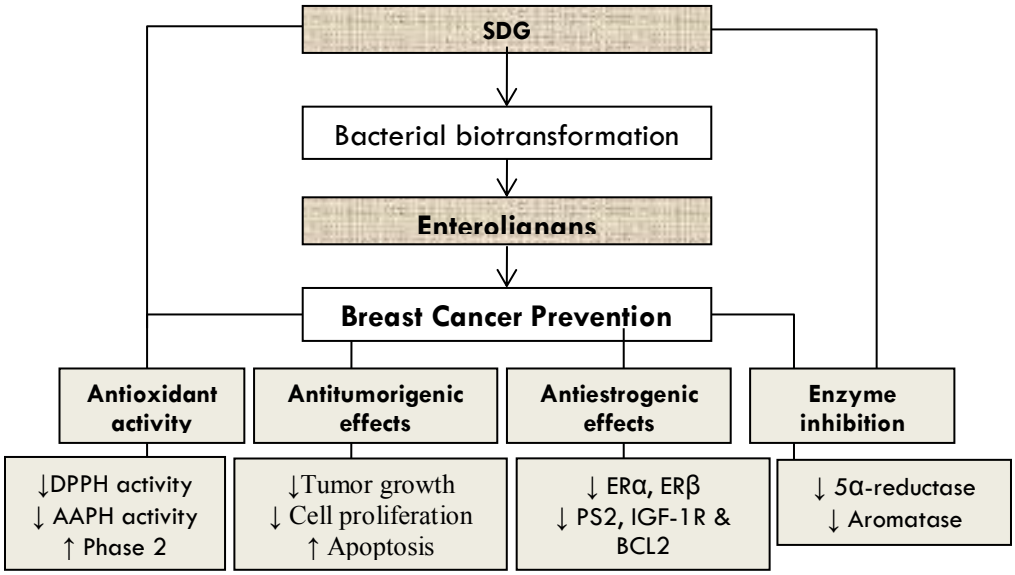


Figure 1. Breast cancer protective mechanisms of SDG. Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; AAPH, 2,2'-Azobis(2-amidinopropane) dihydrochloride; ERα & β, estrogen receptor; BCL2, B-cell lymphoma 2; IGF-1R, insulin-like growth factor 1 receptor.

The study by Saggar et al.[22] revealed that a strong and consistent pattern exists for the effect of SDG and FO on EGFR, IGF-IR, and BCL2. It was found that there was a decrease in all 3 biomarkers when compared to the controls in the presence of SDG alone and FO alone; however, in all cases the reduction was greater for SDG. The only significant drop in EGFR was for SDG, and there was a significantly larger drop for SDG relative to FO for BCL2 too. In the case of IGF-IR, only SDG had a significant main effect. The results suggest that SDG lignans are better tumor growth modulators than FO through involvement of the growth factor signaling pathway. Other studies have shown that FS, ENL, and END can inhibit angiogenesis in ER+ breast tumor growth under high levels of circulating estrogen[29] or in ER– breast tumor.[30] From the results of the study by Saggar et al.[22] it can be concluded that SDG exerts its antitumorogenic activity via modulation of both the ER and growth factor signaling pathway, suggesting that SDG is the most effective anticancer component of flaxseed.

## Antioxidant activity of SDG and its metabolites

One of the well-studied aspects of plant lignans are their antioxidant effects using various *in vitro* antioxidant assays. A study by Hu et al.[6] compared the antioxidant effects of SDG and SECO with ED and EL.[6] It was found that SDG and SECO exhibited strong antioxidant and protective effects in quenching the DPPH (2,2-diphenyl-1-picrylhydrazyl) stable free radical and inhibiting AAPH (2,2'-Azobis(2-amidinopropane) dihydrochloride) peroxyl-radical-mediated damage of plasmid DNA and phosphatidylcholine liposomes at potentially feasible physiological concentrations. However, ED and EL were differentially effective against the free radicals.[6] Therefore, it can be assumed that both the glycosylated and aglycone lignans are likely effective against oxidative damage. However, it will be important in the future to determine whether the glucuronide and sulfate conjugates of these compounds also possess antioxidant activity, since it is these forms that are present in the portal circulation, plasma and urine and thereby most relevant in vivo.

Table 1. Effects of SDG and its metabolites in breast cancer prevention

| Model | Dosage of SDG/ED/EL | Outcome | Reference |
|---|---|---|---|
| ER- human breast cancer cell lines, MDA-MB-435 and MDA-MB-231 | ED and EL at doses of 0.1-10 µM | Reduced cancer cell adhesion, invasion and migration | Chen and Thompson (2003)[13] |
| MCF-7 and MDA-MB-231 cell lines | 25 and 50 µM EL | Reduction in expression of MMP2 in MCF-7 cell line and no change in MMP11 in both cell lines | Mali *et al.* (2012)[14] |
| Pregnant Sprague-Dawley rat dams | 1·5mg/d of SDG | Reduction of terminal end, alveolar bud density, number of estrous cycles & delayed puberty onset | Tou & Thompson (1999)[20] |
| Female Sprague-Dawley rats | 0·7 or 1·4 mg/d of SDG | Reduction in tumor multiplicity by higher dose | Rickard *et al.* (1999)[35] |
| Pregnant Sprague-Dawley rat dams | 20.1 mg SDG/100 g of diet | Exposure during suckling reduced mammary tumor incidence in adulthood | Chen *et al.* (2003)[21] |
| Ovariectomized (OVX) athymic nude mice | 1g SDG/kg of diet | Reduction of palpable tumor area, cell proliferation, mRNA expression of PS2, IGF-1R and BCL2 | Saggar *et al.*(2010)[22] |

## Safety

Both animal and human studies manifest the saftety of flaxseed lignan extracts for human consumption. It was shown that SDG administration at 3 mg/kg body weight for 4 weeks had no adverse health effects in female rats.[31] Dietary supplementation with SDG at levels as high as 200 mg/kg had no adverse effects on growth in mice.[32] Also in a human study that used SDG supplementation at 300 and 600 mg/d for 8 weeks, participants did not report any adverse events.[33]

## Efficacy

Evidence continues to mount to support the role of SDG, or its metabolites SECO, EL and/or ED, in protection against chronic diseases. However, the mechanism(s) through which lignans mediate the purported health benefits and the bioactive lignan form (i.e. SDG, SECO, ED and/or EL) is not known. It is therefore, a challenge to determine whether or not the lignan provided as SDG offers health benefits. This ambiguity arises partly due to the wide variability in study methods used in the literature. Also, the subject/animal model characteristics, sources of SDG and dearth of test product quality assurance, dosage, methods used, data analysis and the study duration vary greatly between published studies.

Another important aspect is that the micromolar concentration required to modulate *in vitro* estrogen receptor activity is much higher than the serum EL and ED levels normally measured in the general population.[34] Therefore, there is the need for more animal and human studies to determine the effective flaxseed dose(s).

## Concluding remarks

It becomes clear from the above research syntheses that flax seed lignan SDG is effective in combating cancers especially breast cancer due to its acknowledged estrogenic activities. However, more research on the pharmacokinetic aspects is required in animal models and humans to validate the efficacy and arrive at optimal dosage levels. Although, the consumption of SDG appears to be safer with no reported major adverse effects, the same has to be established using long term studies together with the measurement of more markers of toxicity. Overall, flaxseed lignan SDG holds greater therapeutic potential for its application as a nutraceutical for the prevention of breast cancers.

## Conflict of Interest

None

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