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Review

The phyto-oestrogens: its anticarcinogenic and antioxidant activity-a review

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SUMMARY

The isoflavonoids comprise a group of phyto-oestrogens that have useful biological activities including oestrogenic, antioxidant and anticancer. As dietary components for humans, they are bioavailable from leguminous vegetables (such as genistein from soybean), and have been well-documented to have numerous health benefits. A wide range of epidemiological studies in humans and limited studies in animals have identified isoflavonoids as potential chemopreventive agents against hormone-dependent cancers. Therefore, an attempt has been made through this review to summarise the information in the mechanisms aspect of isoflavonoid phyto-oestrogens in inhibiting cancer *in vitro* and *in vivo* in the models of human cancers.

Key words: Phyto-oestrogens; Oestrogenic properties; Antioxidant activity; Anticancer activity

INTRODUCTION

Phyto-oestrogens that occur in many plants of dietary significance to humans have been found to have diverse biological effects. They can be divided into two main catagories: the isoflavonoids and the lignans (Kurzer and Xu, 1997) which have similar molecular weights and metabolism as steroids, but clearly different biological effects on the cells (Adlercreutz, 1995). The isoflavonoid phyto-oestrogens are heterocyclic phenols with a close similarity in the structure to oestrogen and a diphenolic character similar to that of lignans. The precusors of the biologically active compounds originate in the soybean products (mainly isoflavonoid), whole-grain cereal products, seeds, clovers, and probably berries and nuts (mainly lignans) (Adlercreutz, 1997). The structure of the most important dietary isoflavonoids and lignans described in human biological samples can be seen in Fig. 1.

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Biological effects of phyto-oestrgens

Previous studies have shown that isoflavonoid phyto-oestrogens have significant oestrogenic effects in animals and humans (Price and Fenwick, 1985; Gavaler *et al*., 1991; Van Thiel *et al*., 1991). The most well-known oestrogenic effect in animals is clover disease in Australian sheep (Price and Fenwick, 1985). When consumed, the plant isoflavonoids and lignans undergo metabolic conversion in the gut, which results in the formation of further hormone-like compounds with oestrogen activity and the ability to bind weakly to oestrogen

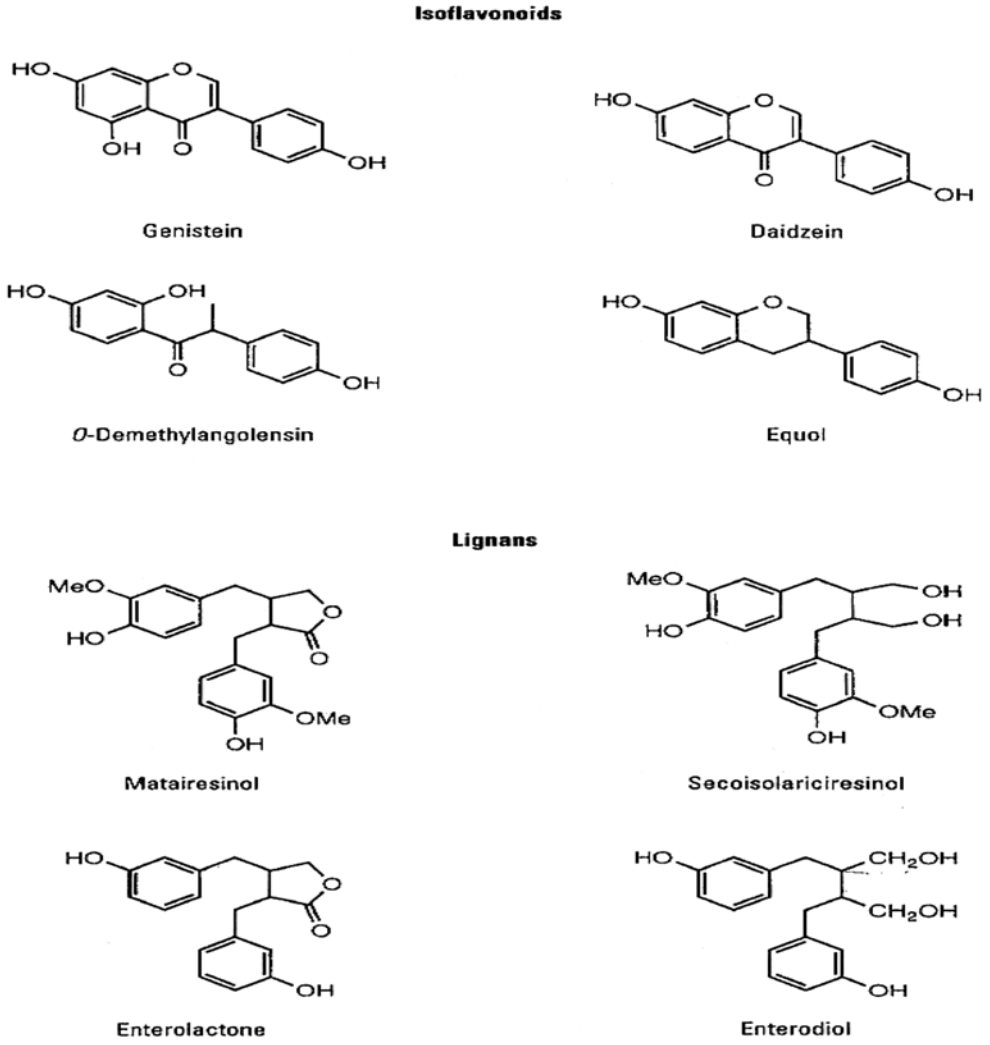

Fig. 1. Structure of the most important isoflavonoids and lignans identified in human biological samples (Kelly *et al*., 1993; Adlercreutz, 1995).

receptors (Setchell and Adlercreutz, 1988; Smith and Yang, 1994; Tham *et al*., 1998), approximately 10^3 to 10^5 fold less than 17-β-oestradiol (Davis *et al*., 1998). However, isoflavonoids may have antioestrogenic potency, when the circulating concentration of the isoflavone aglycones becomes 100-fold greater than oestradiol (Davis *et al*., 1998). Hence the oestrogenic or antioestrogenic potency of isoflavones may depend on the level of natural oestrogens. A recent report also indicates that the metabolite (equol) of an orally consumed isoflavonoid (*e.g*., daidzein) has greater oestrogenic activity than the parent compound (Wiseman, 1999).

Isoflavonoids might counteract endogenous oestrogens, through competitive binding to oestrogen receptors, although Shutt and Cox (1972) reported that the relative binding affinity of isoflavonoids for oestrogen receptor is only 0.05 - 1% of the binding affinity of 17-β-oestradiol. However, Santti *et al*. (1998) reported that dietary isoflavonoids competed

with endogenous oestrogens for active sites of oestrogen biosynthesising and metabolising enzymes and thus altered the concentration of biologically active endogenous oestrogens at the target cell level. Hence, on the basis of both weak oestrogen receptor binding capacity and competition for oestrogen metabolising pathway, isoflavonoids might exert significant modulation of oestrogen-dependent mechanisms.

The isoflavonoids and the lignans have now been shown to influence not only sex hormone metabolism and biological activity (Smith and Yang, 1994; Tham *et al*., 1998), but also intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation, differentiation and angiogenesis (Adlercreutz, 1995; Adlercreutz and Mazur, 1997; Kapiotis *et al*., 1997). It has been reported that some isoflavones (*e.g*., genistein) have the ability to inhibit tyrosine-specific protein kinases in a redox-sensitive manner (Akiyama *et al*., 1987; Adlercreutz, 1995), also topoisomerase II (Adlercreutz, 1990), and protein histidine kinase (Huang *et al*., 1992). Phyto-oestrogens are also antioxidants, as discussed in antioxidant effects of phyto-oestrogens. The potential of isoflavones to modify cell proliferation and cancer is discussed in anticarcinogenic effects of phyto-oestrogens. Hence, the ability of isoflavonoid phyto-oestrogens to influence sex hormone metabolism, biological activity, intracellular enzyme, protein synthesis, growth factor action, malignant cell proliferation, differentiation and angiogenesis in a way to make them strong candidates for a role as natural cancer protective.

Metabolism of phyto-oestrogens

The isoflavonoids genistein and daidzein are derived from the precursors biochanin-A and formononetin present in the plant. When ingested, daidzein can be metabolised by bacteria in the large intestine to form equol (oestrogenic) or O-demethylangolensin (non-oestrogenic) (Wiseman, 1999), whereas genistein is metabolised to the non-oestrogenic p-ethyl phenol. However, not all subjects who consume soy products can metabolise daidzein to equol, or they excrete this isoflavone in a very low amount, thus there is an interindividual metabolic variation between subjects (Morton *et al*., 1994; Kelly *et al*., 1995).

The following isoflavonoid phyto-oestrogens have been identified in human urine: genistein, daidzein, O-demethylangolensin and equol (Axelson *et al*., 1982; Adlercreutz *et al*., 1991; Adlercreutz, 1995). Moreover, studies on absorption and metabolism have found these compounds in plasma (Adlercreutz *et al*., 1993), breast milk (Franke *et al*., 1998) and faeces (Adlercreutz *et al*., 1995). Bannwart *et al*. (1984, 1989) also reported that a small amount of plant lignans (matairesinol, lariciresinol, isolariciresinol, and secoisolariciresinol) have also been identified in human urine.

Anticarcinogenic effects of phyto-oestrogens

Studies of cancer prevention have assessed isoflavonoid phyto-oestrogens for their efficacy in inhibiting cancer *in vitro* and *in vivo* particularly in models of the hormone-dependent human cancers. Interestingly, protection against carcinogens was provided by very low concentrations of isoflavones. The potential mechanisms for inhibition of cancer by isoflavonoid phyto-oestrogens, whether via their oestrogenic properties or their antioxidant activity, will be described below.

A. Oestrogenic and anti-oestrogenic activity

Breast cancer

The soybean isoflavones, genistein and daidzein have been studied for anti-breast cancer activity, because of their oestrogen receptor antagonist and agonist activities. Constantinou *et al*. (1996) reported that injection of genistein and daidzein (0.8 mg daily for six months) had the ability to protect against *N*-methyl-*N*-nitrosourea-induced mammary tumours in rats. In this study, both genistein and daidzein moderately reduced the number of tumours, but only marginally reduced the tumour incidence. Furthermore, Constantinou *et al*. (1998)

demonstrated that the growth of both the oestrogen-receptor positive human breast cancer cell line (MCF-7), or the oestrogen-receptor negative human breast cancer cell line (MDA-MB-468), was inhibited by genistein. In addition, treatment of these cells with genistein prior to implantation into nude mice decreased their growth in the animal (Constantinou *et al*., 1998). These investigators suggested that inhibition of human cancer cell growth by genistein, was unrelated to the oestrogenic activity of this compound.

Considering that neonatal oestrogen was known to inhibit both spontaneous and chemically-induced breast cancer, and that dietary isoflavones in early life have been suspected of playing a role in human breast cancer, it may be asked whether isoflavonoids can inhibit the formation of breast cancer. Lamartiniere *et al*. (1995) have reported that the development of mammary tumours induced by dimethyl-benz(a)anthracene (DMBA), could be delayed by giving 5 mg genistein to neonatal rats on days 2, 4 and 6 *postpartum*. Furthermore, administration of genistein (0.25 and 250 mg/kg diet) from conception to 21 days *postpartum* prior to treatment with DMBA at 50 days *postpartum*, resulted in a dose responsive inhibition of mammary tumours by altering the ontogeny of mammary gland development in rats (Lamartiniere *et al*., 1998). Thus there were fewer terminal end buds and fewer undifferentiated terminal ductal stuctures of mammary glands in the rats at 21 and 50 days of age (Fritz *et al*., 1998), before carcinogen treatment.

In contrast with oestrogen or genistein in early life protecting against breast cancer, further studies determined that genistein (750 ppm in the diet), like oestrogen, when administered during tumour development, enhanced the growth of oestrogen-responsive tumours (Hsieh *et al*., 1998; Allred *et al*., 2001). Genistein (10 nM to 10 μM) was found to enhance the proliferation of MCF-7 human breast cancer cells, both *in vitro* and in ovariectomised athymic mice. Genistein (1 μM) acted as an oestrogen agonist that induced expression of the oestrogen-responsive gene pS2 (Hsieh *et al*., 1998). These findings suggest that caution should be used in considering cancer prevention by soybean isoflavones in humans. This is of particular concern because high-potency isoflavone preparations are now available as dietary supplements.

Endometrial cancer

Studies on endometrial carcinogenesis in mice have shown that administration of genistein or daidzein (1 mg/30 g body weight) significantly decreased the level of oestradiol-induced expression of mRNAs for *c-jun*, *c-fos*, IL-1α and TNF-α in the uterus of ovariectomised mice (Lian *et al*., 2001). These investigators further reported that the incidences of endometrial adenocarcinoma and atypical endometrial hyperplasia, were significantly lower in the group of mice given oestradiol plus genistein or plus daidzein, than the group with oestradiol alone. These findings indicate that both genistein and daidzein have an inhibitory effect on oestrogen-related endometrial carcinogenesis in mice, possibly by suppressing expression of the oestrogen-induced genes *c-fos* and *c-jun*.

Prostate cancer

Mitchell *et al*. (2000) have shown shown that genistein, daidzein, coumerasol and equol inhibited cell growth and DNA damage in the human prostate tumour cell lines, androgen-receptor positive LNCaP and androgen-receptor negative PC-3. Genistein induced DNA damage and inhibited cell growth in both cell lines at < 10 μM. Daidzein inhibited cell growth at 10 - 100 μM and yet had no effect on DNA damage up to 500 μM. Hence, despite their structural similarity, these phyto-oestrogens inhibit prostate tumour cell growth by different mechanisms.

In humans, the effect of isoflavones has been studied on prostate cancer patients (Stephens, 1997). Peroral administration of 160 mg of a phytoestrogen preparation from red clover (*Trifolium pratense*) daily for 7 days prior to operation, was shown to reduce the prostate specific antigen level, PSA, and to

induce apoptosis in the prostatectomy specimen. These results, especially the apoptosis, indicate androgen deprivation and resemble the typical response to oestrogen therapy (Hellstrom *et al*., 1993; Ford *et al*., 1994).

B. Anti-proliferation

Cancer prevention is generally associated with inhibition, reversion, or retardation of cellular hyperproliferation. Isoflavonoids in general appear non-toxic to humans and animals, and have been demonstrated to inhibit proliferation in many kinds of cultured human cancer cell lines. Le-Bail *et al*. (1998) have reported that genistein in high concentrations (50 μM) has anti-proliferative activity in breast-cancer MCF-7 cells through an oestrogen-independent mechanism. Moreover, Kuo (1996) demonstrated that the two most potent isoflavonoids, quercetin and genistein (1 - 100 μM) have dose responsive anti-proliferative potency in human colon carcinoma HT29 and Caco-2 cell lines via an apoptosis induction mechanism. Genistein and synthetic analogues, at 0.1 - 25 μg/ml, were found to have anti-proliferative potency in transformed human (SW620, HT29) intestinal epithelial cell lines and to have induced apoptosis (Booth *et al*., 1999). Genistein and biochanin A have also been shown to inhibit epidermal growth factor in the human prostate cancer cell lines LNCaP and DU-145 (Peterson and Barnes, 1993), with IC_{50} values from 8.0 to 2.4 μg/ml and 4.3 to 15 μg/ml respectively.

Although the anti-proliferative effects of isoflavonoids in cultured cells appear well established, relatively little data have been published regarding anti-proliferative activity *in vivo*. Studies by Wei *et al.* (1998) focused on the ability of the isoflavone genistein to inhibit skin tumorigenesis in mice. These studies demonstrated that topically applied genistein (10 μM), prior to the carcinogen DMBA, reduced tumour multiplicity and tumour incidence. This was associated with genistein blockage of DMBA-induced bulky DNA-adduct formation (Wei *et al*., 1998). Recent study in mouse skin model demonstrated that topically applied equol at 10 μM following ultraviolet radiation (UVR) reduced tumour multiplicity and tumour incidence (Widyarini *et al*., 2005). Furthermore, these researchers found equol protected similarly from UVR-induced ODC activity, indicating that antipromotional effect of equol involved in cancer chemoprevention.

C. Cell cycle arrest and apoptosis

The investigators of the anti-proliferative effects of isoflavonoids noted that these compounds may inhibit the cell cycle or induce apoptosis. It has been demonstrated that genistein, at 5 - 20 μg/ml, produced cell cycle arrest at both the G1/S and G2/M phases in human myelogenous leukaemia HL-60 cell lines and the lymphocytic leukaemia MOLT-4 cell lines (Traganos *et al*., 1992). Moreover, human gastric cancer cells were arrested at G2/M by genistein, up to 60 μM (Matsukawa *et al*., 1993). Studies in a non-small-cell lung cancer cell line, demonstrated that genistein, at 30 μM, induced G2/M arrest and apoptosis induction (Lian *et al*., 1998). Zhou *et al.* (1998) reported that the isoflavones (genistein, daidzein, and biochanin A), at 0 to 50 μ M, inhibited growth of murine and human bladder cancer cell lines, by inducing cell cycle arrest and apoptosis. Cell cycle arrest and induction of apoptosis, could be functionally related to the activation of p53 (Plaumann *et al*., 1996) and the inhibition of cell cycle kinase activity (Kyle *et al*., 1997).

D. Regulation of host immune function

The role of host immune function, has become important in understanding the mechanisms that are involved in cancer prevention. Middleton (1998) has reported that a number of immune cell systems, do not appear to be affected significantly by flavonoids, while they are resting. However, once a cell becomes activated by a physiological stimulus, a flavonoid-sensitive substance is generated and interaction of flavonoids with that substance, alters the outcome of the activation process (Middleton

and Kandaswami, 1992; Middleton, 1998). Zhang *et al.* (1999) have demonstrated that daidzein, genistein and genistein glucuronides in nutritionally relevant concentrations (0.1 to 10 μM), enhanced the activation of NK cells *in vitro.*

In vivo, the isoflavone daidzein administered orally at 20 to 40 mg/kg body weight, stimulated murine nonspecific immunity, activated humoral immunity and enhanced cell-mediated immunity (Zhang *et al.*, 1997). Moreover, daidzein at the physiologically relevant concentrations (0.01 to 10 μM) potentiates lymphocyte activation in murine spleen, suggesting that the immunostimulatory effects of daidzein may be involved in cancer chemoprevention (Wang *et al.*, 1997). Study in mouse skin showed that topically applied equol at 1 to 20 μM markedly reduced UVR-induced inflammation and abrogated the UVR-induced immunosuppression (Widyarini *et al.*, 2001). Furthermore, this study also found equol protected similarly from immunosuppression, induced by the putative epidermal mediator, *cis*-UCA, indicating a potential mechanism of action involving inactivation of this UVR photoproduct.

Antioxidant effects of phyto-oestrogens

The antioxidant properties of genistein and other isoflavones have been demonstrated in several experimental models (Arora *et al.*, 1998; Mitchell *et al.*, 1998), such as protection from 12-O-tetradecanoyl phorbol-13 acetae (TPA)-induced $^1O_2$ or H_2O_2 formation, and particularly from UV-induced oxidative damage to DNA *in vitro* (Wei *et al.*, 1993, 1995, 1996; Cai *et al.*, 1997). In mice, dietary genistein has been shown to stimulate the endogenous antioxidants, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx) and glutathione-s-transferase (GSH-s-T) (Cai and Wei, 1996), with the effects found mainly in the small intestine and the skin. Furthermore, genistein has also suppressed prostaglandin synthesis, both basally and UV-stimulated, in cultured human cells and human skin (Isoherranen *et al.*, 1999), in a manner similar to the antioxidant *N*-acetylcysteine.

The antioxidant activity of isoflavonoids was suggested to be related to the number and position of hydroxyl groups. The active compounds all have 4'-hydroxyl and 5'–hydroxyl groups, which is consistent with other observations indicating that these groups are crucial for antioxidant activity due to their ability to scavenge free radicals (Wei *et al.*, 1995; Arora *et al.*, 1998). In contrast, both biochanin A (4'-O-methyl group) and genistein (4'-hydroxyl group), were found to inhibit UV induction of oxidative lesions in DNA *in vitro*, although the reactive oxygen scavenging properties of the two compounds were shown to be very different (Wei *et al.*, 1996; Ruiz-Larrea *et al.*, 1997). Arora *et al.* (1998) have also reported that equol (4'-hydroxyl group) and genistein (4'-hydroxyl group) were effective scavengers of metal iron radicals such as iron Fe (II) and Fe (III) *in vitro*. However, whereas the chemical structures of phyto-oestrogens may play an important role in providing protection against oxidants by scavenging free radicals, the precise structural requirements remain unclear.

It has been suggested that the oestrogen receptor may activate an antioxidant response, and it is possible that isoflavonoid phyto-oestrogens may posses antioxidant activity dependent on their receptor-binding characteristics. Natural oestrogens have significant radical-scavenging antioxidant activity (Ruiz-Larrea *et al.*, 2000), post-menopausal women had significantly elevated plasma antioxidant thiol levels after 6 months of hormone replacement therapy (Konukoglu *et al.*, 2000) and rabbits receiving transdermal oestrogen for 4 months doubled their total reactive antioxidant potential (Blumel *et al.*, 2000). There is also evidence that intraperitoneal injection of 17-β-oestradiol increased haemoxygenase (HO)-1 activity in the brain of rats (Lu *et al.*, 2002). The lipid protecting antioxidant activity of a diet of increased soy protein content, a source of phyto-oestrogens, was not accompanied by altered urinary sex hormone activity, however (Jenkins *et al.*, 2000). Therefore, it remains to be clarified, whether the protection against oxidative damage by phyto-

oestrogens might be regulated via its oestrogenic action.

CONCLUSION

To conclude, *in vitro* and *in vivo* studies in the models of human cancers shown that isoflavonoid phyto-oestrogens are natural cancer-protective compounds. Very low concentrations of isoflavonoid phyto-oestrogens, have the potential mechanisms in inhibiting cancer progression via their oestrogenic properties, antiproliferation activities, and their ability to regulate host-immune function. Moreover, antioxidant properties of isoflavonoid phyto-oestrogens generally agreed to be involved in the cancer chemoprevention. Epidemiologic investigations strongly support these above studies because the highest level of phyto-oestrogens in the diet are found in the countries or regions with low cancer incidence (Adlercreutz, 1995).

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REFERENCES

Allred CD, Ju YH, Allred KF, Chang J, Helferich WG. (2001) Dietary genistin stimulates growth of estrogen-dependent breast cancer tumors similar to that observed with genistein. *Carcinogenesis* **22**, 1667-1673.

Adlercreutz H, Mazur W. (1997) Phyto-oestrogens and Western diseases. *Annals. Med.* **29**, 95-120.

Adlercreutz H, Fotsis T, Kurzer MS, Wahala K, Makela T, Hase T. (1995) Isotope dilution gas chromatographic-mass spectrometric method for the determination of unconjugated lignans and isoflavonoids in human feces, with preliminary results in omnivorous and vegetarian women. *Annal. Biochem.* **225**, 101-108.

Adlercreutz H, Fotsis T, Lampe J, Wahala K, Makela T, Brunow G, Hase T. (1993) Quantitative determination of lignans and isoflavonoids in plasma of omnivorous and vegetarian women by isotope dilution gas chromatography-mass spectrometry. *Scan. J. Clin. Lab. Inves.* **215**, 5-18.

Adlercreutz H, Honjo H, Higashi A, Fotsis T, Hamalainen E, Hasegawa T, Okada H. (1991) Urinary excretion of lignans and isoflavonoid phyto-oestrogens in Japanese men and women consuming a traditional Japanese diet. *Am. J. Clin. Nutr.* **54**, 1093-1100.

Adlercreutz H. (1995) Phyto-oestrogens: Epidemiology and a possible role in cancer protection. *Environ. Health Perspectives* **103**, 103-112.

Adlercreutz H. (1990) Western diet and Western diseases: Some hormonal and biochemical mechanisms and associations. *Scan. J. Clin. Lab. Inves.* **201**, 3-23.

Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M,. Fukami Y. (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J. Biol. Chem.* **262**, 5592-5595.

Arora A, Nair MG, Strasburg GM. (1998) Antioxidant activities of isoflavones and their biological metabolites in a liposomal system. *Arch. Biochem. Biophys.* **356**, 133-141.

Axelson M, Kirk DN, Farrant RD, Cooley G, Lawson AM, Setchell KD. (1982) The identification of the weak oestrogen equol [7-hydroxy-3-(4'-hydroxyphenyl)-chroman] in human urine. *Biochem. J.* **201**, 353-357.

Bannwart C, Fotsis T, Heikkinen R, Adlercreutz H. (1989) Identification of the isoflavonic phytoestrogen daidzein in human urine. *Clin. Chim. Acta* **136**, 165-172.

Bannwart C, Adlercreutz H, Wahala K, Brunow G, Hase T. (1984) Detection and identification of the plant lignans lariciresinol, isolariciresinol and secoisolariciresinol in human urine. *Clin. Chim. Acta* **180**, 293-301.

Blumel JO, Castelo-Branco C, Gonzalez P, Moyano C, Iturriaga M, Videla L, Sajuan A, Cano A. (2000) Transdermal estrogens do not appears to modify the extent of lesional areas of aortic atherosclerosis in oophoriectomized rabbits on a cholesterol-rich diet. *Atherosclerosis* **148**, 303-308.

Booth C, Hargreaves DF, Hadfield JA, McGown AT, Potten CS. (1999) Isoflavones inhibit intestinal epithelial cell proliferation and induce apoptosis *in vitro*. *Br. J. Cancer* **80**, 1550-1557.

Cai QY, We HC. (1996) Effect of dietary genistein on antioxidant enzyme activities in Sencar mice. *Nutr.*

Cancer **25**, 1-7.

Cai QY, Rahn RO, Zhang RW. (1997) Dietary flavonoids, quercetin, luteloin, and genistein reduce oxidative DNA damage and lipid peroxidation and quench free radicals. *Cancer Lett.* **119**, 99-107.

Constantinou AI, Krygier AE, Mehta RR. (1998) Genistein induces maturation of cultured human breast cancer cells and prevents tumor growth in nude mice. *Am. J. Clin. Nutr.* **68**, 1426S-1430S.

Constantinou AI, Mehta RG, Vaughan A. (1996) Inhibition of N-methyl-N-nitrosourea-induced mammary tumors in rats by the soybean isoflavones. *Anticancer Res.* **16**, 3293-3298.

Davis SR, Murkies AL, Wilcox G. (1998) Phytoestrogens in clinical practice. *Integr. Med.* **1**, 27-34.

Ford LG, Bawley OW, Pearlman JA. (1994) The potential for hormonal prevention trials. *Cancer* **74**, 2726-2733.

Franke AA, Custer LJ, Tanaka Y. (1998) Isoflavones in human breast milk and other fluids. *Am. J. Clin. Nutr***. 68**, 1466s-1473s.

Fritz WA, Coward L, Wang J, Lamartiniere CA. (1998). Dietary genistein: perinatal mammary cancer prevention, bioavailability and toxicity testing in the rat. *Carcinogenesis* **19**, 2151-2158.

Gavaler JS, Galvao-Teles A, Monteiro E, Van Thiel D, Rosenblum E. (1991) Clinical responses to the administration of bourboun phyto-oestrogens to normal postmenoposal women. *Hepatol.* **14**, 87A.

Hellstrom M, Haggman M, Brandstedt S. (1993) Histopathological changes in androgen-deprived localised prostatic cancer. A study of total prostatectomy specimens. *Eur. Urol***. 24**, 461-465.

Hsieh CY, Santell RC, Haslam SZ, Helferich WG. (1998) Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells *in vitro* and *in vivo. Cancer Res*. **58**, 3833-3038.

Huang J, Nasr M, Kim Y, Matthews HR. (1992) Genistein inhibits protein histidine kinase. *J. Biol. Chem***. 267**, 15511-15515.

Issoherranen K, Punnonen K, Jansen C, Outila P. (1999) Ultraviolet irradiation induced cyclooxygenase-2 expression in keratinocytes. *Br. J. Dermatol*. **140**, 1017-1022.

Jenkins DJ, Kendall CW, Garsetti M, Rosenberg-Zand RS, Jacson CJ, Agarawal S, Rao AV, Diamandis EP, Parker T, Faulkner D, Vuksan V, Vidgen E. (2000) Effect of soy protein foods on low-density lipoprotein oxidation and ex-vivo sex hormone receptor activity-acontrolled crossover trial. *Metab. Clin. Exp*. **49**, 537-543.

Kapiotis S, Hermann M, Held I, Seelos C, Ehringer H, Gmeiner BM. (1997) Genistein, the dietary-derived angiogenesis inhibitor, prevents LDL oxidation and protects endothelial cells from damage by atherogenic LDL. *Arteriosclerosis Throm. Vas. Biol*. **17**, 2868-2874.

Kelly GE, Joannou GE, Reeder AY, Nelson C, Waring MA. (1995) The variable metabolic response to dietary isoflavones in human. *Proc. Soc. Exp. Biol. Med.* **208**, 40-43.

Kelly GE, Nelson C, Waring MA, Joannou GE, Reeder AY. (1993) Metabolites of dietary (soya) isoflavones in human urine. *Clin. Chim. Acta* **223**, 9-22.

Konukoglu D, Serin O, Yelke HK. (2000) Effects of hormone replacement therapy on plasma nitric oxide and total thiol levels in postmenopausal women. *J. Toxicol. Environ. Health A* **60**, 81-87.

Kuo SM. (1996) Antiproliferative potency of structurally distinct dietary flavonoids on human colon cancer cells. *Cancer Lett*. **110**, 41-48.

Kurzer MS, Xu Xia. (1997) Dietary Phytoestrogens. *Annu. Rev. Nutr*. **17**, 353-381.

Kyle E, Neckers L, Takimoto C, Curt G, Bergan R. (1997) Genistein-induced apoptosis of prostate cancer cells is preceded by a specific decrease in focal adhesion kinase activity. *Mol. Pharmacol*. **51**, 193-200.

Lamartiniere CA, Moore J, Holland M, Barnes S. (1995) Neonatal genistein chemoprevents mammary cancer. *Proc. Soc. Exp. Biol. Med*. **208**, 120-123.

Lamartiniere CA, Murrill WB, Manzolillo PA, Zhang JX, Barnes S, Zhang X, Wei H, Brown NM. (1998) Genistein alters the ontogeny of mammary gland development and protects against chemically-induced mammary cancer in rats. *Proc. Soc. Exp. Biol. Med.* **217**, 358-364.

Le Bail JC, Varnat F, Nicolas JC, Habrioux G. (1998) Estrogenic and antiproliferative activities on MCF-7 human breast cancer cells by flavonoids. *Cancer Lett.* **130**, 209-216.

Lian FL, Bhuiyan M, Li YW, Wall N, Kraut M, Sarkar FH. (1998) Genistein-induced G2-M arrest, p21WAF1 upregulation, and apoptosis in a non-small lung

cancer cell line. *Nutr. Cancer* **31**, 184-191.

Lian Z, Niwa K, Tagami K, Hashimoto M, Gao J, Yokoyama Y, Mori H, Tamaya T. (2001) Preventive effects of isoflavones, genistein and daidzein, on estradiol-17beta-related endometrial carcinogenesis in mice. *Jpn. J. Cancer Res*. **92**, 726-734.

Lu A, Ran Rq, Clark J, Reilly M, Nee A, Sharp FR. (2002) 17-β-estradiol induces heat shock proteins in brain arteries and potentiates ischemic heat shock protein induction in glia and neurons. *J. Cerebral Blood Flow Metab*. **22**, 183-195.

Matsukawa Y, Marui N, Sakai T, Satomi Y, Yoshida M, Matsumoto K, Nishino H, Aoike A. (1993) Genistein arrests cell cycle progression at G2-M. *Cancer Res*. **53**, 1328-1331.

Middleton E, Kandaswami C. (1992) Effects of flavonoids on immune and inflammatory cell functions. *Biochem. Pharmacol*. **43**, 1167-1179.

Middleton E. (1998) Effect of plant flavonoids on immune and inflammatory cell function. *Adv. Exp. Med. Biol*. **439**, 175-182.

Mitchell JH, Duthie SJ, Collins AR. (2000) Effects of phytoestrogens on growth and DNA integrity in human prostate tumor cell lines: PC-3 and LNCaP. *Nutr. Cancer* **38**, 223-228.

Mitchell JH, Gardner PT, McPhail DB, Morrice PC, Collins AR, Duthie GG. (1998). Antioxidant efficacy of phytoestrogens in chemical and biological model systems. *Arch. Bochem. Biophys*. **360**, 142-148.

Morton MS, Wilcox G, Wahlqvist ML, Griffiths K. (1994) Determination of lignans and isoflavonoids in human female plasma following dietary supplementation. *J. Endocrinol.* **142**, 251-259.

Peterson G, Barnes S. (1993) Genistein and biochanin A inhibit the growth of human prostate cancer cells but not epidermal growth factor receptor tyrosine autophosphorylation. *Prostate* **22**, 335-345.

Plaumann B, Fritsche M, Rimpler H, Brandner G, Hess RD. (1996) Flavonoids activate wild-type p53. *Oncogene* **13**, 1605-1614.

Price KR, Fenwick GR. (1985) Naturally occurring oestrogens in foods-a review. *Food Addit. Contam.* **2**, 73-106.

Ruiz-Larrea MB, Mohan AA, Paganga G, Miller NJ, Bolwell GC, Rice-Evans CA. (1997) Antioxidant acitivy of phytoestrogenic isoflavones. *Free Radic. Res*. **26**, 63-70.

Ruiz-Larrea MB, Martin C, Martinez R, Navarro R, Lacort M, Miller NJ. (2000). Antioxidant activities of estrogens against aqueous and lipophilic radicals; differences between phenol and catechol estrogens. *Chem. Phys. Lipid* **105**, 179-188.

Santti R, Makela S, Strauss L, Korkman J, Kostian ML. (1998) Phyto-oestrogens: potential endocrine disruptors in males.*Toxicol. Industrial Health* **14**, 223-237.

Setchell KDR, Adlercreutz H. (1988) Mammalian lignans and phyto-oestrogens. Recent studies on their formation, metabolism and biological role in the health and diseases. In Role of gut for a in toxicity and cancer, pp. 315-345, Academic Press, London.

Shutt DA, Cox RI. (1972) Steroid and phyto-oestrogen binding to sheep uterine receptors *in vitro*. *J. Endocrinol.* **52**, 299-310.

Smith TJ, Yang CS. (1994) Effects of food phytochemical on xenobiotic metabolism and tumorigenesis. In Food Phytochemicals I: Fruits and Vegetables, pp. 18-41, American Chemical Society.

Stephens FO. (1997) Phytooestrogens and prostate cancer: possible preventive role. *Med. J. Aust.* **167**, 138-139.

Tham DM, Gardner CD, Haskell WL. (1998) Potential health benefits of dietary phytoestrogens: A review of the clinical, epidemiological, and mechanistic evidence. *J. Clin. Endocrinol. Metab*. **83**, 22223-22232.

Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. (1998) Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. *Proc. Natl. Acad. Sci. U.S.A*. **95**, 3106-3110.

Traganos F, Ardelt B, Halko N, Bruno S, Darzynkiewicz Z. (1992) Effects of genistein on the growth and cell cycle progression of normal human lymphocytes and human leukemic MOLT-4 and HL-60 cells. *Cancer Res*. **52**, 6200-6208.

Van Thiel DH, Galvao-Teles A, Monteiro E, Rosenblum E, Gavaler JS. (1991) The phytoestrogens present in de-ethanolized bourbon are biologically active: a preliminary study in a postmenopausal woman. *Alcoholism Clin. Exp. Res*. **15**, 822-823.

Wang W, Higuchi CM, Zhang R. (1997) Individual and combinatory effects of soy isoflavones on the *in vitro* potentiation of lympocyte activation. *Nutr. Cancer* **29**, 29-34.

Wei H, Bowen R, Cai Q, Barnes S, Wang Y. (1995)

Antioxidant and antipromotional effects of the soybean isoflavone genistein. *Proc. Soc. Exp. Biol. Med.* **208**, 124-130.

Wei H, Wei L, Bowen R, Frenkel K, Barnes S. (1993) Inhibition of tumor promotion-induced hydrogen peroxide production *in vitro* and *in vivo* by genistein. *Nutr. Cancer* **20**, 1-12.

Wei HC, Bowen R, Zhang XS, Lebwohl M. (1998) Isoflavone genistein inhibits the initiation and promotion of two-stage skin carcinogenesis in mice. *Carcinogenesis* **19**, 1509-1514.

Wei HC, Cai QY, Rahn RO. (1996) Inhibition of UV light and Fenton reaction-induced oxidative DNA damage by the soybean isoflavone genistein. *Carcinogenesis* **17**, 73-77.

Widyarini S, Husband AJ, Reeve VE. (2005). Protective Effect of Isoflavonoid Equol Against Hairless Mouse Skin Carcinogenesis Induced by UV Radiation Alone or with a Chemical Cocarcinogen. *Photochem. Photobiol.* **81**, 32-37.

Widyarini S, Spinks N, Husband AJ, Reeve VE. (2001) Isoflavonoid compounds from red clover (*Trifolium pratense*) protect from inflammation and immune suppression induced by UV radiation. *Photochem. Photobiol.* **74**, 465-470.

Wiseman H. (1999) The bioavailability of non-nutrient plant factors: dietary flavonoids and phyto-oestrogens. *Proc. Nutr. Soc.* **58**, 139-146.

Zhang R, Li Y, Wang W. (1997) Enhancement of immune function in mice fed high doses of soy daidzein. *Nutr. Cancer* **29**, 24-28.

Zhang Y, Song TT, Cunnick JE, Murphy PA, Hendrick S. (1999) Daidzein and genistein glucoronides *in vitro* are weakly estrogenic and activate human natural killer cells in nutritionally relevant concentration. *J. Nutr.* **129**, 399-405.

Zhou JR, Mukherjee P, Gugger ET, Tanaka T, Blackburn GL, Clinton SK. (1998). Inhibition of murine bladder tumorigenesis by soy isoflavones via alterations in the cell cycle, apoptosis, and angiogenesis. *Cancer Res.* **58**, 5231-5238.