Pharmacognosy Reviews
Vol 2, Issue 3, Jan-Jun, 2008

PHCOG REV.
An official Publication of Phcog.Net

Phcog Rev.: Review Article
Health Benefits of Phyto-oestrogens – A Consensus Review

P. Sunita*[1], S. Jha[1] and S.P. Pattanayak[2]

[1] *Division of Pharmacognosy, Birla Institute of Technology, Mesra, Ranchi - 835 215*
[2] *Division of Pharmacology, Birla Institute of Technology, Mesra, Ranchi - 835 215, India.*
Corresponding author: priyashree_lenka@yahoo.com

ABSTRACT
This paper brings up regarding the plant derived compounds with estrogenic activity. The authors correctly emphasize the need for control of estrogenicity of foods containing phyto-oestrogens in view of the significant health effects of various estrogenic derivatives. This is particularly essential in the light of the current wave of enthusiasm for vegetarian food in general and phyto-oestrogens in particular. Phytoestrogens are plant-derived hormone-like diphenolic compounds of dietary origin that are present at high levels in plasma of healthy subjects. These compounds are weakly estrogenic and appear to influence the hormone dependent cancers (breast cancer, prostate cancer), viral infection, atherosclerosis, chronic heart diseases and osteoporosis, besides that the review concludes with the discussion of the effects of phyto-oestrogen on reproductive system, neuroprotective effect, retinal thickness, dementia and alcohol metabolism.
KEY WORDS : Phyto-oestrogen, health effects, vegetarian food.

INTRODUCTION

Interest in the physiological role of bioactive compounds present in plants has increased dramatically over the past decade. In relation to human health, particular attention has been given to the flavonoids, and especially one of their subclasses, the phytoestrogens. Phytoestrogens embody several groups of plant derived, nonsteroidal compounds with oestrogen like activity. The major classes of dietary phytoestrogens are the flavones and lignans both of which are widely distributed within the plant kingdom (1). Phytoestrogen is a general definition that has been applied to any plant substance or metabolite that induces biological responses in vertebrates and can mimic or modulate the actions of endogenous oestrogens usually by binding to oestrogen receptors (2). Sheep grazing on Australian pastures containing a particular type of clover *(Trifolium* sp) rich in formononetin, which is converted to daidzein in the rumen during fermentation, developed a widespread infertility in the 1940s. This problem was traced to these non-steroidal weak oestrogens (3). Flavonoids, including isoflavones, are naturally components in our diet and, with the burgeoning interest in alternative medicine, are increasingly being ingested by the general population. These compounds have a wide range of hormonal and non hormonal activities in animals or *in vitro*, and these suggest possible mechanisms for potential physiological effects of diets rich in isoflavones in humans (4, 5). In addition, experimental and epidemiological data are available to support the concept that isoflavone-rich diets exert physiological effects in different parts of the body. This article reviews potential molecular mechanisms of action by which dietary isoflavones potentially prevent, hormonal cancers (breast cancer, prostate cancer), viral infection, atherosclerosis, congestive heart diseases and osteoporosis, besides that it also includes the effect of phyto-oestrogen on reproductive system, neuroprotective effect, retinal thickness, dementia and alcohol metabolism.

Classification and Occurrence of Phyto-Oestrogens

Currently, four groups of phenolic compounds are classified as phytoestrogens: the isoflavones, stilbenes, coumestans, and lignans. The main stilbene is resveratrol, found primarily in grapes and peanuts (6). Although there are two isomers, cis and trans, only the latter shows estrogenic activity. Resveratrol is biosynthesized only in grape skin; therefore white wines contain only traces, whereas higher levels of resveratrol are found in red wines, fermented with skins (7, 8). Only a few coumestans are characterized by estrogenic activity, the most important being coumestrol, whose main dietary source is legumes, although it has also been reported in other vegetables, such as Brussels sprouts and spinach (9). 'Lignans' is a general term for a large family of compounds. One important example, matairesinol, is a non-estrogenic dimer, converted by gut microflora to enterolactone, which is estrogenic and easily absorbed (10). The main dietary source of lignans is flax-seed, but they are present also in whole wheat flour, fruits, and tea (6). Isoflavones are a subclass of the flavonoids, which are found almost exclusively in soybeans and in a few other legumes including red clover. The main isoflavones are genistein, daidzein and glycitein, which in soybean and soy-based foods exist as 7-O-glucosides (6). As isoflavones are the most widely investigated phyto-oestrogens.

Mechanisms of Action of Phyto-Ostrogens

ER-mediated mechanisms of action

The oestrogenic activity of isoflavones was first described in the 1940s when the infertility of sheep in Western Australia was proposed to be caused by ingestion of clover pastures rich in the isoflavone precursors formononetin and biochanin A (7). Using an Estrogen receptor (ER)-dependent transcriptional response assay, reported that of the isoflavone precursors, genistein had the highest oestrogenicity compared with daidzein, biochanin A and formononetin (8). It is this oestrogenic activity that is thought to be mainly responsible for several of the beneficial effects of isoflavones in

hormone-dependent processes, such as reducing bone loss associated with osteoporosis, improving menopausal symptoms, and lowering levels of plasma low density lipoprotein (LDL) (which accumulates in blood vessel walls during arteriosclerosis) (1). There are two types of ER, ER-α and ER-ß encoded by distinct genes: ER - α and ER - ß appear to serve distinct biological roles, as judged from the different phenotypes of mice devoid of each gene alone and the fact that they differ in the C-terminal ligand-binding domain and the N-terminal transactivation domain (9,10,11). ER- ß is mainly expressed in non reproductive tissues, such as the vascular system and bone, and seems to mediate in part the impact of oestrogens on the vasculature and the growth-promoting effects of oestrogens on non gonadal tissues (10). By contrast, ER- α is responsible for the classical hormonal effects, such as endometrial proliferation and mammary enlargement. Following ligand binding, the ER dimerises before binding to target genes and modulating transcription. Ctivated ERs regulate transcription of target genes either directly by binding to regulatory DNA elements or indirectly by modulating the expression of other transcription factors, such as AP-1 or NF-κB. Thus, ERs can trans activate AP-1- or NF-κB-responsive genes (9, 10, 11). The oestrogenic potency of isoflavones is low compared with 17- ß oestradiol, as soy isoflavones have ~1/3 and 1/1000 of the affinity of 17- ß oestradiol for ER - ß and ER- α, respectively (9, 11). Since genistein possesses a much higher binding affinity for ER-ß than for ER-α, isoflavones can be regarded as a type of natural 'selective ER modulator' (SERM). However, recent X-ray crystallographic studies examining the interaction of oestrogens, raloxifene and genistein with ERß suggest that the orientation of raloxifene and genistein with ERß is different from that of oestradiol, in particular in the interaction with helix 12 of the receptor (1).Isoflavones lack specific lipophilic regions, which undoubtedly affect their ER-ß-binding ability and the subsequent initiation of cellular events.

Role of phyto-oestrogen in the treatment of breast cancer

In vitro studies have established that phyto-oestrogens are weakly oestrogenic, since they have the ability to bind to mammalian oestrogen receptors to a low degree. Their affinity to receptors (from rabbit, sheep and rat uterine receptors, and a human cancer cell line) has been compared with oestradiol. Coumestrol has the greatest affinity, only ten to twenty times lower than oestradiol, and genistein about 100 times less; daidzein and equol bind about 1000 times less (12).

Oestrogen-dependent mechanisms

Oestrogen receptor α and oestrogen receptor ß -mediated mechanisms. Phyto-oestrogens appear to exert biphasic effects on breast cancer cell growth in vitro implicating differential mechanisms of action. At low concentrations (1-10 mM), cell proliferation is stimulated in ER(þ) cell lines only, suggesting that the phytooestrogens are acting via the ER. This idea is strengthened by the finding that phyto-oestrogens can induce pS2 expression in MCF-7 cells. Both the ER-α and ER-ß forms could be involved in the stimulation of cell proliferation by phyto-oestrogens. At low concentrations genistein and quercetin have been shown to be full agonists for ER-α as well as ER-ß. It is possible therefore that phyto-oestrogens may exert their effects at the cellular level via a similar mechanism of action to that of oestradiol. It is thought that the binding of oestradiol to the ER results in a conformational change, which enables binding of the oestradiol–ER complex to the oestrogen response element on the DNA. Activation of the oestrogen response element may induce the expression of the growth-related proto-oncogene c-fos (13), forming a c-fos–c-jun heterodimer that activates the activation protein-1 site, leading to cell proliferation (14).

Oestrogen independent mechanisms

There are several other anti-cancer effects of isoflavones which are not related to their anti-oestrogenic properties. Genistein is known to inhibit tyrosine kinases, which are responsible for phosphorylating proteins required for the regulation of cell functions, including cell division. Hence, it has been shown to inhibit growth in many cell lines. These lines include those which do not have oestrogen receptors, which suggests that these effects may be independent of any anti-oestrogen effects. Genistein has also been shown to inhibit the DNA repair enzyme topoisomerase, and to act as an antioxidant, thus potentially preventing oxidative DNA damage (15). In one cell line, genistein has been shown to cause changes characteristic of apoptosis, or programmed cell death, a protective mechanism induced in cells that have been damaged in order to prevent the proliferation of harmful mutations and possibly cancer (16). It has also been shown to inhibit ras gene expression in a rat pheochromocytoma cell line (17). In addition, genistein has been shown to inhibit angiogenesis, the formation of new blood vessels, an abnormal event which occurs as part of the growth and expansion of malignant tumours (18). It has been pointed out that many of these effects have been shown with very high concentrations, and not in cells treated with the levels likely to be achieved in plasma of human subjects eating foods containing phyto-oestrogens (19).

Role of phyto-oestrogen in the treatment of prostate cancer

Prostate cancer is the most common hormone-related cancer in men, and incidence has been rising rapidly, by about 3 4 % per year, in the UK (20). High-fat and -meat, low-NSP diets are currently linked to increased risk of the disease, and, like breast cancer, it is comparatively rare in Far Eastern populations consuming soyabean. In animal models, all three studies investigating the effects of soyabean showed reduced tumorigenesis. (21,19).

There has been little investigation of the effect of the lignans, although they too have been proposed to be protective in prostate cancer (22). Like isoflavones, they also inhibit 5α -reductase (23). Of the population groups studied, Portuguese men have markedly higher levels of lignans in prostatic fluid compared with British, Hong Kong and Chinese men (24). The incidence of prostate cancer in Portugal is higher than that in Hong Kong and China, but about half that of Britain (25).

Cesare et al suggested that the possibility that prostate cancer protection may be related to mechanisms such as inhibition of 5α-reductase, responsible for converting testosterone to dihydrotestosterone, or other enzymes regulating steroid hormone biosynthesis (26).

Phyto-oestrogen as anti-viral agent

Phyto-oestrogen prevents the virus attack in the following mechanism *in vitro.*

Inhibition of virus entry

Simian virus 40 (SV-40) has been associated with pleuralmesothelioma (27) and osteosarcoma (28). Treatment of kidney fibroblast cells with genistein has been shown to block SV-40 by inhibiting virus-induced signals that are required for its entry. When treated with genistein the SV-40 virions were shown to be delayed at the mouth of caveolae. This rendered the virions incapable of being internalized, with the SV-40 virus remaining at the cell surface (29). Indeed, genistein may be able to block tyrosine phosphorylation of caveolin-1 resulting in inhibition of SV-40 entry into cells (30). Furthermore, genistein has also been shown to block SV-40-induced upregulation of c-myc and c-jun and to cause a delay in the onset of SV-40 DNA synthesis (31).

Signaling pathway inhibition

It has been demonstrated that the virus triggers intracellular signals that involve phosphorylation of proteins from different signaling pathways of the cell. One of these is the epidermal growth factor receptor pathway substrate clone 15 (eps15) protein. Tyrosine phosphorylation of eps15 is required for viral entry into cells but treatment with genistein is able to significantly inhibit infectious entry of JCV (32) possibly by inhibitingviral induced phosphorylation.

Viral protein synthesis inhibition

Genistein is capable of suppressing replication of Encephalomyocarditis virus(EMC) virus in L929 cells to 0.5% of control level by a mechanism involving inhibition of viral protein synthesis at 50μm, (33). EMC virus can cause encephalitis, myocarditis, and also cause diabetes mellitus (34, 35).

Inhibition of viral enzymes

It has been recognized that flavonoids and isoflavonoids are also capable of inhibiting the activity of other ATP-utilising enzymes such as topoisomerase II (36). Some viruses are known to encode type I topoisomerases (37) that have been shown to be essential for viral replication (38). Since phytoestrogens can act as inhibitors of topoisomerase, this mechanism may also be important. While the investigations of the anti-viral properties of phytoestrogens have concentrated on their ability to inhibit tyrosine-specific protein kinases.

Phyto-oestrogens have antiviral property in vivo which has proved by Greiner et al., 2001 (39). It has been shown that pigs fed a soy genistein enriched diet exhibited reduced replication of porcine reproductive and respiratory syndrome (PRRS) virus when virally challenged.

Effect in cardiovascular diseases and artherosclerosis

A number of epidemiological observations have supported a protective role of phytoestrogens in modulating cardiovascular disease (CVD) risk markers. Consumption of soy products has been associated with reduced serum cholesterol (40) and lignans have also shown a moderately protective effect on triglyceridemia (41). A recent consensus paper (42) indicates that both soy protein and isoflavones may be needed for the maximal cholesterol lowering effect of soy, also recommending a diet low in saturated fat and cholesterol to promote heart health. It is however difficult at present to identify possible mechanisms whereby phytoestrogens may exert any additional effects to those exerted by soy protein. It has been considered that by binding to estrogen receptors, phytoestrogens may stimulate LDL receptors. By using phytoestrogen concentrations elevated far beyond physiological levels, Borradaile et al. (43) show reduced secretion of apolipoprotein B and increased LDL receptor activity in liver cells:

In many animal species, substituting dietary animal protein with soy protein consistently reduces LDL cholesterol and total cholesterol levels (44). Gerbils fed soy-based diets have significantly lower levels of total cholesterol, LDL plus VLDL (very-low-density ipoprotein) cholesterol, and apolipoprotein B concentrations (45). Isoflavone consumption led to a 30% decrease in plasma cholesterol levels and a 50% reduction in atherosclerotic lesion area in a strain of mice with low HDL (high-density lipoprotein) cholesterol (46). Soy protein containing isoflavones decreased LDL cholesterol and increased HDL cholesterol in a group of female monkeys fed a moderately atherogenic diet (47).

Soy phytoestrogens do also exert some vasodilatory activity in special conditions. Acute intravenous administration of genistein or daidzein was evaluated in healthy humans of both sexes (48). However, in a study using phytoestrogens in pill form (86 mg/day) (49), there was no evidence of reduced LDL oxidation. This latter finding suggests a direct antioxidant activity of soy proteins *per se*, an hypothesis also supported by the findings of (50) that showed a powerful hypolipidemic and antiatheromatous activity of an essentially phytoestrogen free soy protein diet in rabbits. Moreover, a very recent study in mildly hypercholesterolemic individuals found very little effect of soy protein or phytoestrogens on plasma antioxidant capacity or biomarkers of oxidative stress (51). Similar inconclusive findings were also observed in a large Dutch study (Dutch Prospect-EPIC cohort) on 16,165 women from 49 to 70 years of age that carefully evaluated phytoestrogen intake. In this study phytoestrogens were not associated with decreased CVD risk. However when stratifying for 'ever' *versus* 'never' smokers, CVD risk did decrease with increasing intake of lignans in 'ever' smokers (52).

Oestrogens exert potent effects on the arterial wall, and this was first recognised more than 40 years ago. The endothelial wall of the blood vessel has been found to have almost equal proportions of ERα and ERß (Refs 13, 46) (11, 53), and these two receptors play an important role in the vasoreactivity of the blood vessels. ERα rapidly activates endothelialderived NO synthase (eNOS), the key enzyme responsible for NO-induced dilation of the blood vessels (54). This is a nongenomic and rapid event that is reduced in atherosclerotic arteries. Studies of the ERα knockout and ERß knockout mice attest to the important role that these two receptors play in vascular events related to CVD; recent data show ERß-

deficient mice have numerous functional abnormalities in vascular smooth muscle cells and blood vessels, suggesting an essential role for ERß in the regulation of blood pressure and vascular function (55). Therefore, perhaps the greatest benefits of a diet rich in isoflavones might be their effects on improving the quality of blood vessels, rather than effects on blood cholesterol levels per se. Vascular constriction is associated with an increased risk of arteriosclerosis and hypertension. The few animal and clinical studies conducted to date suggest that isoflavones can improve vascular compliance. Isoflavones increase blood vessel dilation and improve blood flow in rhesus monkeys (56).

Effect on bone health

Estrogen plays an important role in maintaining bone density by regulating the formation and resorption of bone (57). Coxam 2003, (58) has recently reviewed twenty-two studies involving ovariectomised rats, six studies of other rat models (including male rats) one study of mice and two studies of ovariectomised monkeys. The studies, which used various doses, different phyto-oestrogen-rich products and purified compounds (soyabean, soya milk, soyabean protein, genistein, daidzein, rutine, zearalenone and ipriflavone, as well as several phyto-oestrogen-rich medications used in traditional medicine) and different end points (BMD, bone strength, bone mass, histomorphometry and bone formation and resorption markers), all showed an effect in rats (except in one case) but not in monkeys. Muhlbauer & Li have demonstrated that consumption of a variety of salad vegetables, herbs and cooked vegetables commonly found in the human diet can increase bone mineral content, mean cortical thickness and mineral density of trabecular bone in male rats, and they attributed this effect to flavanols (59). Most of the studies suggest that phytoestrogens are somewhat effective in maintaining bone mineral density (BMD) in postmenopausal women (60, 61).

Specific effects of phyto-oestrogen on bone cells have been shown both in osteoblasts and osteoclasts. In osteoblasts genistein stimulates a concentration-dependent increase in alkaline phosphatase activity. In osteoblastic MC3T3-E1 cells genistein or daidzein at concentrations of 10^{-7}–10^{-5} mol increase protein content, DNA content and alkaline phosphatase activity (62). In femoral metaphyseal tissue from elderly female rats genistein (10^{-6}–10^{-5} mol) increases Ca content and alkaline phosphatase activity. This increase is blocked by tamoxifen, indicating an ER-mediated pathway, and also by cycloheximide, suggesting it is mediated by overall increased protein synthesis (63). On the other hand, genistein can also cause apoptosis of osteoblasts by activating caspase-3 and cleaving adhesion molecules such as cadherins and catenins (64). In normal fetal osteoblast cells genistein increases the progesterone receptor and alkaline phosphatase gene expression and inhibits osteopontin and interleukin 6 gene expression (65). Gao & Yamaguchi observed that genistein is able to reduce the formation of osteoclast like mononuclear cells induced by dibutyryl cAMP (cAMP pathway), but not the formation of mononuclear cells induced by phorbol 12-myristate 13-acetate (protein kinase C pathway) (66). Phyto-oestrogens may also act on osteoclast production through inhibition of macrophage colony stimulating factor (67). Hughes et al (68) showed that oestrogens cause osteoclasts to undergo apoptosis through increased production of transforming growth factor-ß1 by osteoblasts.

On reproductive system

Phytoestrogens are recognized to act as either estrogenic agonists or antagonists that rely on different factors such as the relative expression of estrogen receptor (ER) subtypes (69) or the estrogenic environment in the target tissue (70,71,72). Indeed, phytoestrogens administered in the presence of high physiological concentrations of endogenous estradiol (E_2) may act as an estrogen antagonist; whereas their administration in a hypo estrogenic environment (similar to that found in postmenopausal women or in the male) produces estrogenic effects (71, 72). Moreover, some studies assessing the in vitro effects of phyto-oestrogens demonstrate an increase in androgen production from adrenal cortical cells (73). E_2 and environmental estrogens such as genistein can significantly stimulate mammalian sperm capacitation, acrosome reactions and fertilizing ability, with the environmental estrogens being more potent than E_2 (74). Currently, some authors suggest that when oligospermia is caused by partial maturation arrest of spermatic cells it can be easily and successfully treated through a therapy consisting of a very low dose of ethinyl estradiol and testosterone (75, 76). Casini et al (77) published a case study of the therapeutic effect of phyto-oestrogens on an infertile couple suffering from oligospermia by partial sperm maturation arrest they have concluded in that phytoestrogen treatment for a long period strongly influenced spermatogenesis and improved sperm parameters (i.e. sperm count and morphology) in a patient with severe oligospermia, leading to successful intrauterine insemination and a term pregnancy. This pleasing result suggests a possible therapeutic role for phytoestrogens in the treatment of oligospermia.

Jaroenporn and her co - workers in 2006 (78) worked on *Pueraria mirifica*, an herb containing Phytoestrogens, on Reproductive organs and fertility of adult male mice and they investigated The *Pueraria mirifica* -10 mg/kg and *Pueraria mirifica* -100 mg/kg treatments had no effect on testicular weight, sperm number, and serum LH, FSH, and testosterone levels. Only the *Pueraria mirifica* -100 mg/kg treatment reduced weights of epididymes and seminal vesicle and the sperm motility and viability. Histopathological examination demonstrated that testis, epididymis, and seminal vesicle were normal in all doses of *Pueraria mirifica* treatment. *Pueraria mirifica* -treated males showed no alterations in mating efficiency and on causing pregnancy of their female partners. So no effects were detected on endocrine parameters, testicular volume or semen parameters (79). In conclusion it can be stated that phyto-oestrogens have no therapeutic role in the reproduction system of healthy subjects.

Neuroprotective effects

In the nervous system, soy phytoestrogens have the potential to act as estrogen receptor agonists (80, 81) or antagonists (82). Soy phytoestrogens have been shown to influence

learning and memory (83), affect aggressive and social behavior (84), produce anxiolytic effects (85), regulate serotonin and cholinergic neurotransmission (81, 86) and affect the stress response of the hypothalamic-pituitary-adrenal axis (87). Furthermore, several studies have shown neuroprotective effects of soy hytoestrogens. Low doses of genistein reduce neuronal apoptosis and damage induced by hapsigargin (88), oxidative stress (89, 90), glutamate excitotoxicity (91) and b-amyloid protein *in vitro* (92, 93). However, genistein may induce apoptosis in neuronal cultures at high doses (94). *In vivo* studies have shown that soy phytoestrogens protect cholinergic neurons and reduce age-related cognition decline in male rats (95). In addition, chronic administration of genistein reduces brain lesion after experimental stroke in mice (96) and delays the onset of the disease and mortality in a murine model of familial amyotrophic lateral sclerosis (96), although chronic treatments with high doses of genistein may induce cytotoxicity and apoptosis in the rat brain *in vivo* (97).

Mechanism involved in the neuroprotection

Both estrogen receptors, α and ß, have been shown to be involved in neuroprotective effects of estradiol (98, 99, 100, 101, 102) and to be expressed in the adult rat hippocampus (103, 104, 105, 106, 107). Therefore, the neuroprotective effects of soy extract and genistein in the hippocampus *in vivo* may be mediated by estrogen receptors. Although soy isoflavones may activate both estrogen receptor α - and estrogen receptor ß -mediated transcription, they are more potent activators of estrogen receptor ß (108, 109, 110, 111). Therefore, it is possible that the neuroprotective effects of soy extract and genistein may be mainly mediated by the activation of estrogen receptor ß, which is highly expressed in neurons and glial cells in the adult rat hippocampal formation (103). In addition, estrogenic compounds may have neuroprotective effects that are independent of the activation of estrogen receptors (112). Indeed, genistein has estrogen receptor-independent effects, affecting the activity of enzymes such as protein tyrosine kinases, mammalian DNA topoisomerase I and II, and ribosomal S6 kinase (113). By its action on tyrosine kinases, genistein may alter phosphorylation events associated with the activation of neurotransmitter receptors (114). In addition, genistein may directly interact with neurotransmitter receptors (115) and, in consequence, alter neuronal function and neuronal response to injury. Finally, genistein and soy extracts have antioxidant properties (116), which may contribute to their neuroprotective effects.

Effect on retinal thickness

Lund et al 2002 (117) worked on dietary soy phytoestrogens effects on retinal thickness in rats and they reached in a conclusion that phytoestrogens influences rat retinal characteristics in a sexually dimorphic manner (more robust effect in males vs. females).

Genistein, via its mechanism as a tyrosine kinase inhibitor, has been found to completely abolish the effects of (a) carbamylcholine (a cholinergic agonist), which has been shown to increase retinal ganglion cells survival and (b) veratridine (a depolarizing agent which promotes neuronal survival and cellular proliferation (118) *in vitro*. Additionally, genistein was found to cause significant inhibition of and to induce apoptosis in the choriocapillaris and the retinal pigment epithelium (119). While the concentration of genistein is an important factor to consider in interpreting these data, it does suggest that phytoestrogens can alter retinal characteristics by neuroprotective and apoptotic pathways (118, 119). Furthermore, phytoestrogens can selectively bind the estrogen receptor (ER) system with a greater affinity for ER- ß vs. ER-α (11), (120), suggesting that the dimorphism in retinal morphology may be regulated by ER-ß.

Pigment epithelium of the retina contains the aromatase enzyme responsible for estrogen biosynthesis (121). Also, data revealed that the aromatase enzyme in brain is not significantly altered by dietary phytoestrogens (122), suggesting that the most likely action of phytoestrogens in neural tissue is at the ER system. However, the effects of the diet treatments on aromatase enzyme activity were not tested in this study. The dynamics of the dimorphic changes in retinal parameters in this study were similar to that of estrogenic hormone-dependent brain dimorphisms. Phytoestrogens may also have a significant influence on the visual system, including visual perception.

Miscellaneous

Dietary soy phytoestrogens may influence the differentiation, signalling and actions of numerous cells of the immune system as the receptor(s) have been identified on many cell types including lymphocytes and antigen presenting cells. This is further supported by the correlation between oestrogen levels and susceptibility to certain infectious agents in addition to the mounting evidence linking gender bias in cytokine responses (123). Several studies have noted altered gender-specific Th1/Th2 immune responses attributable in part to signalling via oestrogen or oestrogen-like compounds. In general, females are considered more prone towards a Th2 response and males a Th1 response (124,125). The action of phytoestrogens in the diet is likely complex with the potential to act either as agonists or antagonists on signalling through ER pathways. Additionally, higher concentrations can inhibit tyrosine kinases, constituents of many signalling pathways in immune cells (126). Genistein has also been shown to modulate metabolism of E2 (127). Collectively, signalling by dietary phytoestrogens through these pathways may influence the innate and adaptive immune responses to infection via alteration of cytokine responses.

Curran and his co- workers in 2003 (128) worked on dietary soy phytoestrogens and ER- α signalling modulate interferon gamma production in response to bacterial infection collectively, they suggest that dietary soy isoflavones, such as genistein, may impact resistance or susceptibility to infection. Dietary supplementation of daidzein, or phytoestrogen in soy, resulted in enhanced thymus weight and phagocytic activity by macrophages (129). They have suggested that dietary estrogens play a role in modulation of cell-mediated immunity and type I inflammatory responses and may be a factor in disease resistance and susceptibility.

Dementia and alcohol metabolism

Hormone-replacement therapy is thought to improve cognitive function and perhaps reduce the onset of dementia (130). As a consequence of the probable similarities between hormone-replacement therapy and isoflavones, a possible role for isoflavones may emerge. The isoflavones also inhibit the human aldehyde and alcohol dehydrogenase isoenzymes which are responsible for the metabolism of alcohol and detoxification of acetaldehyde, raising the possibility that they could be used as a remedy for the treatment of alcohol abuse (131, 132). In hamsters, alcohol consumption can be suppressed by daidzein, although in rats the reduction in alcohol intoxication did not appear to be mediated by changes in liver dehydrogenase activity. (133,134).

REFERENCES

1. K.D. Setchell, A. Cassidy. Dietary isoflavones: biological effects and relevance to human health. *J. Nutr.* 129(1):758-67 (1999).
2. Food Standards Agency (2002) Report of the COT Working Group on Phytoestrogens; available at http://www.food.gov.uk
3. D.A. Shutt. The effects of plant oestrogens on animal reproduction. *Endeavour* **35 (126):**110- 13(1976).
4. A. Cassidy, M. Faughnan. Phytooestrogens through the life cycle. *Procced. Nutr. Soc.* **59,(2)**: 489-96 (2000).
5. M. Bingham, G. Gibson, N. Gottstein, S.D. Pascual-Teresa, A.M. Minihane, G. Rimbach. Gut metabolism and cardioprotective effects of dietary isoflavones. *Current Topics on Nutra Res.* **1(1)**: 31-48 (2003).
6. K. Verdeal, D.S. Ryan. Naturally occurring estrogens in plant foodstuffs-a review. *J. Food Protec.* **42(7)**, 299-3 (1979).
7. H.W. Bennets, E.J. Underwood, F.L. Shier. A specific breeding problem of sheep in subterranean clover pastures in western Australia. *Aust. Vet. J.* **22(1)**: 2-12 (1946).
8. R.J. Miksicek. Interaction of naturally occurring nonsteroidal estrogens with expressed recombinant human estrogen receptor. *J. Steroid Biochem. Mol Biol.* **49(2-3)**: 153-60 (1994).
9. Kuiper, G.G. (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology* **138(3)**: 863-870.
10. G G Kuiper, E Enmark, M Pelto-Huikko, S Nilsson, and J A Gustafsson. (1996) Cloning of a novel receptor expressed in rat prostate and ovary. *Procc. Nat. Acad. Sci. U S A* **93(12)**, 5925-30.
11. G. G. Kuiper, J.G. Lemmen, B. Carlsson, J.C Corton, S.H. Safe, P.T. van der Saag, B. van der Burg, J.A. Gustafsson. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinol.* **139(10)**: 4252-63 (1998).
12. M. Maggiolini, D. Bonofiglio, S. Marsico, M.L. Panno, B. Cenni, D. Picard, S. Ando. Estrogen receptor alpha mediates the proliferative but not the cytotoxic dose-dependent effects of two major phytoestrogens on human breast cancer cells. *Mol. Pharmacol.* **60(3)**: 595–02 (2001).
13. B. V. D. Burg, J. P. Adrie, V. S. Miltenburg, P. V. Maurik, G. R. Rutteman, W. Misdorp, W. Siegfried, E. de Laat, J. J. van Zoelen. Isolation of autonomously growing dog mammary tumor cell lines cultured in medium supplemented with serum treated to inactivate growth factors. *J. nat can inst.* **81(20):**1545-1551 (1989)
14. X. Wang, S.M. Srinivas, A.Ahmed, E.S. Alnemi. Prevention of apotosis by Bcl-2: release of cytochrome c from mitochondria blocked. *Science* **21(5503):** (1997).
15. G. Peterson. Evaluation of the biochemical targets of genistein in tumour cells. *J. Nutr.* **125(3)**: 788-789 (1995).
16. M.C. Pagliacci, M. Smacchia, G. Migliorati, F. Grignani, C. Riccardi, I. Nicoletti. Growth inhibitory effects of genistein in MCF-7 human breast cells. *Euro. J. Can.* **30(A)**: 1675-82 (1994).
17. M. Nakafuku, T. Satoh, Y. Kaziro. Differentiation factors, including nerve growth-factor, fibroblast growth-factor, and interleukin-6, induce an accumulation of an active ras.GTP complex in rat pheochromocytoma PC12 cells. *J. Biol. Chem.* **267(27)**: 19448-54 (1992).
18. T. Fotsis, M. Pepper, H. Adlercreutz, G. Fleischmann, T. Hase, R. Montesano, L. Schweigerer. Genistein a dietary derived inhibitor of in vitro angiogenesis. *Proc. Nat. Acad. Sci. USA* **90(7)**: 2690-94 (1993).
19. D. Barnes. Effect of genistein on in vitro and in vivo models of cancer. *J. Nutr.* **125(3)**: 778-83 (1995).
20. M.P. Coleman, J. Esteve, P. Damieki, A. Arslan, H. Renard. Trends in Cancer Incidence and Mortality. *International Agency for Research on Cancer Scientific Publication no.121. Lyon, France: IARC Scientific Publications* (1993).
21. M.J. Messina, V. Persky, K.D.R. Setchell, S. Barnes. Soy intake and cancer risk A review of the *in vitro* and *in vivo* data. *Nutr. Can.* **21(2)**: 113-31 (1994).
22. V. Adlercreutz. Western diet and western diseases: some hormonal and biochemical mechanisms and associations. *Scand. J. Clin. Lab.Inv.* **201(1)**: 3-23 (1990).
23. B.A.J. Evans, K. Griffiths, M.S. Morton. Inhibition of 5% reductase and 178 hydroxysteroid dehydrogenase in genital skin fibroblasts by dietary lignans and isoflavonoids. *J. Endocrinol.* **147(7)**: 295-02 (1995).
24. Morton M, Wilcox G, Wahlquist M & Griffiths K. Determination of lignans and isoflavonoids in human female plasma following dietary supplementation. *J. Endocrinol.* **142(2)**: 251-9 (1994).
25. D.M. Parkin, C.S. Muir, S.L. Whelan, Y.T. Gao, J. Ferlay, J. Powell. Cancer Incidence in Five Continents, vol 5, *International Agency for Research on Cancer Scientific Publication* no. 88. Lyon, France: IARC Scientific Publications (1992).
26. R.S. Cesare, A. Anna, K.J. Stuart. Phytoestrogens: End of a tale. *Annal. Med.* **37(6)**: 423–38 (2005).
27. M. Carbone, H.I. Pass, P. Rizzo, M. Marinetti, M. DiMuzio, D.J. Mew, A.S. Levine, A. Procopio. Simian virus 40-like DNA sequences in human pleural mesothelioma. *Oncogene* **9(6)**: 1781–90 (1994).
28. S.M. Medoza, T. Konishi, C.W. Miller. Integration of SV40 in human osteosarcoma. *Oncogene* **17(19)**: 2457–62 (1998).
29. Y. Chen, L.C Norkin. Extracellular simian virus 40 transmits a signal that promotes virus enclosure within caveolae. *Exptl. Cell. Res.* **246(1)**: 83–90 (1999).
30. L. Pelkmans. A. Helenius. Endocytosis via caveolae. *Traffic* **3(5)**: 311–20 (2002).
31. N.S. Dangoria, W.C. Breau, H.A. Anderson, D.M. Cishek, L.C. Norkin. Extracellular simian virus 40 induces an ERK/MAP kinase-independent signaling pathway that activates primary response genes and promotes virus entry. *J. Gen. Viriol.* **77(12)**: 2173–82 (1996).
32. W. Querbes, A. Benmerah, D. Tosoni, P.P. Di Fiore, W.J Atwood. A JC virus-induced signal is required for infection of glial cells by a clathrin- and eps15-dependent pathway. *J. Viriol.* **78(1):** 250–6 (2004).
33. K. Hirasawa, A. Kim, H.S. Han, J. Han, H.S. Jun, J.W. Yoon. Effect of p38 mitogen-activated protein kinase on the replication of encephalomyocarditis virus. *J. Viriol.* **77(10)**: 5649–56 (2003).
34. J.W. Yoon, M.M. Rodrigues, C., Currier, A.L. Notkins. Longterm complications of virus-induced diabetes mellitus in mice. *Nature* **296(1)**: 566–9 (1982).
35. J.E. Craighead, M.F. McLane. Diabetes mellitus: induction in mice by encephalomyocarditis virus. *Science* **162(3856)**: 913–4 (1968).
36. A. Constantinou, R. Mehta, C. Runyan, K. Rao, A. Vaughan, R. Moon. Flavonoids as DNA topoisomerase antagonists and poisons: structure–activity relationships. *J. Natl. Prod.* **58(2)**: 217–25 (1995).
37. N.D. Klemperer, J. Lyttle, D. Tauzin, P. Traktman, A.J. Robinson. Identification and characterization of the orf virus type I topoisomerase. *Virol.* **206(1)**: 203–15 (1995).
38. S. Shuman, M. Golder, B. Moss. Insertional mutagenesis of the vaccinia virus gene encoding a type I DNA topoisomerase: evidence that the gene is essential for virus growth. *Virol.* **170(1)**: 302–306 (1989).
39. L.L. Greiner, T.S. Stahly, T.J. Stabel. The effect of dietary soy genistein on pig growth and viral replication during a viral challenge. *J. Animal Sci.* **79(5)**: 1272–9 (2001).
40. C.R. Sirtori, F. Pazzucconi, L. Colombo, P. Battistin, A. Bondioli, K. Descheemaeker. Double-blind study of the addition of high-protein soya milk v. cow's milk to the diet of patients with severe hypercholesterolaemia and resistance to or intolerance of statins. *Brit. J. Nutr.* **82(2)**: 91–6 (1999).
41. M.J. De Kleijn, Y.T. van der Schouw, P.W Wilson, D.E Grobbee, P.F. Jacques. Dietary intake of phytoestrogens is associated with a favorable metabolic cardiovascular risk profile in post-menopausal U.S. women: the Framingham study. *J. Nutr.* **132(2)**: 276–82 (2002).
42. J.W. Erdman. Soy protein and cardiovascular disease – A statement for healthcare professionals from the Nutrition Committee of the AHA. *Circulation* **102(11)**: 2555–9 (2000).
43. N.M. Borradaile, L.E. de Dreu, L.J. Wilcox, J.Y. Edwards, M.W. Huff. Soya phytoestrogens, genistein and daidzein, decrease apolipoprotein B secretion from HepG2 cells through multiple mechanisms. *Biochem. J.* **366(2)**:531–9 (2002).
44. C.R., Sirtori, R. Even, M.R. Lovati. Soybean protein diet and plasma cholesterol: from therapy to molecular mechanisms. *Annals New York Acad Sci.* **676(1)**: 188-201 (1993).
45. C. Tovar-Palacio. Intake of soy protein and soy protein extracts influences lipid metabolism and hepatic gene expression in gerbils. *J. Nutr* **128(5)**: 839-42 (1998).
46. A. Elizabeth, K. P Sutherland**, A.** Shari, A. C. Wang**,** R. C. LeBoeuf. Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. *J. Nutr.* **128(1)**, 954-959 (1998).
47. M. S Anthony, T. B.Clarkson, C. L. Hughes, T. M. Morgan, G. L. Burke. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. *J. Nutr.* **126(1)**: 43-50 (1996).
48. H.A. Walker, T.S. Dean, T.A.B. Sanders, G. Jackson, J.M. Ritter, P.J. Chowienczyk. The phytoestrogen genistein produces acute nitric oxide-dependent dilation of human forearm vasculature with similar potency to 17b-estradiol. *Circulation* **103(3)**: 258–62 (2001).
49. S. Samman, P.M. Lyons Wall, G.S. Chan, S.J. Smith, P. Petocz. The effect of supplementation with isoflavones on plasma lipids and oxidizability of low density lipoprotein in premenopausal women. *Atheroscl.* **147(2)**: 277–83 (1999).

50. S. Castiglioni, C. Manzoni, A. D'Uva, R. Spiezie, E. Monteggia, G. Chiesa. Soy proteins reduce progression of a focal lesion and lipoprotein oxidability in rabbits fed a cholesterol rich diet. *Atheroscl.* **171(2)**:163–70 (2003).
51. S. Vega-Lopez, K.J. Yeum, J.L. Lecker, L.M. Ausman, E.J. Johnson, S. Devaray. Plasma antioxidant capacity in response to diets high in soy or animal protein with or without isoflavones. *Ame. J. Clin.l Nutr.* **81(4)**: 43–9 (2005).
52. Y.T. Van der Schouw, S. Kreikamp-Kaspers, P.H.M. Peeters, L. Keinan-Boker, E.B. Rimm, D.E. Grobbee. Prospective study on usual dietary phytoestrogen intake and cardiovascular disease risk in Western women. *Circulation* **111(1)**: 465–71 (2005).
53. T.C. Register, M.R. Adams. Coronary artery and cultured aortic smooth muscle cells express mRNA for both the classical estrogen receptor and the newly described estrogen receptor beta. *J. Steroid Biochem Mol. Biol.* **64(3-4)**: 187- 91 (1998).
54. C.C. Chen, J.K. Wang, S.B. Lin. Antisense oligonucleotides targeting protein kinase C-alpha, -beta I, or -delta but not –eta inhibit lipopolysaccharide-induced nitric oxide synthase expression in RAW 264.7 macrophages: involvement of a nuclear factor kappa B dependent mechanism. *J. Immunol.* **161(11)**: 6206-14 (1998).
55. Y. Zhu, Z. Bian, P. Lu, H. Richard, K.L. Bao, D.Cox, J. Hodgin, P. W. Shaul, P. Thorén, O. Smithies,J.Å. Gustafsson, M. E. Mendelsohn. Abnormal vascular function and hypertension in mice deficient in estrogen receptor beta. *Science* **295(55554)**: 505-8 (2002).
56. E.K Honore, J.K Williams, M.S. Anthony, T.B. Clarkson. Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques. *Fertil Steril* **67(1)**:148-154 (1997).
57. S. Nilsson, J.A. Gustafsson. Biological role of estrogen and estrogen receptors. *Crit. Rev. Biochem. Mol. Biol.* **37(1)**: 1–28 (2002).
58. V. Coxam. Prevention of osteopaenia by phyto-oestrogens: animal studies. *Brit. J. Nutr.* **89(1)** S75–S85 (2003).
59. R.C. Muhlbauer, F. Li. Effects of vegetables on bone metabolism. *Nature* **401(1)**: 343–4 (1999).
60. F.S. Dalais, G.E. Rice, M.L. Wahlqvist, M. Grehan, A.L. Murkies, G. Medley, R. Ayton, B.J. Strauss. Effects of dietary phytoestrogens in postmenopausal women. *Climac.* **1(2):**124–9 (1998).
61. A.F.M. Kardinaal, M.S. Morton, I.E.M. Bruggemann-Rotgans, E.C.H. van Beresteijn. Phyto-estrogen excretion and rate of bone loss in postmenopausal women. *Euro. J. Clin. Nutr.* **52(11)**: 850– 855 (1998).
62. M. Yamaguchi, E. Sugimoto. Stimulatory effect of genistein and daidzein on protein synthesis in osteoblastic MC3T3-E1 cells: activation of aminoacyl-tRNA synthetase. *Mol. Cell. Biochem.* **214(1-2)**: 97–102 (2000).
63. M. Yamaguchi, Y.H. Gao. Anabolic effect of genistein on bone metabolism in the femoral-metaphyseal tissues of elderly rats is inhibited by the anti-estrogen tamoxifen. *Res. Exptl Med.* **197(2)**: 101–107 (1997).
64. I. Hunter, D.P. McGregor, S.P. Robins. Caspase-dependent cleavage of cadherins and catenins during osteoblast apoptosis. *J. Bone Min. Res.* **16(3)**: 466–477 (2001).
65. D.J. Rickard, D.G. Monroe, T.J. Ruesink, S. Khosla, B.L. Riggs, T.C. Spelsberg. Phytoestrogen genistein acts as an estrogen agonist on human osteoblastic cells through estrogen receptors alpha and beta. *J. Cell Biochem.* **89(3)**: 633–646 (2003).
66. Y.H. Gao, M. Yamaguchi. Inhibitory effect of genistein on osteoclast-like cell formation in mouse marrow cultures. *Biochem. Pharmacol.* **58(5):** 767–772 (1999).
67. S. Srivastava, M.N. Weitzmann, R.B. Kimble, M. Rizzo, M. Zahner, J. Milbrandt, F.P. Ross, R. Pacifici. Estrogen blocks MCSF gene expression and osteoclast formation by regulating phosphorylation of Egr-1 and its interaction with Sp-1. *J. Clin. Invest.* **102(10)**: 1850–1859 (1998).
68. D.E. Hughes, A. Dai, J.C. Tiffee, H.H. Li, G.R. Mundy, B.F. Boyce. Estrogen promotes apoptosis of murine osteoclasts mediated by TGFβ. *Nat. Med.* **2(10)**: 1132–6 (1996).
69. L. O'Donnell, K.M. Robertson, M.E. Jones, E.R. Simpson. Estrogen and spermatogenesis. *Endocrine Rev.* **22(3)**: 289–18 (2001).
70. U.A. Kayisli, C.A. Aksu, M. Berkkanoglu, A. Arici. Estrogenicity of isoflavones on human endometrial stromal and glandular cells. *Journal of Clin. Endocrinol. Metab.* **87(12)**:5539–44 (2002).
71. J. Mei, S.S. Yeung, A.W. Kung. High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women. *J. Clin. Endocrinol. Metabol.* **86(11)**:5217–5221 (2001).
72. V. Unfer, M.L. Casini, L. Costabile, M. Mignosa, S. Gerli, G.C. Di Renzo. High dose of phytoestrogens can reverse the antiestrogenic effects of clomiphene citrate on the endometrium in patients undergoing intrauterine insemination: a randomized trial. *J. Soc. Gynecol. Invest.* **11(5)**: 323–8 (2004).
73. W.S. Mesiano, S.L. Katz, J.Y. Lee, R.B. Jaffe. Phytoestrogens alter adrenocortical function: genistein and daidzein suppress glucocorticoid and stimulate androgen production by cultured adrenal cortical cells. *J.Clin. Endocrinol. Metabol.* **84(7)**:2443–8 (1999).
74. S.A. Adeoya-Osiguwa, S. Markoulaki, V. Pocock, S.R. Milligan, L.R. Fraser. 17β-Estradiol and environmental estrogens significantly affect mammalian sperm function. *Human Reprod.* **18(1)**:100–7 (2003).
75. P. Sah. Role of low-dose estrogen–testosterone combination therapy in men with oligospermia. *J. urol.* **70(5)**:780–1 (1998).
76. P. Sah. Oligospermia due to partial maturation arrest responds to low dose estrogen–testosterone combination therapy resulting in live-birth: a case report. *Asian J. Androl.* **4(5)**: 307–8 (2002).
77. M. L. Casini, G. Sandro, U. Vittorio. An infertile couple suffering from oligospermia by partial sperm maturation arrest: Can phytoestrogens play a therapeutic role? A case report study. *Gynecol. Endocrinol.* **22(7)**: 399–01 (2006).
78. S. Jaroenporn, S. Malaivijitnond, K. Wattanasirmkit, H. Trisomboon, G. Watanabe, K. Taya, W. Cherdshewasart. Effects of *Pueraria mirifica*, an Herb Containing Phytoestrogens, on reproductive organs and fertility of adult male mice. *Endocrine* **30 (1)**, 93–101 (2006).
79. J.H. Mitchell, E. Cawood, D. Kinniburgh, A. Provan, A.R. Collins, D.S. Irvine. Effect of a phytoestrogen food supplement on reproductive health in normal males. *Clin. Sci.* **100(6)**:613–8 (2001).
80. D.A. Schreihofer. Transcriptional regulation by phytoestrogens in neuronal cell lines. *Mol. Cell. Endocrinol.* **231(1-2)**:13–22 (2005).
81. Y. Pan, M. Anthony, T.B. Clarkson. Effect of estradiol and soy phytoestrogens on choline acetyl transferase and nerve growth factor mRNAs in the frontal cortex and hippocampus of female rats. *Proceed. Soc. Exptal. Biol. Med.* **221(2)**: 118–125 (1999).
82. H.B. Patisaul, M. Melby, P.L.Whitten, L.J. Young. Genistein affects ER β- but not ER α-dependent gene expression in the hypothalamus. *Endocrinol.* **143(6)**:2189–97 (2002).
83. T.D. Lund, T.W. West, L.Y. Tian, L.H. Bu, D.L. Simmons, K.D. Setchell, H. Adlercreutz, E.D. Lephart. Visual spatial memory is enhanced in female rats (but inhibited in males) by dietary soy phytoestrogens. *BMC Neurosci.* **2(1)**:20 (2001).
84. N.G. Simon, J.R. Kaplan, S. Hu, T.C. Register, M.R. Adams. Increased aggressive behavior and decreased affiliative behaviour in adult male monkeys after long-term consumption of diets rich in soy protein and isoflavones. *Horm. Behav.***45(4)**: 278–84 (2004).
85. T.D. Lund, E.D. Lephart. Dietary soy phytoestrogens produce anxiolytic effects in the elevated plus-maze. *Brain Res.* **913(2)**:180–4 (2001).
86. C.A. Shively, S.J. Mirkes, N.Z. Lu, J.A. Henderson, C.L. Bethea. Soy and social stress affect serotonin neurotransmission in primates. *Pharmacogen. J.* **3(2)**: 114–21(2003).
87. E.D. Lephart, E. Galindo, L.H. Bu. Stress (hypothalamic–pituitary–adrenal axis) and pain response in male rats exposed lifelong to high vs. low phytoestrogen diets. *Neurosci. Lett.* **342(1-2)**: 65–8 (2003).
88. N.J. Linford, D.M. Dorsa. 17β-Estradiol and the phytoestrogen genistein attenuate neuronal apoptosis induced by the endoplasmic reticulum calcium-ATP ase inhibitor hapsigargin. *Steroids* **67(2)**:1029–40 (2002).
89. K.P. Ho, L. Li, L. Zhao, Z.M. Qian. Genistein protects primary cortical neurons from iron-induced lipid peroxidation. *Mol. CellBiochem.* **247(1-2)**: 219–22 (2003).
90. M. Sonee, T. Sum, C. Wang, S.K. Mukherjee. The soy isoflavone, genistein, protects human cortical neuronal cells from oxidative stress. *Neurotoxicol.* **25(5)**:885–891 (2004).
91. L. Zhao, Q. Chen, R. Diaz Brinton. Neuroprotective and neurotrophic efficacy of phytoestrogens in cultured hippocampal neurons. *Exp Biol Med* **227(7)**: 509–19 (2002).
92. O.Y. Bang, H.S. Hong, D.H. Kim, H. Kim, J.H. Boo, K. Huh, I. Mook-Jung. Neuroprotective effect of genistein against β amyloid-induced neurotoxicity. *Neurobiol. Disorder* **16(1)**:21–28 (2004).
93. H. Zeng, Q. Chen, B.Zhao. Genistein ameliorates β-amyloid peptide (25–35)-induced hippocampal neuronal apoptosis. *Free Rad. Biol. Med.* **36(2)**:180–8 (2004).
94. N.J. Linford, Y. Yang, D.G. Cook, D.M. Dorsa. Neuronal apoptosis resulting from high doses of the isoflavone genistein: role for calcium and p42/44 mitogen-activated protein kinase. *Journal Pharmacol. Exptal Therap.* **299(1)**:67–75 (2001).
95. Y.B. Lee, H.J. Lee, M.H. Won, I.K. Hwang, T.C. Kang, J.Y. Lee, S.Y. Nam, K.S. Kim, E. Kim, S.H. Cheon. Soy isoflavones improve spatial delayed matching-to-place performance and reduce cholinergic neuron loss in elderly male rats. *J. Nut.* **134(7)**:1827–31 (2004).
96. V.N. Trieu, F.M. Uckun. Genistein is neuroprotective in murine models of familial amyotrophic lateral sclerosis and stroke. *Biochem. Biophy. Res. Comm.* **258(3)**: 685–8 (1999).
97. E.J. Choi, B.H. Lee. Evidence for genistein mediated cytotoxicity and apoptosis in rat brain. *Life Sci.* **75 (4)**: 499–509 (2004).
98. I. Azcoitia, A. Sierra, L.M. Garcia-Segura. Neuroprotective effects of estradiol in the adult rat hippocampus: interaction with insulin-like growth factor-I signalling. *Journal of Neuroscience and Research* **58(6)**:815–822 (1999).
99. J. Veliskova, L. Velisek, A.S. Galanopoulou, E.F. Sperber. Neuroprotective effects of estrogens on hippocampal cells in adult female rats after status epilepticus. *Epilepsia* **41(Suppl 6)**:S30–S35 (2000).
100. D.B. Dubal, H. Zhu, J. Yu, S.W. Rau, P.J. Shughrue, I. Merchenthaler, M.S. Kindy, P.M. Wise. Estrogen receptor α, not β, is a critical link in estradiol-mediated protection against brain injury. *Proceed. Nata.l Acad. Sci. USA* **89(1-3)**:1952–7 (2001).
101. J.L. Fitzpatrick, A.L. Mize, C.B. Wade, J.A. Harris, R.A. Shapiro, D.M. Dorsa. Estrogen-mediated neuro protection against β amyloid toxicity requires expression of

estrogen receptor α or β and activation of the MAPK pathway. *J. Neurochem.* **82(3)**:674–82 (2002).

102. E. Vegeto, S. Belcredito, S. Etteri, S. Ghisletti, A. Brusadelli, C. Meda, A. Krust, S. Dupont, P. Ciana, P. Chambon. Estrogen receptor-α mediates the brain anti-inflammatory activity of estradiol. *Proceed. Nat.l Acad. Sci. USA* **100(3)**: 9614–19 (2003).
103. I. Azcoitia, A. Sierra, L.M. Garcia-Segura. Localization of estrogen receptor β-immunoreactivity in astrocytes of the adult rat brain. *Glia* **26(3)**: 260–7 (1999).
104. G.P. Cardona-Gomez, L. DonCarlos, L.M. Garcia-Segura. Insulin-like growth factor I receptors and estrogen receptors colocalize in female rat brain. *Neurosci.* **99(4)**: 751–60 (2000).
105. P.J. Shughrue, I. Merchenthaler. Evidence for novel estrogen binding sites in the rat hippocampus. *Neurosci.* **99(4)**: 605–12 (2000).
106. S.A. Hart, J.D. Patton, C.S. Woolley. Quantitative analysis of ER α and GAD colocalization in the hippocampus of the adult female rat. *J. Comp. Neurol.* **440(2)**:144–55 (2001).
107. T.A. Milner, B.S. McEwen, S. Hayashi, C.J. Li, L.P. Reagan, S.E. Alves. Ultrastructural evidence that hippocampal a estrogen receptors are located at extranuclear sites. *J. Comp. Neurol.* **429(3)**:355–371(2001).
108. G.G. Kuiper, J.G. Lemmen, B. Carlsson, J.C. Corton, S.H. Safe, P.T. van der Saag, B. van der Burg, J.A. Gustafsson. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. *Endocrinol.* **139(10)**:4252–63 (1998).
109. J.M. Hall, K.S. Korach. Analysis of the molecular mechanisms of human estrogen receptors α and β reveals differential specificity in target promoter regulation by xenoestrogens. *J. Biol. Chem.* **277(46)**:44455–61 (2002).
110. H.B. Patisaul, M. Melby, P.L. Whitten, L.J. Young. Genistein affects ER β - but not ER α - dependent gene expression in the hypothalamus. *Endocrinol.* **142(7)**:2189–97 (2002).
111. R. Ise, D. Han, Y. Takahashi, S. Terasaka, A. Inoue, M. Tanji, R. Kiyama. Expression profiling of the estrogen responsive genes in response to phytoestrogens using a customized DNA microarray. *FEBS Lett.* **579(7)**:1732–40 (2005).
112. J.W. Simpkins, S.H. Yang, R. Liu, E. Perez, Z.Y. Cai, D.F. Covey, P.S. Green. Estrogen-like compounds for ischemic neuroprotection. *Stroke* **35(11)**:2648–51 (2004).
113. T. Akiyama, J. Ishida, S. Nakagawa, H. Ogawara, S. Watanabe, N. Itoh, M. Shibuya, Y. Fukami. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J. Biol. Chem.* **262(12)**: 5592–95 (1987).
114. J.D. Sweatt. The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J. Neurochem.* **76(1)**:1–10 (2001).
115. R.Q. Huang, M.J. Fang, G.H. Dillon. The tyrosine kinase inhibitor genistein directly inhibits GABA A receptors. *Brain Res. Mol. Brain Res.* **67(1)**:177–183 (1999).
116. P. Foti, D. Erba, P. Riso, A. Spadafranca, F. Criscuoli, G. Testolin. Comparison between daidzein and genistein antioxidant activity in primary and cancer lymphocytes. *Ach. Biochem.Biophy.* **43(2)**:421–7 (2005).
117. T.D. Lund, D.E. Fleming, J.R. Dayton, D.E. Lephart, D.L. Salyer. Dietary soy phytoestrogens effects on retinal thickness in rats. *Nutr. Neurosci.* **6 (1)**:47–51 (2003).
118. S.P. Pereira, S.V. Medina, E.G. Araujo. Cholinergic activity modulates the survival of retinal ganglion cells in culture: the role of M_1 muscarinic receptors. *Int. J. Developmental Neurosci.* **19(6)**, 559–567 (2001).
119. G. Lutty, J. Grunwald, A.B. Majji, M. Uyama, S. Yoneya. Changes in choriocapillaris and retinal pigment epithelium in age-related macular degeneration. *Mol. Vis.* **5(1)**: 35 (1999).
120. J.E. Thigpen, K.D.R. Setchell, K.B. Ahlmark, J. Locklear, T. Spahr, G.F. Caviness, M.F. Goelz, J.K. Haseman, R.R. Newbold, D.B. Forsythe, "Phytoestrogen content of purified,open- and closed-formula laboratory diets", *Lab. Animal Sci.* **49(3)**, 530–536. (1999).
121. D.L. Salyer, T.D. Lund, D.E. Fleming, E.D. Lephart, T.L. Horvath. Sexual dimorphism and aromatase in the rat retina. *Brain Res. Developmental Brain Res.* **126(1)**: 131–6 (2001).
122. E.D. Lephart, H. Adlercreutz, T.D. Lund. Dietary soy phytoestrogen effects on brain structure and aromatase in Long-Evans rats, *Neuroreport* **12(16)**: 3451–55 (2001).
123. B. Styrt, B. Sugarman. Estrogens and infection. *Rev. Inf. Diseases* **13(6)**:1139–50 (1991).
124. A. Friedman, Y. Waksman. Sex hormones and autoimmunity. *Israel J. Med. Sci.* **33(4)**:254–7 (1997).
125. S. Ansar Ahmed, N. Talal. Sex hormones and autoimmune rheumatic disorders. *British Med. J* **18(2)**: 69–76 (1989).
126. T. Akiyama, J. Ishida, S. Nakagawa, H. Ogawara, S. Watanabe, N. Itoh, M. Shibuya, Y. Fukami. Genistein, a specific inhibitor of tyrosine specific protein kinases. *J. Biol. Chem.* **262(12)**: 5592–95 (1987).
127. M.C. Martini, B.B. Dancisak, C.J. Haggans, W. Thomas, J.L. Slavin. Effects of soy intake on sex hormone metabolism in premenopausal women. *Nutr. Can.- an Int. j.* **34(1)**:133–9 (1999).
128. E.M. Curran, B. M.Judy, L.G. Newton, D.B. Lubahn, G.E. Rottinghaus, R.S. Macdonald, C. Franklin, D.M. Estes. Dietary soy phytoestrogens and ER a signalling modulate interferon gamma production in response to bacterial infection. *Clin. Exptal Immol.* **135(2)**: 219–25 (2004).
129. R.Q. Zhang, Y.P. Li, W.Q. Wang. Enhancement of immune function in mice fed high doses of soy daidzein. *Nutr.Can.- an Int. j.* **29(1)**:24–8 (1997).
130. R. Erkkola. Female menopause, hormone replacement therapy, and cognitive processes. *Maturitas* **23(May)**: S27-30 (1996).
131. W.M. Keung. Biochemical studies of a new class of alcohol dehydrogenase inhibitors from *radix puerariae*. *Alcoholism – Clin. Exptal Res.* **17(6):** 1254-60 (1993).
132. W.M. Keung, B.L. Vallee. Daidzin: a potent selective inhibitor of human mitochondria1 aldehyde dehydrogenase. *Proceed. National Acad. Sci. USA* **90(4):** 1247-51 (1993).
133. C.I. Xie, R.C. Lin, V. Antony. Daidzin, an antioxidant isoflavonoid, decreases blood-alcohol levels and shortens sleep time induced by ethanol intoxication. *Alcoholism – Clin. Exptal. Res.* **18(6):** 1443-47 (1994).
134. W.M. Keung, A.A. Klyosou, B.L. Vallee. Daidzin inhibits mitochondria1 aldehyde dehydrogenase and suppresses ethanol intake of Syrian golden hamsters. *Proceed. National Acad. Sci. USA* **94(5):** 1675-79 (1997).