An insight on genistein as potential pharmacological and therapeutic agent

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ABSTRACT

Genistein recognized as phytoestrogens is one of the most extensively studied isoflavones. It comprises of significant portion of Asian diet including Japanese and Chinese cuisine in the form of Soy food products. Evidence showed that geinstein increases osteoblasts formation as well as decreases osteoclast production. It plays an important role in immunity; such as suppression of delayed hypersensitivity and increases host resistance to B16F10 tumor by proliferating cytotoxic T and NK cells. It also decreases the activity of lipoprotein lipase which in turn inhibits lipogenesis and prevents the uptake of glucose in type 2 diabetic in rats. Geinstein play important role in reproductive system where it regulates the productive of oestrogen and progesterone. Moreover Geinstein has the ability to inhibit the tumor and cancer cell proliferation. Numerous beneficial effect of Geinstein including cancer treatment and function in immunity, obesity, diabetes and reproductivity Geinstein proves the potentiality of phytoestrogens as a source of bioactive substance.

1. Introduction

Ethnopharmacological tradition followed by pharmacognosy and aided by modern analytical techniques provided numerous examples of plant derived compounds which exhibit selective toxicity or distinct biological activity. Secondary plant metabolites not only provided the foundation of folk medicine and generations of traditional drugs, but also continue to be an inspiration for studies towards modern medicinal applications1.

Isoflavones constitute a sub-class of flavonoids is a large family of secondary plant metabolites, shares common structural feature: a C6–C3–C6 sequence of the carbon skeleton, which split into several variants of heterocyclic ring substitution pattern and diversified by a plethora of further modifications2.

Genistein (4,5,7-trihydroxyisoflavone) is one of the most extensively studied isoflavones, are generally recognized as phytoestrogens. In fact, they can exert estrogenic as well as anti-estrogenic action, which is sometimes connected with animal reproduction problems3. It has been identified as the predominant isoflavone in soybean enriched foods which comprises a significant portion of the Asian diet, and provides 10% of the total per capita protein intake in Japan and China4. Individuals who consume soy as a dietary staple have elevated blood concentrations of genistein when compared to those who consume a Western style red meat based diet. This difference in genistein concentrations has been associated with differential outcomes in a variety of clinical contexts including cholesterol regulation, osteoporosis, and cancer. Interest about genistein as a potential therapeutic agent has recently risen in the field of oncology as population based studies have shown; genistein consumption decrease risk of mortality from several types of cancer, most notably prostate and breast cancer5.

We attempted herein to summarize the effects of genistein in bone metabolism, immunity, obesity, diabetes, reproductive process, cancer cell proliferation and genetic abnormalities underlying the chemopreventive and therapeutic actions of genistein.

Genistein and Bone Metabolism

Bone metabolism is regulated by osteoblasts and osteoclasts localize in bone tissues. Osteoblasts stimulate
bone formation and calcification while osteoclasts promote bone resorption. The anabolic effects of genistein on bone metabolism have been investigated in tissue culture using the femoral metaphyseal (trabecular bone) tissues obtained from elderly female rats in vitro[6]. The presence of genistein (10^{-6} or 10^{-5} M) was found to induce a significant increase in calcium content, alkaline phosphatase activity, which is a marker enzyme of osteoblasts and DNA content, which is an index of bone cell number in bone tissues, in rat femoral metaphyseal (trabecular bone) tissues. Anti–estrogen agent tamoxifen was shown to inhibit completely the genistein induced increase in bone components, although tamoxifen itself had no effect on bone components[6]. The anabolic effects of genistein on bone metabolism may be partly mediated through binding of genistein to the receptor of estrogen in osteoblastic cells and resulting from newly synthesized protein components. Phosphogenistein also increases bone components in tissue culture in vitro. But the anabolic effect of genistein on bone calcification is weakened by the phosphorylation[8].

Impressive data from the many studies of cultured bone cells and rat models of postmenopausal osteoporosis support a significant bone sparing effect of the soy isoflavone genistein[7]. Genistein have been found to have a stimulatory effect in protein synthesis and alkaline phosphatase release by various types of osteoblast cells in vitro[8]. This effect is blocked by adding actinomycin or cycloheximide, suggesting isoflavones influence transcriptional or translational events. Osteoprotegerin (OPG), a member of the tumor necrosis factor receptor superfamily, prevents bone resorption by a paracrine mechanism[9]. It is now apparent that osteoclast activity is modulated through osteoblasts via OPG. The cytokine receptor/activator of nuclear factor–K (RANKL) [10] stimulates osteoclast differentiation and function with higher levels of RANKL expression leading to increased bone resorption. OPG is a ligand for this cytokine and blocks its expression. Ovariectomy, or the pure antiestrogenICI 182 780 decreases, and estrogen increases expression of OPG mRNA and protein by human fetal osteoblastic cell line (hFOB/ER–9) transfected with estrogen receptors (ER) α[11]. More recently, genistein has been found to stimulate the production of osteoprotegerin by human paracrine osteoblasts, providing a further mechanism for the bone–sparing effects of soy isoflavones.

Parathyroid hormone (PTH), prostaglandin E2 (PGE) and lipopolysaccharide (LPS) have stimulatory effects in bone resorption in in vitro culture system[2]. The effects of bone resorbing factors were completely inhibited in the presence of genistein, indicating inhibition of bone resorption. The inhibitory effects of genistein on bone resorption are partly related to the prevention of lactic acid production by bone tissues[6]. Osteoclasts or bone resorbing cells, are formed from bone marrow[13]. In vitro presence of PTH, PGE, LPS or 1,25–dihydroxyvitamin D induce a remarkable increase in osteoclast like multinucleated cells. These increases were significantly inhibited in the presence of genistein[6]. The cellular mechanism by which genistein inhibits osteoclast like cell formation from marrow cells may be involved in cyclic AMP signaling. Genistein was also found to induce cell death (apoptosis) of osteoclasts isolated from rat femoral tissues[6]. Genistein may partly induce apoptosis of osteoclasts through a mechanism that inhibits protein tyrosine kinases in the cells. It has been shown that tyrosine kinase Src is implicated in the process of osteoclast induced bone resorption in vitro and in vivo[14]. Genistein can directly activate protein tyrosine phosphatase in osteoclasts. It has been reported that protein tyrosine phosphatase (Src homology 2 domain containing tyrosine phosphatase) is a negative regulator of osteoclastogenesis and osteoclast–resorbing activity in mutant mice[14]. Presumably, the suppressive effect of genistein on mature osteoclasts is partly mediated through the activation of protein tyrosine phosphatase in the cells. Genistein also may be involved in apoptosis of osteoclasts through the Ca^{2+} mediated signal pathway[15].

Genistein suppress osteoclast activity by a number of possible mechanisms, including induction of apoptosis, activation of protein tyrosine phosphatase, inhibition of cytokines, changes in intracellular Ca^{2+} and membrane depolarization, highlighting the level of complexity in the mechanism of estrogens and phytoestrogens in bone turnover[16,17].

There is ample of evidence from the animal model studies for the effectiveness of isoflavones in conserving bone. Blair et al., (1996) were the first to test pure genistein added to the diet, as opposed to an isoflavone–rich soy protein, in ovariectomized Sprague–Dawley rats and found that it increased bone mineral density (BMD) by 12% over a 30–d period following surgery[18]. This observation was subsequently confirmed by others working with the pure isoflavones and dose–response effects was noted for genistin[7,18]. A low dose of genistein (0.5 mg/d) was considerably more effective than higher doses (> 1.6 mg/d) and comparable to Premarin’s effects on bone in a lactating, ovariectomized and calcium–stressed rat modell[7]. Other finding showed that delaying administration of genistein until long after ovariectomy was less effective in conserving bone than if it was given immediately on loss of ovarian estrogen[7].

The combination of genistein and zine was found to have a synergistic effect in bone components in femoral tissue from elderly female rats in vitro and in vivo[19]. Also the combination of genistein and casein phosphopeptides (CPP) in dietary supplementation may be a good tool for the prevention of bone loss in aging[20].

2. Genistein and Immunity

Genistein is one of the most extensively studied isoflavones for its effect of immunity. A relatively high concentration of genistein inhibits lymphocyte proliferation response induced by mitogen or alloantigen in vitro[21]. The tyrosine kinase signaling cascade plays a pivotal role in the activation of various inflammatory cells. Genistein is known to be an inhibitor of protein tyrosine kinase, and its activity may contribute to the suppressive effect in vitro. However, genistein inhibits tyrosine kinases only at supraphysiologic concentrations (typically 100 μmol/L), which are many fold greater than concentrations that can be obtained through consumption of dietary genistein or even through the use of supplements containing genistein[22].

A number of recent studies addressed the potential effects of genistein in vivo in immune system, especially the thymus. The thymus is largest during fetal and early
postnatal life and developmental toxicological insults have the greatest potential to depress immune function and/or increase susceptibility to autoimmune disease[22]. Genistein decreases thymocyte numbers by up to 86% and doubles apoptosis. Increased apoptosis is involved in the mechanism by which genistein causes loss of thymocyte. Administration of genistein to mice caused decreases percentages of thymic CD4⁺CD8⁺ and double positive CD4⁺CD8⁺ thymocytes, providing evidence that genistein may affect early thymocyte maturation and maturation of CD4⁺CD8⁺ helper T cells. Treatment of genistein administered mice with anti—estrogen ICI 182,780 partially restored thymic weight. Therefore, the effect of genistein on thymic weight is mediated in part by the estrogen receptor. Genistein reduces the numbers of peripheral CD4⁺ and CD8⁺ T cells, and this reduction might come from thymic atrophy[23]. In case of thymic gene expression several 17β—estradiol (E2) responsive genes involved in thymic development and thymocyte signaling during selection and maturation, E2 up regulated more genes than genistein, whereas genistein down regulated more genes than E2. Although each treatment regulated several genes not altered by the other, there was substantial overlap in the genes regulated by E2 and genistein. Changes in transcription factors and cell cycle factors were consistent with decreases in cell proliferation induced by both genistein and E2[22].

Delayed type hypersensitivity (DTH) reaction is classified as type IV allergy response and is mainly mediated by T cells and macrophages. Genistein suppresses DTH reaction to oxazolone and granulocyte mediated response[24]. In addition to cellular immune response, genistein also suppresses antigen (Ag) induced antibody (Ab) production. In ovalbumin (OVA) immunized mice, genistein suppresses OVA specific Immunoglobulin G (IgG) levels. Interestingly, an inhibitory effect of genistein on Ab production was not observed when thymus independent Ag TNP ficoll was used [25], suggesting that the suppressive effect of genistein on Ag specific Ab response is not a result of a direct inhibitory effect on B cells. In addition, genistein did not affect the expression of major histocompatibility complex (MHC) class II, CD80 and CD86 and the Ag presenting capacity of CD11c+ dendritic cells[25]. Although genistein inhibits OVA specific T cell proliferation and cytokine responses, production of Interferon gamma (IFN-γ) and Interleukin 4 (IL-4) from T cells of genistein treated mice is increased upon stimulation with anti-CD3 mAb[25,26].

It has been reported that genistein increased host resistance to B16F10 tumor and induced a dose dependent increase in cytotoxic T cell and natural killer (NK) cell activities[27]. However, genistein did not inhibit growth of tumor cells in athymic nude mice[28]. These conflicting findings in euthymic and athymic mice suggest that genistein inhibits growth of a tumor not by direct inhibition but by enhancing immune function. The finding support that tumor cells cultured with serum from genistein treated mice did not suppress their growth ability suggests the speculation that genistein enhances antitumor immunity [21]. At high concentrations in vitro genistein also inhibits cytotoxic T cell mediated tumoricidal activity, alters leukocyte adherence, impairs T cell motility and inhibits the activation of NK cells in response to lipopolysaccharide or fixed bacteria[22]. However, studies showing genistein effects in immune cells or functions in vitro have typically used genistein concentrations higher than that would be obtained in vivo; thus, it is unclear whether similar effects occur at physiologic concentrations of genistein in vivo.

NC/Nga mice have been shown to develop spontaneous severe dermatitis when kept in conventional conditions[29]. Oral administration of genistein suppresses the development of dermatitis but does not suppress serum immunoglobulin E (IgE) levels in NC/Nga mice. The mechanism underlying the suppressive effect of genistein for the development of dermatitis is not known, but little contribution of Thl/Th2 (T helper cell 1/2) balance has been reported[26].

Allergic asthma is a chronic airway inflammatory disease that manifests itself as recurrent reversible acute bronchoconstriction and airway hyperresponsiveness (AHR). Duan et al., (2003) examined anti—inflammatory effects of genistein in guinea pig model of asthma[30]. Genistein markedly inhibited OVA induced and methacholin—induced acute bronchoconstriction. In addition, genistein reduced OVA induced increases in total cell counts and eosinophils recovered in bronchoalveolar lavage fluid, and attenuated OVA induced airway hyperrehyper responsiveness to inhaled methacholine. The authors speculated that the inhibitory effect of genistein on AHR is attributed to the block of protein tyrosine kinase signaling cascades.

Estrogen receptor dependent and independent mechanisms have been proposed for the immune modulating effect of genistein since genistein is structurally similar to estrogen. Indeed, expression of the estrogen receptor in thymocytes, lymphocytes and macrophages has been reported[21]. Thymus expresses both ERα and ERβ and normal thymic development is dependent in E2/ERα signaling pathway, as shown by reduced thymic size in neonatal mice lacking ERα[22]. Estrogen is known to suppress the activity of immune cells and to suppress the development of DTH reaction[31]. CIA induced arthritis[32] and experimental autoimmune encephalomyelitis[33] in animal models. It is possible that genistein has estrogen like action and modulates immune function mediated by the estrogen receptor. However, several studies have shown that blockade of the estrogen receptor pathway partially abolishes the action of genistein. Genistein is known to be a broad spectrum protein tyrosine kinase inhibitor[34], and its activity may contribute to one of the estrogen receptor independent mechanisms. In vitro experiments have shown that genistein at a dose of 100 μmol/L inhibits both tyrosine phosphorylation and binding of the nuclear factor to the specific promoter region, resulting in inhibition of proliferation response and cytokine production. Genistein decreases nitric oxide production in cultured mouse macrophages, suggesting that it could modulate immune responses[22]. In addition, genistein at high doses inhibited the proliferation of cultured T cells in response to CD28 monoclonal antibody, and also inhibited production of leukotriene B4, interleukins, and the interleukin receptor [22]. Curran et al., (2004) reported that dietary genistein or soy could inhibit the amount of IFN—γ normally produced in response to a bacterial infection in mice both in juvenile and aged animals[35,36]. This study is especially noteworthy in that these effects were obtained with dietary genistein or soy supplementation that produced circulating serum genistein concentrations of 0.4 and 1 μmol/L. Genistein
injections typically produced the greatest effects, but effects were also detectable when genistein was given in the diet at levels that produced serum genistein concentrations that can be obtained in humans, raising the concern that high levels of genistein exposure in humans may at least have the potential to produce immune changes[22].

However higher genistein concentrations which are effective in vitro to suppress immune system and may not have great significance in vivo, may be beneficial or desirable in certain situations (e.g., autoimmune diseases or after organ grafting). For example, O’Connor et al., (2002) showed that a high isoflavone diet or i.v. genistein produced immune inhibition, as reflected by delayed rejection of cardiac allografts in rats[37]. The effects of genistein in organ rejection were also additive with those produced by cyclosporine, the drug used for suppressing rejection in transplant recipients.

3. Genistein and Obesity

There are two types of fat depots: brown adipose tissue (BAT) and white adipose tissue (WAT); only WAT is important in terms of obesity. Excess fat consumption can stimulate enlargement of existing adipocytes (adipocyte hypertrophy) and induce differentiation of dormant preadipocytes in the adipose tissue into mature adipocytes (adipocyte hyperplasia) to accommodate the demand for extra storage[38]. Hormones, including estrogen, growth hormone, thyroid hormone, glucocorticoids, catecholamines, glucagons, insulin, and insulin-like growth factor are regulators of adipogenesis[39]. 17β-Estradiol (E2), the most ubiquitous estrogen, is a major regulator of adipocyte development and adipocyte number in females and males[40]. Binding of E2 to ERs inhibits lipogenesis primarily through decreasing activity of lipoprotein lipase (LPL), an enzyme that regulates lipid uptake by adipocytes[41] and the isoflavone genistein has recently been shown to cause decreases in LPL mRNA in adipose tissue with concomitant decreases in lipid filling of adipocytes[42,43]. Peroxisome proliferator-activated receptor (PPAR) families exist in three subclasses: PPARα, PPARβ/δ, and PPARγ. PPARα is important for β-oxidation of fatty acids and is mainly expressed in tissues such as liver, kidney, heart, and muscle, where lipoprotein metabolism is important. Little is known about PPARβ/δ, but it has been shown that PPARβ/δ activation stimulates fatty acid oxidation and loss of adipose mass in genetically obese mice[44]. PPARγ is mainly expressed in adipose tissue and is considered the master regulator of adipogenesis. It is also involved in lipid storage and glucose and cholesterol metabolism[45]. Three different isoforms of PPARγ are known: PPARγ1, which is the form expressed in virtually all tissues; PPARγ2, which is specific to adipose tissue; and PPARγ3, which is expressed in macrophages, large intestine, and WAT. The expression of PPARγ2 and LPL is considered to be an early marker of adipocytes[46]. It has recently been shown, that genistein can bind directly to and activate both PPARα[47] and PPARγ[48,49], and a study by Harmon et al., (2002) showed that genistein can also down regulate the expression of PPARγ and CCAAT/enhancer binding protein α (C/EBPα) in 3T3–L1 cells by inhibiting C/EBPβ activity[50].

Adipocytes and osteoblasts arise from the same bone marrow mesenchymal precursor cells[46], different strains of osteoprogenitor cells from bone marrow are commonly used to investigate the regulation of differentiation to adipocytes. Experiments involving mouse bone marrow cells and KS483 osteoprogenitor cells cloned from mouse calvaria have demonstrated that E2 through the ER regulates two key genes for osteogenic and adipogenic commitment: the gene encoding the core–binding factor α-1 (Cbfa–1) and the gene encoding PPARγ2, respectively[51]. Interestingly, in human bone marrow cells, genistein has also been demonstrated to be a regulator of these lineage determining genes[43]. The results showed that 0.1 to 10 μM genistein stimulated osteogenesis with a maximum effect at 1 μM, whereas concentrations of 25 to 50 μM inhibited osteogenesis. At the same time 0.1 to 1 μM genistein inhibited adipogenesis, whereas concentrations of 10 to 50 μM increasingly stimulated adipogenesis. Although it may not be physiologically relevant, this finding indicated that, at a concentration of approximately 10 μM, the effect of genistein switches from estrogenic to antiestrogenic, and a PPARγ binding assay suggested that the antiestrogenic effect of genistein is due to a direct activation of PPARγ2, leading to the downregulation of ER mediated transcriptional activity and osteogenesis. This is consistent with other results that showed that genistein is a ligand for PPARγ[52] and that activation of PPARγ downregulates osteogenesis and upregulates adipogenesis[53]. Further investigations have also revealed that a direct interaction of genistein with PPARα plays an important role in adipogenesis[47,52]. The important role of the ER in the effect of genistein was supported when genistein failed to reduce adipose weight in ERα knockout (αERKO) mice[42]. On the basis of results from experiments involving ERβ knockout (βERKO) mice, another group recently suggested that the antilipogenic action of genistein and downregulation of adipogenic genes require the expression of ERβ[54]. These results indicated that interaction and cooperation between ERα and ERβ in the downregulation of estrogen dependent genes is crucial for the regulation of adipose tissue. In addition to the ability of isoflavones to interact with ERs and PPARs, genistein in high concentrations is a potent inhibitor of protein tyrosine kinase, DNA topoisomerases 1 and 2, and ribosomal S6 kinase. Studies of 3T3–L1 cells have strongly suggested that genistein concentrations of 50 μM and higher inhibit proliferation of preconfluent and postconfluent preadipocytes and mediate the inhibition of adipocyte differentiation by mechanisms that involve the decreased expression of PPARγ and the activation of the intracellular AMP activated kinase (AMPK) signaling cascades[55]. One experiment demonstrated that genistein concentrations of 10, 100, and 1000 μM significantly restricted lipogenesis and contemporarily induced lipolysis at the 100 and 1000 μM concentrations, whereas E2 at the same concentrations did not have any significant effects[56]. Furthermore, lipogenesis was inhibited when glucose and acetate were used as substrates. This result suggests not only that genistein not only obstructs glucose uptake by inhibiting glucose transporter 1 (GLUT1) and glucose transporter 4 (GLUT4) but also that this phytoestrogen is capable of inhibiting pathways of lipogenesis after acetyl-CoA formation[56].

Induction of apoptosis may contribute to the reducing...
effect of isoflavones on adipose tissue. As little as 10 μM genistein significantly increases apoptosis of mature adipocytes after 24 hrs but not of preadipocytes. Recent results have demonstrated that genistein also induces apoptosis of HepG2 cells. As HepG2 cells do not express ERs, this result is consistent with those in other reports that genistein has multiple effects and induces apoptosis by mechanisms that do not involve ERs. Recent reported that a dose of 1500 mg genistein per kilogram diet increases DNA fragmentation in the ING fat pad by 290% in ovariectomized adult mice, a result that further confirms that adipose apoptosis contributes to the genistein-mediated reduction of adipose tissue. DNA fragmentation was not detected in the retroperitoneal (RP) and PM fat pads; this absence suggests that apoptosis by genistein may be fat depot-specific. This hypothesis has also been supported by results of studies of human and rat preadipocytes.

Isoflavones are subject to extensive first-pass clearance by the liver; therefore, it is possible that hepatocytes in vivo may be exposed to isoflavone concentrations higher than 10 μM. This hypothesis is interesting because a recent study reports that 10 μM genistein activates PPARα, and subsequently induces expression of PPARα target genes involved in both mitochondrial and peroxisomal fatty acid β-oxidation in HepG2 cells. These results suggest that genistein treatment increases energy expenditure through enhanced fatty acid catabolism in liver cells, which may contribute to a beneficial effect of isoflavones on hyperlipidemia in obese animals and humans. The liver is also the major site of LDL cholesterol synthesis, and is closely associated with hyperlipidemia, cardiovascular disease and obesity. Of 29 metabolism related genes altered by the high fat diet (HFD), the expression of 21 genes was normalized or reversed by genistein supplementation. This result suggests that the hypolipidic effect of genistein could be ascribed in part to the upregulation of genes involved in fatty acid catabolism in liver. Others have reported that genistein supplementation can decrease triglyceride, total cholesterol and LDL cholesterol levels in the serum and liver of mice. It has been reported that 50 and 100 μM of genistein, through multiple mechanisms that include inhibition of cholesterol synthesis and esterification, significantly decrease HepG2 secretion of apolipoprotein B, the primary lipoprotein of LDL particles. Furthermore, genistein increase LDL receptor expression and activity, which is in agreement with results from other recent reports. 20 μM genistein increase the abundance of the mature form of SREBP-2 and the expression of sterol regulatory element (SRE) regulated genes in HepG2 cells, and these increases in turn result in increases in the surface LDL receptor expression; thus, plasma cholesterol levels decrease.

Another factor that seems to be very important is the difference between sexes. This is supported by the fact that the pharmacokinetics of genistein have been reported to be faster in males that in females. In an experiment, genistein was demonstrated to increase epididymal and renal fat pads and adipocyte size at daily oral doses up to 50 mg/kg body weight in male mice, whereas this effect was not seen in females. The results may be relevant to the sex specific regulation of adipose development, deposition, function and metabolism during growth and adulthood.

Dietary genistein at 1000 mg/kg diet does not affect body weight. Other study also found that body weights in ovariectomized mice were not affected by a diet containing 300 to 1000 mg genistein per kilogram. Other experiments in rats have demonstrated that dietary isoflavones significantly decrease body weight and adipose tissue.

4. Genistein and Diabetes

Soybean and its components have beneficial effects on lipid concentrations in healthy and type 2 diabetic subjects. However, it was not clear whether this beneficial effect of lipids was due to soy protein, isoflavones or cotyledon fiber, because high-fiber diets are known to have beneficial effects in lipid metabolism. Soy isoflavones may be beneficial for diabetic subjects because of their estrogenic activity and their ability to prevent glucose induced lipid peroxidation and inhibit intestinal glucose uptake by decreasing sodium dependent glucose transporter, which results in a reduction in postprandial hyperglycemia. In vitro studies have shown that a soybean phytochemical containing the isoflavones genistein inhibits glucose uptake into rabbit intestinal brush border membrane vesicles in a dose dependent manner and also protects against glucose induced oxidation of human LDL. Experimental evidence suggests that protein tyrosine kinases play a permissive role in the regulation of insulin secretion from pancreatic β cells. Several in vitro studies using the isoflavone genistein as a protein tyrosine kinase inhibitor have shown that this compound exerts multiple actions releasing insulin from pancreatic islet cells. For example, in cultured islets of Langerhans, genistein (at a concentration of 100 μmol/L) was shown to increase basal insulin secretion, but this dose of genistein also reduced islet cell proliferation. In additional studies shows that genistein was inhibited islet tyrosine kinase activities and glucose and sulfonlurea stimulated insulin release without affecting glucose metabolism. However, other groups have reported that genistein inhibits glucose stimulated insulin secretion. Genistein was also reported to decrease the number of high-affinity insulin receptors in the livers of ovariectomized rats. Similarly, incubation of isolated rat adipocytes with increasing doses of genistein (0.01, 0.3, 0.6, and 1 mmol/L) resulted in inhibition of glucose conversion to total lipids both absence and presence of insulin.

5. Action in reproductive processes

Observations from 1940 to 1970 reported that the ingestion of high levels of phytoestrogens by animals led to relatively constant adverse effects on reproduction which were more marked in females than males. The consumption of high
levels of genistein, daidzein and coumestrol led to infertility in sheep, cattle and rodent. Direction and degree of genistein in vivo effects appear to be dependent on species, reproductive status of a subject, individual phytoestrogen metabolism and even a blood sampling schedule[76]. In vitro studies concerning phytoestrogen action on steroidogenesis were performed mostly in granulosa cells. Genistein was found to inhibit basal and stimulated progesterone (P4) production by human, rat and bovine granulosa cells as well as porcine theca and luteal cells[77]. The P4 inhibition was demonstrated for genistein concentration ranging from 0.5 to 100 μM. A biphasic mode of genistein action was observed in bovine (185 – 1850 nmol/l) and rat (0.3 μM – 100 μM[76] granulosa cells. These authors found that low concentrations of genistein stimulated and high concentrations inhibited P4 production. In contrast, Makarevich et al. (1997) demonstrated that genistein (37 nM – 37 μM) stimulated P4 production by bovine and rabbit granulosa cells[76]. Estradiol production was either stimulated (rabbit granulosa cells[76] or inhibited (rat and human granulosa cells[78]) by genistein. In pigs, genistein was found to stimulate E2 production by whole follicles[76] and inhibited P4 production by theca and luteal cells[79]. Genistein and daidzein did not affect basal and LH stimulated E2 production. However genistein—stimulated basal production of E2 by granulosa cells originated from GRO follicles[80]. Estrogen receptor β is a predominant ER in granulosa cells of humans, rats and pigs[26]. Preliminary data indicated that genistein decreased the amount of ERβ mRNA in granulosa cells from GRO porcine follicles[76]. And this may be the mechanism by which genistein exert its effect in granulosa cells.

Harrison et al. (1999) demonstrated that dietary genistein increased E2 level during advanced pregnancy and delivery in pregnant thesus monkeys without altering the P4 level[81]. Dietary genistein can also modify the expression of the estrogen–regulated P4 receptor in the uterus during pregnancy and lactation in rats and, consequently, may have long-term reproductive health consequences[82]. Pregnancy demands motherly adaptation of metabolism and endocrine system suitably for the developing fetus. This adaptation may be disturbed by dietary factors which influence hormone and metabolic status[76]. In an experiment genistein was administered in the diet of Wistar rats throughout the entire pregnancy. Females were divided into two groups: 1/ genistein–fed rats (100 mg/kg of feed), and 2/controls with no genistein in diet. Genistein did not change the serum insulin concentration in pregnant rats[83]. The results of semiquantitative RT–PCR analysis and western blotting also indicated a lack of significant effects of dietary genistein action on insulin receptor expression in liver, adrenal and thyroid gland during pregnancy[76]. During late pregnancy and on the first day after delivery, serum leptin (regulator of food intake, energy expenditure and is also involved in the regulation of reproductive processes) concentration was reduced in genistein treated rats in spite of non altered serum insulin level[83]. Progesterone, an important gestation hormone is also an endogenous substrate for 21–hydroxylase in corticosterone synthesis. Genistein as a competitive inhibitor of 21–hydroxylase in vitro[84] is known to decrease adrenal steroidogenesis. During pregnancy, however, genistein did not manifest its inhibitory action on serum corticosterone concentration[83] probably due to the higher concentration of P4. Adrenocorticotrophic hormone (ACTH) level in pregnant rats was also not affected by genistein.

Exposure to dietary genistein during gestation and lactation of female rats resulted in a lower plasma T concentration in their adult male offspring[85]. The aberrant or delayed spermatogenesis was observed in male rats exposed to dietary genistein during their development[86]. On the other hand, neither serum testosterone (T) concentration nor sperm counts in male rats were changed by neonatal oral administration of genistein[87]. Genistein showed the strong effect on testicular secretion[88]. Genistein decreased T secretion by Leydig cell in roosters. Moreover, long-term dietary administration of genistein inhibited Leydig cell steroidogenesis ex vivo[89], but without altering the serum level of either LH or T.

Human fetuses and infants can be exposed to genistein during critical periods of development through soy consumption of mothers during pregnancy and lactation and through soy based infant formulas and other soy products that children consume. Recent studies have shown that developmental exposure to genistein can cause alterations in the development of the female reproductive tract of the rodent, including altered estrous cyclicity, altered ovarian function, subfertility and infertility[90]. The effects of genistein on the developing ovary following neonatal exposure at doses of 0.5, 5, and 50 mg kg−1 day−1 included the presence of multi–oocyte follicles (MOFs) similar to those reported following neonatal exposure to E2 or diethylstilbestrol (DES)[80,91]. Genistein alters ovarian differentiation during neonatal development. Ovaries from neonatal mice treated with genistein have more oocytes not enclosed in follicles, more oocytes persisting in nests, and retention of oocyte intercellular bridges. Retention of intercellular bridges between oocytes also demonstrates that genistein inhibits oocyte nest breakdown in neonatal mice[92]. In addition, neonatal genistein treatment influenced oocyte survival as shown by decreased oocyte apoptosis and increased oocyte numbers[90]. Other studies demonstrated that neonatal injections or oral administration of genistein altered adult estrous cycles of mice and rats[93]. Neonatal genistein treatment caused increased relative uterine weight and down–regulation of progesterone receptor in uterine epithelia. Estrogenic effects of genistein were also seen in the neonatal ovary and thymus, which had an increase in the incidence of multi–oocyte follicles (MOFs) and a decrease in thymic weight relative to body weight, respectively. The increased incidence of MOFs persisted into adulthood for neonatally treated genistein females and estrous cycle abnormalities were seen at 6 months of age despite normal fertility in these mice[93]. Jefferson et al., (2007) showed ovarian function and estrous cyclicity was disrupted in genistein treated mice with increasing severity over time[94]. Reduced fertility was observed in mice treated with genistein (0.5, 5, or 25 mg/kg) and infertility was observed at 50 mg/kg. Females generated from genistein 25 mg/kg females bred to control males have increased MOFs suggesting these effects can be transmitted to subsequent generations. Thus, neonatal treatment with genistein at environmentally relevant doses caused adverse consequences on reproduction in adulthood in mice.

A continuous exposure to low combined doses of genistein and vinclozolin affects male reproductive health by inducing
reproductive developmental anomalies, alterations in sperm production and quality, and fertility disorders. The low-dose mixture and high-dose vinclozol produced the most significant alterations in adults: decreased sperm counts, reduced sperm motion parameters, decreased litter sizes, and increased postimplantation loss. Testicular mRNA expression profiles for these exposure conditions were strongly correlated. Functional clustering indicated that many of the genes induced belong to the “neuroactive ligand–receptor interactions” family encompassing several hormonally related actors (e.g., follicle–stimulating hormone and its receptor). All exposure conditions decreased the levels of mRNAs involved in ribosome function, indicating probable decreased protein production. The long-term deleterious consequences of chronic exposures to a low-dose mixture of genistein/vinlozolin suggest complex modes of action. Transcriptome analysis may uncover specific factors with potentially major effects on numerous gene targets, such as Cbx3[3,95]. In male mice Gestational exposure to genistein contributes to hypospadias by altering pathways of tissue morphogenesis, cell proliferation and cell survival. In particular, genes in the MAPK and TGF–β signaling pathways and those controlled by FOXO, HOX and ER transcription factors are disrupted[96].

Genistein also have effect on reproductive tissues of ovariectomized gilts. Treatment groups received vehicle, estradiol benzoate (2 mg/d), or genistein (50, 100, 200, or 400 mg/day) via intramuscular injection at 12-h intervals for 10 days. Uterine and cervical tissue mass, as indicated by wet, dry, and protein weights and total DNA content, increased as the dosage of genistein increased. Uterine and cervical wet weights were increased by a dosage of 200 mg of genistein/day but not by 100 mg of genistein/day compared with those of control gilts. Height of epithelial cells lining the uterine glands and the lumen of uterus and cervix increased when gilts were treated with estradiol benzoate or 400 mg of genistein/day. Estrogen–sensitive tissues of the ovariectomized gilt, such as the cervix and uterus, are affected by injection of large dosages of the phytoestrogen genistein[97].

Researchers found that orally administered genistein had no effect on tonic luteinising hormone (LH) values in ovariectomized gilts. Treatment groups received vehicle, the period preceding puberty reduces later susceptibility to develop cancers like breast cancer and prostate cancer[5,99]. Genistein has therefore become one of the most widely studied small-molecule inhibitors of both cancer cell growth and metastasis and has the potential to be of substantial clinical benefit to patients with various types of cancer. Genistein has the ability to inhibit primary tumor growth, regulation of later stages of the metastatic process, including cell adhesion, migration and invasion.

Several reports have demonstrated that genistein can induce cell cycle arrest and that it can therapeutically modulate key regulator cell cycle proteins at concentrations ranging from 5 to 200 μM[100]. It is important to note that these concentrations are greater than the blood levels that are observed with dietary consumption, indicating that this is likely not the primary mechanism by which genistein inhibits metastasis. Although studies have described genistein’s ability to induce cell cycle inhibition, an exact mechanism of action has not been identified. Most studies indicate that genistein potentially targets proteins involved in the G2/M checkpoint, as it has been shown to induce arrest in this checkpoint in breast cancer[5], prostate cancer[13], and other cancers[14].

Scientist has demonstrated that genistein increases human prostate cancer cell adhesion both in vitro and in prostate cancer cells orthotopically implanted into mice[101], breast cancer and melanoma cells[102] in vitro at submicromolar concentrations. This indicates that genistein can act to inhibit cell detachment, which is an early step in the metastatic cascade. Genistein mediated increases in cell adhesion are time and concentration dependent and can be observed at low nanomolar concentrations of genistein. Focal adhesion kinase (FAK) plays a central role in integrin signaling, cell adhesion, migration, and invasion[103]. Increased FAK expression has been reported in several different cancers and can lead to decreased apoptosis and increased cell motility[104,105]. Genistein increases FAK translocation to the focal adhesion complexes[106]. Thus genistein consistently inhibits FAK activation and alter of its effect upon adhesion[106,107]. Studies have shown that genistein treatment can decrease cell migration in rat prostate carcinoma cells in a dose dependent manner, over the nontoxic concentration range of 1 to 10 μM[108]. Over a similar concentration range, genistein can inhibit the migration of murine breast cancer and melanoma cells[102]. Though the exact mechanism by which genistein can alter these processes is not known but studies have also demonstrated that genistein inhibits phosphorylation of FAK on Y387 which is necessary for increases in cell migration[5]. MMP–2, along with MMP–9, makes up the gelatinase family of matrix metalloproteinases (MMPs). MMPs cleave extracellular matrix (ECM) proteins and thereby contributing to a variety of functions involved with normal homeostatic cell movement and tissue remodeling. However, MMPs also play a role in aberrant cell growth and tumor formation, since they provide space for the tumor to grow and release various growth factors that drive tumor proliferation[5]. In addition, MMP–2–deficient mice show decreased rates of cancer progression and angiogenesis in melanoma cells. Huang et al., has shown that genistein is able to decrease MMP–2 expression in a panel of human prostate cancer cell lines ranging from primary noncancerous cells to established metastatic variant cells, although little to no effect on MMP–9 was observed in that study. Effects were observed with genistein concentrations down to 10 nM. Reduction of MMP–2 by genistein treatment has also been confirmed by other groups in prostate cancer, breast cancer, and
cell death known as ‘apoptosis’. The data showed that genistein could induce apoptosis in MDA–MB–231, MDA–MB–435 and MCF–7 breast cancer cells; PC3 and LNCaP prostate cancer cells; H460 and H322 non–small cell lung cancer cells; HN4 head and neck squamous carcinoma cells, and pancreatic cancer cells[4,115,116]. Estrogen receptors (ER) stress, caspase activation, inhibition of proteosome, down–regulation of Bcl–2, Bcl–xL, and HER–2/Neu may partly represent the molecular mechanism by which genistein induces apoptosis, and the existing evidence suggests that many of these cascades may also be regulated either directly or indirectly by nuclear factor–κB (NF–κB). Genistein has also been found to potentiate the antitumor activity of chemotherapeutic agents through regulation of NF–κB[4]. Akt signaling is another important transduction pathway that plays a critical role in controlling the balance between cell survival and apoptosis. Evidence suggests that Akt also regulates the NF–κB pathway via phosphorylation and activation of molecules in the NF–κB pathway[117]. Thus, strategies to block the activity of Akt would ideally lead to the inhibition of proliferation and the induction of apoptosis. Study data demonstrates that genistein inhibits the activation of Akt, which may result in the inhibition of survival signals ultimately leading to induction of apoptotic signals[118]. In addition to inhibiting cellular proliferation, genistein also increases the rate of cancer cell death. It can induce apoptosis in a variety of epithelial cancers[4,100]. These proapoptotic effects occur at genistein concentrations of 10–200 μM which is higher than the measured blood concentrations of high–soy consumers suggesting that genistein’s ability to inhibit apoptosis may not be the primary mechanism by which it regulates metastasis[5]. Although the exact molecular mechanism of genistein’s effect on apoptosis has not been identified, most studies indicate that genistein acts by altering the expression of the various proapoptotic and antiapoptotic proteins. Specifically, Bcl–xL, an antiapoptotic protein, has been shown to be decreased in breast cancer, prostate cancer, and other cancers upon treatment with genistein. Conversely, proapoptotic proteins Bax, caspase 3 and caspase 9 have all been shown to increase in vitro with genistein treatment[119,120].

The activation of telomerase is crucial for cells to gain immortality and proliferation ability. In prostate cancer cells, genistein plays role in the regulation of telomerase activity and inhibition cell proliferation[121]. Reverse transcriptase PCR and hTERT promoter activity assays showed that genistein decreased hTERT expression and transcriptional activity dose–dependently. Using various deleted hTERT promoter constructs, it was observed that the hTERT core promoter is enough to observe the genistein–induced repression of hTERT transcriptional activity. Because c–Myc is involved in transcriptional regulation of hTERT, c–Myc expression was also examined. A dose–dependent decrease in c–Myc message and proteins was observed with genistein treatment. These results indicate that genistein represses hTERT transcriptional activity via the down–regulation of c–Myc expression[122]. However, genistein–induced repression of hTERT transcriptional activity was not blocked by the mutation of c–Myc at the hTERT promoter, suggesting that additional factors are involved in genistein–dependent repression of telomerase activity. Interestingly,
genistein down—regulates the activation of Akt thereby phosphorilation of hTERT and inhibits its translocation to the nucleus. These results show for the first time that genistein represses telomerase activity in prostate cancer cells not only by repressing hTERT transcriptional activity via c−Myc but also by posttranslational modification of hTERT via Akt[121]. The prostaglandin (PG) pathway as a novel target for the treatment of prostate cancer (PCA) using a combination of calcitriol and genistein. Calcitriol inhibits the PG pathway in PCs in 3 separate ways: by decreasing cyclooxygenase−2 (COX−2) expression, stimulating 15−hydroxyprostaglandin dehydrogenase (15−PGDH) expression, and decreasing EP (PGE) and FP (PGF2α) receptors. These actions of calcitriol result in reduced levels of biologically active PGE, leading ultimately to growth inhibition of the PCs cells. Genistein is a potent inhibitor of the activity of CYP24, the enzyme that initiates the degradation of calcitriol. This leads to increased half life of bioactive calcitriol, thereby enhancing all of calcitriol’s actions including those on the PG pathway. In addition to inhibiting CYP24 enzyme activity, genistein has its own independent actions on the PG pathway in PCs cells[122].

Genistein inhibits Brca1 mutant tumor growth through activation of cell cycle arrest, DNA damage checkpoints and mitotic catastrophe. Tominaga et al., (2007) treated Brca1 mutant mammary tumor cells with genistein[123]. They showed that genistein treatment depleted the G1 population of cells, which was accompanied by an accumulation of cells at G2. Some genistein−treated cells entered mitosis; however, they exhibited chromosome abnormalities and maintained tetraploidy owing to abortive mitotic exit. A fraction of G2 cells underwent endoreduplication and became polyploid, which was accompanied by increased cell death through activating DNA damage response. Furthermore, data indicated that Brca1 mutant cells were more sensitive to genistein than some other types of cancer cells, highlighting a good therapeutic potential of genistein for BRCA1−associated breast cancer[123,124].

Inactivating mutations or deletions of the PTEN gene are among the most common changes found in human cancers, particularly in prostate and endometrial cancers. A study carried out utilizing rats found that genistein promotes apoptosis in mammary epithelial cells by inducing PTEN. These changes were accompanied by a decrease in mammary tumorigenesis[113].

In recent years, novel combination treatments with conventional cancer therapies and chemopreventive agents have received much attention in cancer research. Many studies have shown that isoflavone genistein could potentiate the antitumor effects of chemotherapeutic agents in various cancers in vitro and in vivo in preclinical studies[40]. In vitro genistein potentiates growth inhibition and apoptotic cell death caused by cisplatin, erlotinib, docetaxel, doxorubicin, gemcitabine, and CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) in cancers of prostate, breast, pancreas, and lung and lymphoma. Dietary genistein in vivo could enhance the antitumor activities of gemcitabine and docetaxel in a tumor model, resulting in apoptotic cell death and the inhibition of tumor growth. Similar observations has been reported by other investigators showing that the antitumor effects of chemotherapeutics, including 5−fluorouracil (5−FU), adriamycin, cytosine arabinoside, tamoxifen and perifosine could be potentiated by genistein. Genistein also enhanced the antitumor effect of bleomycin in HL−60 cells[4].

7. Genistein and Genetic Diseases

CF is one of the most common fatal genetic disease in U.S.A. and Europe. The clinical problems in CF arise from dysfunction of a single gene. The gene of affected patients is CFTR, which encodes CF transmembrane conductance regulator protein. CFTR play role as an epithelial Cl− channel and its activity is regulated by cAMP dependent protein kinase A and ATP. Reduction of functional activity of CFTR, the transport of Cl− ions is impaired, which results dehydration of endobronchial secretions and cripples mucociliary clearance. Although several hundred mutations in the CFTR gene have been described to date, but between 70 and 90% of CF patients (depending on population) bear the most frequent defective allele of this gene, called ∆F508[125]. The product of the mutant allele has severe problems of proper folding of this protein. This causes its recognition by the ERQC (endoplasmic reticulum quality control) system, and exoposion to proteolytic degradation. Therefore the mutated protein is not able to reach its proper location in the cells and to perform its biochemical functions[126]. Genistein can enhance the Cl− ion channel activity not only in ∆F508 CFTR, but also the product of another CFTR allele, G551D present in 2−6% of CF patients[125]. Moderate concentrations of genistein augmented CFTR maturation and increase its localization to the cell surface[127].

Sanfilippo (mucopolysaccharidosis type III, MPS III) is a severe metabolic disorder caused by accumulation of heparan sulfate (HS); a glycosaminoglycans (GAGs), due to genetic defect resulting deficiency of GAG hydrolysis. This disorder charaterized as the most severe neurological form of MPS, revealing rapid deterioration of brain functions[128]. SRT (substrate−reduction therapy), is an impairment of production of substrate(s) that unable to degraded due to defects of particular enzyme(s), might be a solution for patients suffering from MPSs, particularly from their neuronopathic forms[129]. Genistein has been demonstrated to be an inhibitor of GAG synthesis in fibroblasts of patients suffering from various types of mucopolysaccharidoses, including MPS III. In pilot clinical studies, it was demonstrated that treatment of patients suffering from MPS IIIA and MPS IIIB with a genistein−rich isoflavone extract resulted in statistically important improvement of all tested parameters including cognitive functions. The effects of genistein on neurological parameters in MPS animals and humans were assumed to be due to an ability of this isoflavone to cross the blood−brain−barrier[128]. The mechanism of the genistein−mediated SRT was proposed as an inhibition of phosphorylation of epidermal growth factor receptor (EGFR) by this isoflavone[129].

8. Genistein as Potential Therapeutics

Low solubility in both aqueous and lipid environments limits genistein’s potential to be developed as a drug. Attempts taken to alter its bioavailability using derivatization
with water–soluble molecules included the synthesis of sugar glycosides from genistein. Conjugation of genistein with a sugar derived peracetylated lactose resulted into a compound. ITB–301 (also called G21) is a novel lipophilic glycoside that has been shown to possess potent anti–proliferative activity in cancer cells through induction of microtubule depolymerization and subsequent mitotic arrest. Ram3, a glyconjugates of genistein exhibit antimitic activity is a potent agent affecting mitotic spindle and leading to apoptotic cell death. Rayalam, et al. (2008) demonstrated that 1,25(OH)2D3 and genistein in combination were capable of inducing apoptosis and decreasing lipid accumulation in adipocytes. But later on Park, et al. (2008) show that lower concentrations of combined treatments with several natural compounds (genistein, quercetin and resveratrol) may be useful for treatments for obesity through the suppression of adipogenesis and enhanced adipocyte apoptosis. Kohen, et al., (2007) obtained a novel genistein derivative exhibiting significantly higher antiproliferative activity than the parent by attaching an N–tert–butoxycarbonylo–1,6–diamino–hexane group to C2 of genistein. Gentile, et al., (2003) used genistein–monoclonal antibody approach to treat SW–620 and HT–29 colon cancer cells. Beside the conjugate of genistein and monoclonal antibody recognizing an epithelial membrane antigen expressed in colon cancer significantly inhibited cell growth in vitro and in vivo, and induced apoptosis.

Pre–clinical studies with genistein have provided a basis for clinical trials with cystic fibrosis (CF) patients, and a Phase II clinical study is currently underway. So far, genistein derivatives for potential treatment of cystic fibrosis have not been studied extensively. In vivo study on spontaneously hypertensive rats had shown, that genistein reduced systolic blood pressure and enhanced endothelium–dependent aortic relaxation. Genistein may prevent progresses of mild disease osteopenia, to its severe form osteoporosis. Which suggest genistein can be effective rather in chemoprevention to treatment of osteopenia. It was recently shown that dietary intake of foods containing genistein improves diabetes in both experimental systems. However, the potential anti–diabetic mechanisms of genistein acutely stimulate insulin secretion in pancreatic β–cells through a cAMP–dependent protein kinase pathway. It also prevents hyperglycemia–induced monocyte adhesion to human aortic endothelial cells through preservation of the cAMP signaling pathway.

The effect of genistein on immunity is immune cell dependent. Genistein suppress antigen specific immune response in vivo and lymphocyte proliferation response in vitro. However genistein enhances cytotoxic response mediated by NK and cytotoxic T cells and the cytokine production from T cell. It also inhibits allergic inflammatory response.

Alongside these influential activities of genisteine; also function as a bacteriostatic, rather than a bactericidal agent. Zhang et al., (2008) reported the derivatization of genistein leading to an increased antibacterial and antifungal activity. Li et al., (2008) synthesized and tested 14 new deoxystilbeno derivatives of genistein and found that dimeric forms were generally more active than genistein or deoxystilbeno against selected microorganisms. Those collective descriptions of genisetein as a potential biomedical component provide an insight on ganistenin for its direct application or other genistenin based combinational remedy for harmless and efficient treatment of ordinary and complicated diseases as well as disorders that may lead to give emphasis on ganistenin by researchers to develop noble genistein based therapeutics.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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