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Review article

Uses of soybean isoflavonoids in dentistry: A literature review

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Abstract Soybean isoflavones including genistein, daidzein and glycitein have excellent therapeutic and health properties. In this article, we reviewed soy isoflavones with a specific focus on the role they play in dentistry. In the present article, we reviewed English published articles up to December 2020 and summarized their effectiveness in inflammation, bone effects, disease prevention, and treatment of periodontal tissue and its related diseases, as well as their anti-microbial activity against oral bacteria, oral, head and neck cancers. This study shows that the anti-inflammatory effect of soy isoflavones in periodontal disease is through its inhibitory effect on the production of inflammatory cytokines and inhibition of mitogen-activated

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protein kinase (MAPK) activity. It has been observed that isoflavones can stop cell division in *Staphylococcus aureus* and may be helpful to treat salivary gland disorders caused by estrogen deficiency. Genistein and daidzein increase mineral content in bones and protect against bone loss and genistein may be beneficial as preventive chemical agents for head and neck cancers.

Introduction

Herbs have been used in many parts of the world as a traditional therapeutic agent for many human diseases such as dental problems. About 80% of people in developing countries utilize traditional medicines for maintaining health. Therefore, the search for appropriate products continues and phytochemicals derived from plants used as herbal medicines seem to be a suitable alternative source.[1] One of these plants that scientists have long considered is soybean (see Table 1).

Soybeans are from the order Rosaceae and family Leguminosae, Fabaceae or Papilionaceae and subfamily Papilionoideae and genus Glycine and cultivar *Glycine max*.[2] Soy is native to China and is widely used because it is an essential source of protein and oil.[3] So far, many studies have been done on the functional components of soy and their positive effects on body health.[4–6] The critical functional components in soy include proteins, peptides, linoleic acid, α-linolenic acid, lectins, lecithins, isoflavones and phytosterols.[3] Until now, many properties have been mentioned for soy protein, including reducing heart disease and lowering blood cholesterol.[7,8] Soy fiber also has anti-inflammatory and anti-cancer properties.[9] Functional soy components with anti-inflammatory, anti-cancer, anti-microbial, etc. effects can be helpful in dental treatments such as anti-periodontal and anti-cancer treatments.[10,11] Soy is a natural source of large amounts of isoflavones. Isoflavones are a subset of the phenolic class of heterocyclic plants called flavonoids. The soy isoflavones include three classes: daidzein, genistein and glycitein. Positive functions of soy isoflavones include: hypocholesterolemic, improve lipid metabolism and digestive tract function, prevents breast, colon and prostate cancer and bone health.[3] The positive benefits of soy isoflavones in the field of maxillofacial and dentistry have long been considered. So far, review studies on the effects of herbs in dental treatments have been published,[12] but to our knowledge, no study has been conducted to review the effects of soy in dentistry. Therefore, the purpose of this article is to review the effects of soy isoflavones in the field of dentistry.

Materials and methods

This article reviewed data relevant to soybean isoflavones and their effects on oral, head and neck diseases. We used English published articles in various journals up to December 2020 by using databases including Pubmed, Web of Science, Scopus, and Google Scholar. The references lists of extracted articles was searched manually. Then relevant articles were summarized.

Results

Soybean isoflavonoids' uses could be investigated in various dentistry fields, including periodontal effects, bone effects, disease prevention, use for treatment, anti-microbial activity against oral microbes, oral cancer, and Salivary effects, which are introduced in the following.

Periodontal effects

Anti-inflammatory

Periodontal tissues are composed of the gingiva, cementum, alveolar bone and periodontal ligament (PDL) and their objective is to support and protect the tooth.[13]

Periodontal disease is an inflammatory process and includes infections that lead to demolition the gingival connective tissue, periodontal tissue, involvement of the alveolar bone and eventually the loss of teeth.[14] Accumulation of biofilm on teeth is the leading cause of most periodontal diseases. It has been shown that the presence of certain bacteria is involved in the development and progression of these diseases; among these bacteria are *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, and *Bacteroides forsythus*.[15] Host immune-inflammatory responses triggered by bacterial antigens and lipopolysaccharides (LPS) play an important role in periodontal destruction. Therefore, host modulation therapies are used to regulate the host response.[16]

One of the crucial processes in the development of periodontal disease is regulating the extracellular matrix revenue of gingival connective tissue. Growth factors such as epidermal growth factor (EGF) regulate urokinase-type plasminogen activator (uPA), which contributes to cellular events in regulating the extracellular matrix revenue.[17] Phytoestrogens, such as genistein, have been shown to modulate EGF-related signaling pathways in human gingival fibroblast culture, followed by uPA. In fact, it has been observed that genistein more than curcumin has inhibited EGF-stimulated urokinase production by reducing JNK phosphorylation.[18] Genistein acts as a ligand for estrogen receptors in inhibiting IL-1β-induced MAPK activity and it plays a vital role in regulating MAPK activity from the GPR 30 pathway by delaying p38 MAPK, ERK and JNK activation in human ligament periodontal cells[19] and it blocks inflammation induced by LPS from *P. gingivalis* in H9c2

Table 1 A summary of the potential effects of soy isoflavones on oral, head and neck diseases.

Author	Soy isoflavone	Type of disease	Method	Model	Outcomes
Johnson et al.[74]	Genistein	oral squamous cell carcinoma	cell line treatment	In Vitro: SCC15 and SCC25 cell lines	↓Cell growth (IC50 = 50 μM), ↓ERK and Akt phosphorylation
Alhasan et al.[72]	genistein	head and neck squamous cell carcinoma	cell line treatment	In Vitro: HN4 cell line of SCC	↑$P21^{WAF1}$, ↓Cdkl (24 h), ↓CyclinB1, ↓Cdc25C
Alhasan et al.[71]	genistein	head and neck squamous cell carcinoma	cell line treatment	In Vitro: HN4 cell line of SCC	↓c-erbB-2, ↓MMP-2, ↓MMP-9 secretion, ↓NF-κB DNA binding activity, inhibition of 14-3-3 protein, telomerase shortening
Ye et al.[10]	genistein	head and neck squamous cell carcinoma	cell line treatment	In Vitro: SCC-25 cell line	↓Cell proliferation (IC50 = 200 μM), ↓PCNA, ↓PGE_2
Elattar et al.[75]	genistein	oral squamous cell carcinoma	cell line treatment	In Vitro: SCC-25 cell line	↓Cell proliferation (concentration dependent)
Liu et al.[84]	genistein	salivary adenoid cystic carcinoma	Injection of the treated ACC-M cells	In Vivo: nude mice	↓number of metastases, ↑Apoptosis index, ↓VEGF, ↓MMP-9
Ma et al.[81]	genistein	salivary adenoid cystic carcinoma	cell line treatment	In Vitro: SACC-83 cell line	Inhibited cell growth, ↓cells' volume ↑suspended cells, Arrested cell cycle (G2/M), induced cell apoptosis
Ma et al.[80]	genistein	salivary adenoid cystic carcinoma	cell line treatment	In Vitro: SACC-83 cell line	↑bax protein, ↓BCL-2 protein, ↓survivin protein
Ma et al.[82]	genistein	salivary adenoid cystic carcinoma	cell line treatment	In vitro: SACC-83 cell line	↓survivin
Katz et al.[79]	genistein and daidzein	oral carcinoma	cell line treatment	In vitro: human salivary gland (HSG) cell line	↑p53 and $p21^{CIP1}$ phosphorylation
Kuzumaki et al.[92]	genistein	melanoma	cell line treatment	In vitro: mouse B16–F1 melanoma cells	↓ cyclin E/CDK2, ↑ $p21^{Cip1/WAF1}$
Casagrande et al.[89]	genistein	melanoma	cell line treatment	In vitro: Human choroidal melanoma cells (spindle-shape OCM-1)	↑$p21^{CIP1}$, ↓CDK1 activity, ↓CDK1 Tyr15 dephosphorylation
Darbon et al.[90]	genistein	melanoma	cell line treatment	In vitro: Human choroidal melanoma cells (spindle-shaped OCM-1)	Impaired Cdc25C-dependent Tyr-15 dephosphorylation for CDK1
Morris et al.[91]	genistein	Osteosarcoma	cell line treatment	In vitro: Human MG63 osteosarcoma osteoblast cells	More organized cytoskeleton, exposure of phosphatidylserines on the external plasmalemma, high level of collagen and alkaline phosphatase synthesis
Ryo et al.[99]	Genistein, Daidzein & Equol (in form of one single tablet)	Dry mouth	–	In vivo: 15 patients (SS and non-SS)	↑ Salivary flow; Saliva concentration: ↑Daidzein & ↑Genistein; Improvement of dry mouth symptoms

(*continued on next page*)

Table 1 (*continued*)

Author	Soy isoflavone	Type of disease	Method	Model	Outcomes
Carvalho et al.[98]	40% Isoflavone extract	Estrogen deficiency	Induced by ovariectomy	In vivo: 120 female rats	↓Acini percentage; Maximum acini percentage for isoflavone group (after 5 & 8 weeks); Isoflavone effect on treatment: Slight increase after primary phase
Kouidhi et al.[100]	genistein	—	—	In vivo: 40 female offspring rats	↓ Striated ducts; Wider striated ducts; ↓ Ki-67 & ↓Acinar proliferation; ↑ BW
Choi et al.[21]	genistein	inflammatory response	Induced by *P. intermedia* LPS (10 ng/ml)	In vitro: RAW264.7 cells	↓NO & IL-6; ↓iNOS and IL-6 mRNA expression
		Periodontitis	Ligator-induced	In vivo: 24 male Sprague—Dawley rats	↓Alveolar bone height destruction; ↓BVF reduction; Preservation of Tb.Th, BMD, Tb.Sp & SMI
Tanaka et al.[48]	Genistein & Daidzein (isoflavone intake)	Periodontal disease	—	Descriptive study: 3956 female students	Reverse relationship between isoflavone intake & periodontal disease
Wang et al.[64]	0.08% Soybean isoflavone	—	—	In vitro: *S. aureus*	↓Nucleic acids by preventing their production; ↑Supercoiled DNA; ↓Topoisomerase I & II activity stop cell division in bacteria
Laodheerasiri et al.[60]	Isoflavones extract of Raw soybean, soybean flour and roasted soybean (3.125—75%)	Ethanol-hexane extraction	—	In vitro: *E. coli* and *S. aureus*	Growth inhibition: Flour > Roasted > Raw; *E. coli* more inhibited than *S. aureus* (in all extracts); Ethanol-hexane extraction found to be a suitable method for extracting isoflavone compounds
Katz et al.[79]	genistein	—	—	In vitro: HSG cells	Cell arrest in G2/M phase; ↓DNA synthesis (dose-dependently); ↑$p21^{CIP1}$ & ↑phos-p53 (dose-dependently); ↓p27 (at 10, 30 μg/ml) and ↑Skp2 expression (at 30 μg/ml)
Noël et al.[105]	genistein	Cystic fibrosis		In vivo: $Cftr^{+/+}$ and $Cftr^{-/-}$ mice	↑Salivary secretion in $Cftr^{+/+}$ mice; No effect on $Cftr^{-/-}$ mice
Choi et al.[23]	daidzein	inflammatory periodontal disease	—	—	↓NO & IL-6; inhibit nuclear translocation and DNA-binding activity of NF-κB p50 subunit & degradation of IκB-α & STAT1 phosphorylation
Choi et al.[21]	genistein	alveolar bone loss in periodontitis	ligature-induced periodontitis	In vivo: rat model of experimental periodontitis	↓microstructural parameters of trabecular; bone destruction
Nakaya et al.[22]	genistein and daidzein at physiological	nitric oxide production and inducible nitric	Induced by Erα & LPS	In vitro: RAW264.7 cells	↑iNOS, NO and TNF-α; inhibit NG-nitro-L-arginine methyl ester and ERs antagonist

Table 1 (*continued*)

Author	Soy isoflavone	Type of disease	Method	Model	Outcomes
	concentrations (10^8 to 10^6 M)	oxide synthase activity			
Luo et al.[19]	genistein	periodontal tissue destruction in periodontitis	induced by IL-1β	In vitro: human periodontal ligament cells	Delay p38 MAPK, ERK and JNK activation
Smith et al.[18]	genistein and curcumin	periodontal disease	Induced by EGF	In vitro: human gingival fibroblasts	inhibit EGF-stimulated urokinase production; ↓JNK phosphorylation;
Gutiérrez-Venegas et al.[20]	genistein and luteolin and quercetin	inflammatory responses	Induced by LPS	In vitro: H9c2 cardiomyoblasts	inhibit p38 phosphorylation block IkBα degradation inhibit the expression of COX-2 inhibit the activation of MAPK & IκB-α
Gutiérrez-Venegas et al.[25]	genistein	Periodontal disease	Induced by LPS	In vitro: human gingival fibroblasts	block phosphorylation of ERK1/2 inhibit p38 phosphorylation inhibit COX-2 expression
Lee et al.[29]	genistein	osteoclast differentiation	Induced by RANK-L	In vitro: Murine macrophage RAW 264.7 cells	↓ROS; Inhibit Osteoclast Differentiation & Nox1; ↑Nrf2-Mediated Translation
Bhattarai et al.[35]	genistein	Periodontitis	Induced by LPS/ ligature	In vivo: mouse	Inhibit tissue damages; Inhibit loss and destruction of alveolar bone; ↓COX-2 and ICAM-1
		osteoclastic differentiation of macrophages	Induced by RANKL	In vitro: RAW 264.7 macrophages	↓osteoclast formation; ↓osteoclast differentiation & Multinucleated osteoclasts formation
Bae et al.[46]	daidzein	periodontitis	Induced by ligature	In vivo: male Sprague Dawley rats	Inhibit loss of alveolar bone height; ↓Bone volume fraction; ↑Trabecular thickness & bone mineral density & structure model index & trabecular separation
Li et al.[28]	genistein (At both the low and high doses)	bone homeostasis	Induced by ER-selective binding phytoestrogen	In vitro: osteoblasts of rat mandibular condyle	low-dose: ↑expression of ALP, OC, OPG, ERβ and Erα; ↓expression of RANKL and the RANKL/OPG ratio high-dose: ↓expression of ALP, OC, OPG and Erα; ↑expression of ERβ

SCC: Squamous cell carcinoma, IC50: Inhibitory Concentration 50%, ERK: extracellular signal-regulated kinases, HN: Head and Neck, WAF: Wild-type Activating Fragment, CDK: Cyclin-Dependent Kinase, Cdc25C: Cell Division Cycle 25C, MMP: Matrix Metalloproteinase, NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells, DNA: Deoxyribonucleic acid, PCNA: Proliferating Cell Nuclear Antigen, PGE2: Prostaglandin E2, ACC: Adenoid Cystic Carcinoma, VEGF: Vascular Endothelial Growth Factor, SACC: Salivary Adenoid Cystic Carcinoma, BCL: B-Cell lymphoma, BAX protein: BCL2-Associated X Protein, HSG: Human salivary gland cells, OCM: Ocular Choroidal Melanoma, SS: Sjogren's syndrome, BW: Body weight, *P. intermedia* LPS: *Prevotella intermedia* lipopolysaccharide, LPS: Lipopolysaccharide, NO: Nitric oxide, IL-6: Interleukin-6, iNOS: inducible nitric oxide synthase, mRNA: Messenger RNA, BVF: Bone volume fraction, Tb.Th: Trabecular thickness, BMD: Bone mineral density, Tb.Sp: Trabecular separation, SMI: Structure model index, Skp2: S-phase kinase-associated protein 2, Cftr: Cystic fibrosis transmembrane conductance regulator, Neu5Ac: N-acetylneuraminic acid, PND: Postnatal day, AR: Androgen receptor, PR: Progesterone receptor, EGF: Epidermal growth factor, TGF-α: transforming growth factor α, IκB-α: inhibitor of kappa B-alpha, STAT1: phosphorylation of signal transducer and activator of transcription 1, Erα: estrogen receptor α, TNF-α: tumor necrosis factor α, ERs: estrogen receptors, IL-1β: interleukin-1β, MAPK: mitogen-activated protein kinase, JNK: c-Jun N-terminal kinases, uPA: urokinase-type plasminogen activator, RANK-L: receptor activator of nuclear factor-κB ligand, ROS: reactive oxygen species, Nox1: nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 1, Nrf2: nuclear factor erythroid 2-related factor 2, COX-2: cyclooxygenase-2, ICAM-1: Intercellular Adhesion Molecule 1, ALP: alkaline phosphatase, OC: osteocalcin, OPG: osteoprotegerin, Erβ: estrogen receptor β

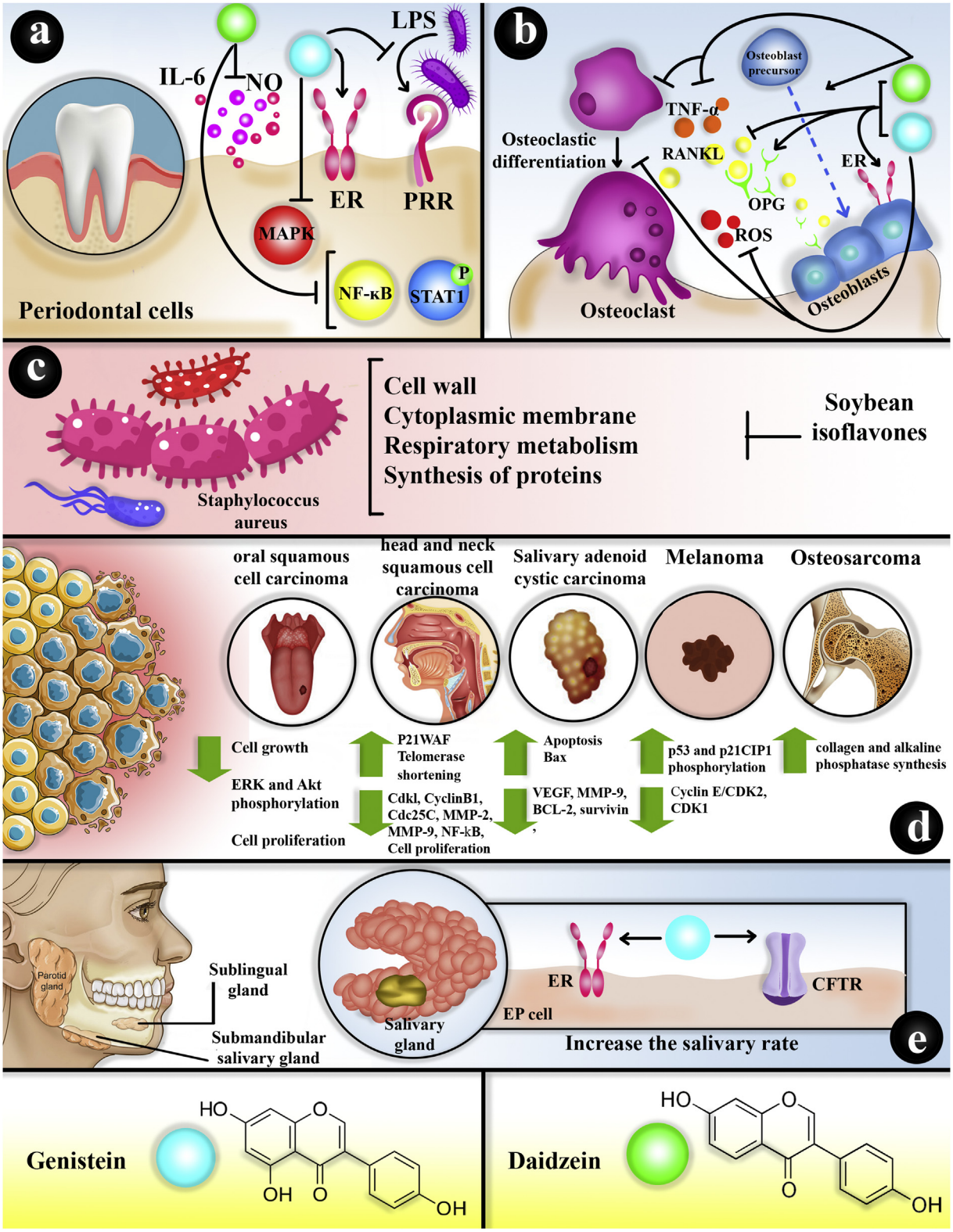

Figure 1 Soybean isoflavones effects on various target cells and tissue. a) Periodontal Anti-inflammatory b) Periodontal Bone effects c) Antimicrobial activity against oral microbes d) Oral cancer e) salivary effect.

cardiomyoblast.[20] the inflammation is reduced through inhibition of p38 phosphorylation, blocking IkBα degradation, inhibition the expression of COX-2, inhibition the activation of MAPK & IκB-α.[20]

A rat model experiment showed that genistein inhibits the reduction in alveolar bone height and volume, which was shown in the reduction of microstructural parameters of trabecular in inflamed periodontal tissue due to ligature placement in the gingival sulcus.[21]

Nitric oxide produced by macrophages plays an important role in host defense and killing bacteria. Inducible NO synthase (iNOS) is expressed in macrophages following lipopolysaccharide induction by gram-negative bacteria such as *Porphyromonas*. It has been observed in an experimental study that physiological concentrations of genistein and daidzein in soybean increase NO production, iNOS and TNF-α in RAW264.7 cells. The effect of these isoflavones was identified through iNOS activation by inhibition of NG-nitro-L-arginine methyl ester and ERs antagonist.[22] It was found that daidzein inhibits the production of NO and IL-6, IjB-α degradation in RAW264.7 cells treated with LPS from *Prevotella intermedia,* and block NF-jB transcriptional activity and STAT1 phosphorylation. Therefore, daidzein can be a periodontal anti-inflammatory therapeutic agent.[23] In an animal model study reported that isoflavones are capable of decreasing TNF-α and IL-6 activity with strengthened adhesion molecules.[24] Generally, flavonoids have inhibitory properties on the production of interleukins such as interleukin-1β (IL-1β), prostaglandin E2 (PGE2) through inhibition of cyclooxygenase pathways and mitogen-activated protein kinase 8 (MAPK) activity through inhibition of p38 phosphorylation and block phosphorylation of ERK1/2 (Fig. 1a).[25]

Bone effects

Soybean isoflavones, genistein and daidzein, affect subprocesses of bone remodeling: bone formation by osteoblasts and bone resorption by osteoclasts.[26]

Genistein

Osteogenic effects: genistein stimulates the increase in the number and rate of maturation of osteoblasts. It is a phytoestrogen and binds to estrogen receptors including estrogen receptor alpha (Erα) and estrogen receptor beta (Erβ) on osteoblasts. It binds to Erβ with greater affinity but the interaction of genistein with ERα is more important to enhance osteogenic differentiation.[27] A low dosage of genistein increased the expression of Erα, alkaline phosphatase (ALP), osteocalcin (OC), osteoprotegerin (OPG), estrogen receptor α (Erα) and bone formation but a high dosage of genistein acted oppositely. However, both doses have been shown to increase the expression of estrogen receptor β (ERβ).[28] Moreover, low concentration of genistein inhibited adipogenesis and stimulated osteogenesis in bone marrow mesenchymal stromal cells (BMSCs) but genistein at high concentration showed an opposite result (Fig. 1b).[27]

Anti-bone resorption effects: genistein suppressed the osteoclastic differentiation of precursors (monocyte/macrophage) and resorption of bone tissues. Nuclear factor-κB (NF-κB) pathway has a main role in them. Receptor activator of nuclear factor-kappa-B ligand (RANKL) and osteoprotegerin (OPG) are two main proteins of the NF-κB pathway. Osteoblasts produce them. RANKL induces osteoclastogenesis but OPG decreases it.[27] Reactive oxygen species (ROS) level is also important for Osteoclastogenesis.[29] Genistein stimulated the expression of OPG and reduced the production of RANKL and the level of ROS, Nox1 and osteoclast differentiation but increase Nrf2-Mediated Translation.[27,29] According to an *in vitro* study (osteoblasts of rat mandibular condyle), by both low dosage and high dosage of genistein, expression of RANKL decreased and genistein did not have a cytotoxic effect on RAW 264.7 cells (monocyte/macrophage-like cells).[28] Inflammatory factors like interleukin-1 (IL-1) and tumor necrosis factor α (TNF-α) induce alveolar bone loss in periodontitis.[30] Genistein also suppressed TNFα-induced osteoclastogenesis and IL-1-induced activation of osteoclasts (Fig. 1b).[27]

Animal studies on rats and mice showed that genistein increased mineral content in bones and protected against bone loss in both genders.[27,28,31–33] Studies on the mandibular bone of female rats showed that genistein increased thickness and the mineral density in mandibular bone.[28,34] An *in vivo* study on C57B7/6 male mice showed that genistein inhibited lipopolysaccharide/ligature-induced alveolar bone loss and decreased the number of osteoclasts in the inflamed region of the periodontium.[35]

Daidzein

Osteogenic effects: daidzein enhanced the osteoblastic differentiation of mesenchymal stem cells (MSCs).[36] It promoted the activation of the bone morphogenetic protein (BMP)-2/Smads pathway. Runt-related transcription factor 2 (Runx2), BMP-2, and Smads are the main proteins of this pathway. Daidzein enhanced their expression and led to stimulating the osteoblastic differentiation of osteoblast precursor cells.[37] It also enhanced the activity of ALP, the marker of osteogenesis.[37,38] Daidzein, like genistein, is a phytoestrogen and stimulates the osteogenic process through binding to ERs, too.[37–39]

Anti-bone resorption effects: daidzein suppressed the osteoclastic differentiation of precursors by affecting the NF-κB pathway. It reduced the expression of RANKL and increased the expression of OPG in osteoblasts.[38,39] Daidzein also inhibited TNF-α-induced osteoclastic differentiation of RAW 264.7 cells[40] and it downregulated TNF-α by inhibiting the increase in TNF-α producing cells, $CD4^{+}$CD28null T Cells and B lymphopoiesis.[41] According to an *in vivo* study, it upregulated the production of calcitonin, a hormone that restrains bone resorption, in thyroid C cells.[42]

Daidzein increased bone mineral content and suppressed bone loss in male and female rats/mice.[38,41–45] Reported daily use of daidzein inhibited induced periodontitis and alveolar bone loss in male Sprague–Dawley rats.[46] It is observed that trabecular thickness, bone mineral density, structure model index and trabecular separation increased; however, bone volume fraction decreased.[46]

Disease prevention

Periodontal diseases are known to have a bacterial cause. Therefore, eradication or controlling the bacteria could be

a reasonable approach toward periodontal diseases. Bioflavonoids, including soy isoflavones, can prevent periodontal diseases through their ability to decrease the population of bacterial pathogens, inhibit plaque accumulation, and accelerate gingival and periodontal healing.[47,48]

Avocado and soy unsaponifiables (ASU) which contain isoflavones in their extract, may prevent periodontal diseases by stimulating regenerative factors expression such as TGF-b1, TGF-b2, and BMP-2 and reverse the destructive effects of IL-1B.[49,50] Furthermore, it showed that alveolar bone resorption was inhibited with the use of ASU in ligature-induced periodontitis model.[51]

Use for treatment

Genistein, a potent tyrosine kinase inhibitor, can be beneficial in the treatment of periodontal diseases.[52] Its administration suppressed the LPS activated proinflammatory response caused by *P. intermedia* in murine macrophages and decreased the destructive consequences of ligature placement including alveolar bone loss and bone volume fraction.[21] Moreover, it inhibits the production of IL-1β and prostaglandin E2, two key proinflammatory cytokines in periodontitis, by human gingival fibroblasts prompted by *P. gingivalis*.[25] Additionally, isoflavones have the bioactive potential to induce osteoblasts proliferation.[53] The effects of ASU on periodontal repair in rats with ligature induced periodontitis showed a reduction in inflammation and osteoclast formation (assessed by measuring RANKL and TRAP expression) and improved the bone volume.[54]

Due to soybean's mentioned favorable effects, novel approaches have been made to use soybean derivatives as biodegradable and bioactive bone filler in regenerative treatments.[55] A relatively straightforward and low-priced thermosetting of soybean curd can result in the production of adaptable bone material with satisfying physicochemical and biological features, which make it an appropriate bone material for maxillofacial and periodontal regenerations.[56] Implantation of soybean granules in a rabbit caused production of new and increasingly maturing bone trabeculae when analyzed by backscattered electron microscope.[57]

Anti-microbial activity against oral microbes

Dental plaque as the initiator of periodontitis has a pivotal role in it.[58] Different forms of soybean have shown antibacterial activity against oral pathogens. For example, soybean extract,[59] Soybean flour,[60] altered soybean cellulose nanofibers,[61] and *Streptococcus salivarius*-fermented soy.[62,63] Soybean isoflavones had antibacterial activity, too. Researches on *Staphylococcus aureus* showed 0.08% soybean isoflavones affected cell wall, cytoplasmic membrane, respiratory metabolism, and synthesis of proteins and DNA by inhibiting the unwinding of DNA by affecting topoisomerase I and II enzymes, reducing nucleic acid production and increase supercoiled DNA so stopped cell division in bacteria (Fig. 1c).[64]

Oral cancers

Carcinoma

Epidemiological studies suggest a lower incidence of breast, oral cavity, skin, pancreas and colon cancers are related to a high intake of soybean-rich diets.[65–68] It has been shown in researches that dietary flavonoids play a protective role in oral diseases like oral cancer. Genistein is considered a natural anti-cancer agent and anti-tumor ingredient of soy.[69] Examination of the effect of flavonoid glycosides on cancer cell proliferation by using squamous cell carcinoma-9 (SCC-9) cell line demonstrated that thanks to a rapid uptake of 7-glucosides of genistein and its hydrolysis into genistein, this had a significant effect on downregulation of cellular proliferation.[68]

Recent studies have shown a relation between genistein intake and reduction in the proliferation of cancerous cells in human head and neck cancers.[70,71] Alhasan et al. reported genistein causes an inhibition in the growth of cancerous cells by stopping the cells at the S/G2-M phases, likewise an increase in the apoptotic death of these cells in HN4 squamous cell carcinoma of head and neck (HNSCC) cell line. In another study, they illustrated that these changes would be regarded as the downregulation of cyclin-dependent kinase 1 (CDK1) and cyclin B1 and, in contrast, the upregulation of the cyclin-dependent kinase (CDK) inhibitor, p21WAF1. Furthermore, these results supported by cleavage of apoptosis hallmarks such as poly-ADP-ribose polymerase (PARP) accompanied with an increase of pro-apoptosis molecules compared to anti-apoptosis molecules.[70,72] In another study by the same authors two years later, this study fundamentally supported the former, and in addition, new findings were added to the previous ones. For instance, down-regulation of c-erbB-2 expression and matrix metalloproteinase 2 (MMP-2) and matrix metalloproteinase 9 (MMP-9) secretion also inhibition of tumor cell invasion was reported. They also observed that genistein restricts the translocation of telomerase catalytic subunit [human telomerase reverse transcriptase (hTERT)] to the nucleus, which as a result, telomerase shortening might happen (Fig. 1d). However, the activity of it would not be affected, which in conclusion, they suggested that genistein might be beneficial as a preventive and therapeutic chemical agent for head and neck cancers.[71] However, Ye et al. stated in their study in 2004 that genistein may have only chemopreventive effects rather than chemo-therapeutic effects. Although they illustrated that application of genistein on SCC-25 line inhibits cell growth at G2/M phase and in addition a significant reduction of proliferating cell nuclear antigen expression in the same cells will occur, they did not report any significant changes in apoptotic cells' number. On the other hand, they found a significant relationship between the application of genistein and suppression of PGE2 levels in SCC-25 cells, which resulted from the direct inhibition of cyclooxygenase 2 (COX-2) activity by genistein. However, they reported much weaker anti-cancer activity for genistein than indomethacin, celecoxib and baicalein (flavonoid isolated from *Scutellaria baicalensis*).[73] Another similar study in this regard was conducted by Johnson et al., in 2008, which suggested genistein may be a more effective but less toxic chemo-therapeutic agent compared to

conventional chemotherapy drugs thanks to their inhibition of cell proliferation in oral SCC cell lines as well as mitogen-activated protein (MAP) kinase and Ak strain transforming (AKT) signaling pathways (Fig. 1d).[74] In an *in vitro* study which was conducted by Elattar et al., in 2000, which aimed to compare the ability of oral cancer cell growth inhibition in curcumin, genistein, quercetin and cisplatin, they counted cell numbers using a hemocytometer after 72 h in order to measure cell growth also they determined cell proliferation by measuring DNA synthesis due to the incorporation of [3H]-thymidine in nuclear DNA. Finally, significant dose-dependent inhibition was found between curcumin and cisplatin with the mentioned variables; However, there was a biphasic effect based on their concentration regarding genistein and quercetin.[75]

A study that examined the effect of genistein on angiogenesis and its chemo-preventive benefits in hamsters was shown that applied 7,12-dimethylbenz [a]anthracene (DMBA) to the cheek pouch of the golden hamster expecting to produce SCC and premalignant lesions based on previous studies, which are histologically similar to the human lesions and administration of genistein suspension for 12 weeks has an insignificant decrease in incidents of oral cancer in the hamsters compared to the control group. Furthermore, poorly differentiated fibro-sarcoma occurred in three hamsters from the genistein-treated group. As a result of this study, no inhibitory effect of genistein on the chemically-induced post-initiation stage of oral carcinogenesis was found and conversely, it appeared to promote oral submucosa stroma tumorigenesis related to DMBA.[76]

Salivary tumors

Genistein affects radiation sensitivity. $P21^{CIP1}$, p53, and p27 are major cell cycle inhibitory proteins (cyclin-dependent kinase inhibitor G0/G1 and G2/M), tumor suppressor gene, cell cycle progression inhibitor (C0/C1), respectively. Although genistein dose-dependently increases S-phase kinase-associated protein 2 (Skp2) expression, $p21^{CIP1}$, p53 phosphorylation may occur, although it does not affect the expression of p53 in the human salivary gland cell line (HSG). P21CIP1 and P53 expression increase leading to cell cycle suppression.[77,78] In contrast, p27 (cell cycle regulator) expression decreased. These are cell cycle-related elements. In addition, genistein with or without the combination of gamma irradiation could use as head and neck radiation therapy.[79]

In previous studies, genistein effect on the salivary adenoid cystic carcinoma cell line, SACC-83, proliferation, apoptosis, and metastases were also identified. Genistein could be a tyrosine kinase inhibitor and inhibit SACC-83 proliferation. Furthermore, it could decrease survivin expression and BCL-2 protein in addition to upregulation of Bax. These cell cycle proteins can play roles in apoptosis. Apoptosis could happen because of the G2/M cell cycle arresting.[80–82] This arresting occurs due to Cdk1, Cdk4, CyclinB1, and CyclinD1 protein expressions decrease.[83] Moreover, a study showed that genistein inhibited the salivary adenoid cystic carcinoma (ACCM) metastasis moderately in nude mice. Due to the inhibition of vascular endothelial growth factor VEGF and matrix metallopeptidase 9 (MMP-9) expression, it may happen (Fig. 1d).[84]

Other tumors

Cell cycle checkpoints are critical pathways in eukaryotic cells to assess and ensure flawless and precise cell progression and DNA replication.[85] Multiple checkpoints are available, including three major ones; The G1 checkpoint, the G2/M checkpoint and the metaphase to anaphase transition also known as spindle checkpoint. These mechanisms are mainly harmonized by heterodimeric protein kinases called cyclin-dependent kinases (CDKs) associated with cyclins. CDK2 and cyclin E regulates G1/S transition while G2/M transition is mainly modulated by CDK 1 with cyclins A and B. Various CDK inhibitors, including $p21^{CIP1}$ and $p27^{KIP1}$ modulate CDK activity. The p21 family can broadly deactivate CDK-cyclin complexes and consequently inhibit cell proliferation.[86–89] Darbon et al. also showed that genistein could inhibit G2 arrest escapes by damaging the Cdc25C-dependent Tyr-15 dephosphorylation of Cdk1. They compared the genotoxicity properties of genistein with etoposide and adriamycin. It was concluded that genistein causes more negligible DNA damage and G2 checkpoint activation of these agents follow distinct paths.[90]

Genistein, a major soy isoflavone, which has been demonstrated to be one of the most potent cell proliferation inhibitors by affecting estrogen receptors,[91] blocks G1/S transition by stimulating $p21^{CIP1/WAF}$ production, thus suppressing CDK2/cyclin E complex in a dose-dependent manner.[92] However, a later study suggested that genistein administration did not affect CDK2 considerably while CDK1 activity was decreased. Hence, it proposes that the genistein-induced increase in p21 is not sufficient for CDK2 inhibition. Genistein inhibits OCM-1 melanoma cells proliferation by CDK1 tyrosine 15 dephosphorization provoked by Cdc25c at the G2 stage.[89] Additionally, genistein downregulates DNA topoisomerase II, which is in charge of breaking and rejoining DNA strands during replication (Fig. 1d).[27]

Salivary effects

Dry mouth could cause cervical caries, gingivitis, tooth mobility, calculus, and halitosis.[93] In literature, the effect of estrogen on the oral mucosa, gingiva, and salivary glands flow rate was shown.[94,95] Estrogens affect the electrolyte and flow rate.[96] Isoflavones present in soy showed to could be estrogen agonists or antagonists.[97] Carvalho et al. ovariectomized some female rats to induce estrogen deficiency. This condition led to negative histopathological changes of salivary glands like acini percentage reduction. Treatment with estrogen or isoflavones alone or in combination showed good effectiveness in decreasing these adverse effects. This finding could be helpful as a future therapeutical approach for salivary glands dysfunction in the estrogen deficit condition.[98] Another study demonstrated that 25 mg per day of genistein increases salvation after one and two months. Oxidative stress markers hexanoyl-lysin (HLE) and 8-hydroxy-2- deoxyguanosine (8-OHdG) decreased in contrast to propanoyl-lysine (PRL), but none of these were significant. Furthermore, the salivary concentration of genistein was significantly increased.[99] In another study, it was shown that exposure to genistein, vinclozolin, or genistein plus vinclozolin mixture (each 1 mg/kg) could significantly diminish striated duct numbers in immature female rats. The number

of ducts reduced because of increases in their area. It also decreases acinar proliferation. The mixture was significantly more effective than each substance alone. It can occur due to the transforming growth factor α, epidermal growth factor, and nerve growth factor expression suppression. Sex hormones receptors expression profiles were different among groups. Androgen receptor was the most abundant versus estrogen receptor α (ERα) and progesterone receptors (PR). Estrogen receptors β (ER β) was not detected. the effect of genistein was more than the mixture, but both were not too significant. Thus, genistein and vinclozolin may affect glandular structure and endocrine gene expression mRNA of prepubertal submandibular salivary gland (SSG) in the female rat.[100]

The cholinergic and adrenergic autonomic systems regulate salivary secretion. Cystic fibrosis (CF) occurs by a defect in chloride channels.[101] CF transmembrane conductance regulator (CFTR) is believed to express in epithelial and non-epithelial cells. It also plays a role in CF pathogenesis.[102–104] Sabrina Noël et al. investigated the effect of genistein on Cftr $+/+$ and Cftr $-/-$ mice. Their evidence demonstrated that 50 μM genistein plus isoproterenol (β-adrenergic agonist) and ethanol (used for solubility of non-soluble genistein) could increase the salivary rate of Cftr $+/+$ mice in comparison with isoproterenol alone. This mixture does not affect the Cftr $-/-$ mice.[105]

Conclusion

Finally, it is concluded that soybean contains compounds of isoflavonoids that have been shown to have beneficial effects, including anti-inflammatory and bone formation stimulants that can be useful in preventing and treating inflammatory diseases, including periodontal disease. The known antibacterial effect of soy isoflavonoids on *S. aureus* has opened the window to their anti-microbial effects on other microorganisms, including bacteria that cause tooth decay, which need further investigation. The anti-cancer effects of isoflavonoids have been observed in numerous laboratory and animal studies and appear promising in head and neck cancers. The effect of soy isoflavonoids on the function of the salivary glands is promising because it can be potent in treating some types of salivary disorders. Soy isoflavonoids can be a suitable treatment option for many head and neck diseases due to their appropriate therapeutic effects. However, what combination of isoflavonoid extracts should be used and what amount and duration and biocompatibility and bioefficiency in human studies require further studies and carefully controlled trials.

Declaration of competing interest

The authors have no conflicts of interest.

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