Isoflavones Protective Mechanisms Against Cardiovascular Diseases

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ABSTRACT
Isoflavones are polyphenolic compounds and a class of phytoestrogens naturally present in plants belongs to legume family and also quantified in fruits, vegetables and beverages. Soybean is rich source of isoflavones. Their chemical structure is similar to endogenously available female reproductive hormonal substance estradiol, and cellular targets are estrogen receptors. After bind to the estrogen receptors isoflavones exert estrogenic and anti-estrogenic action based upon circulatory levels of estradiol. Cardiovascular diseases are leading cause of death in most of the developing countries and they may occur due to the structural and functional changes in either cardiac muscle or smooth muscle of the vasculature and both. Common cardiac diseases are heart attack, coronary heart disease, hyperlipidaemia, angina pectoris, hypertension. Many epidemiological studies data revealing that consumption of soy protein and soy enriched diet correlate with preventive chances of cardiovascular disease. The United States Food and Drug Administration (USFDA) and other countries declared that everyday consumption of food enriched soy along with low fat may decrease the risk of cardiovascular disease. In this review we attempt the mechanism based cardioprotection of isoflavones in different cardiovascular diseases.

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INTRODUCTION
Phytoestrogens are polyphenolic compounds mainly present in legumes. The term phytoestrogen was derived from Greek word phyto means plant and estrogen means a hormone regulates female reproductive functions such as fertility in vertebrates. Phytoestrogens are available in the form of flavonoid and nonflavonoid groups, flavonoids include isoflavones, coumestans, etc. and nonflavonoid includes lignans as shown in Figure 1. Especially soybean is the richest source of isoflavones such as daidzein, genistein and glycitein and also found in flowers, fruits, vegetables, cereals and beverages (Viggiani et al., 2019). Phytoestrogens are able to produce potential health benefits on different systems of human body by exerting agonist and antagonistic effects on estrogen receptors (ERs) such as ERα (Estrogen Receptor α) and ERβ (Estrogen Receptor β), because of their structural similarity with endogenous female reproductive hormone 17β-estradiol (Kitajewska et al., 2014; Ko, 2014).
Cardiovascular disease (CVD) is the major cause of mortality in developing countries. Atherosclerosis is the major cause of coronary heart disease (CHD), and it is a common form of cardiovascular disease, and is initiated by inflammatory response of the vascular endothelial cells following injury (Krizova et al., 2019; Pabich and Materska, 2019). Similar to endogenous estrogens, phytoestrogens may produce beneficial effects on cardiovascular diseases through the estrogen receptors present in the vascular endothelium, vascular smooth muscle cells, intracellular lipid metabolism, extracellular matrix synthesis and vascular inflammation mediates through estrogen receptors (Sirotkin and Harrath, 2014; Rizzo and Baroni, 2018).

Epidemiological studies reveal that premenopausal women are less prone to development of CVD when compared to men. Female protection against CVD is associated with sex hormone levels as the incidence and severity of CVD increases in postmenopausal women. Worldwide women are suffering with reduced quality of life by menopausal symptoms. Estrogen levels are declined in postmenopausal condition, leads to vasomotor symptoms combine with increased probabilities of bone fractures, cardiovascular problems and changes in blood cholesterol levels (Sathyapalan et al., 2018). Current treatment for vasomotor symptoms is hormone replacement therapy (HRT) but this therapy increases risk of cancer in uterus, breast and ovaries because of they are rich in ERα (Poluzzi et al., 2013). Estradiol show equal affinity towards both estrogen receptors this results in cancer of tissues rich in ERα because of overexpression of cells, but isoflavones have strong affinity to ERβ and weak affinity to ERα. Through ERβ selectivity isoflavones produce beneficial effects and minimise the risk of cancer. Phytoestrogens have been used in the Population that consumes soy-based food products less affected with cardiovascular disease based on this Asian people less affected with cardiovascular disease compared with western population because of their higher intake of soy-based diet (Krizova et al., 2019; Abdelrazek et al., 2019).

**Classification of Phytoestrogens**

Phytoestrogens may be classified as flavonoids and non-flavonoids. The flavonoids include Isoflavones (genistein, daidzein, glycitein, biochanin A and formononetin) and Coumestans (coumestrol, wedelolactone, plicadin), and nonflavonoids include lignans as shown in Figure 1. (Krizova et al., 2019; Pabich and Materska, 2019).

**Absorption, Distribution, Metabolism and Excretion of Isoflavones**

Mostly isoflavones are available as inactive glycoside conjugates in diet. After ingestion these are hydrolysed partly by the β-glucosidase present in the small intestine and mainly by bacterial β-glucosidase present in large intestine to form aglycones (Ko, 2014). These aglycones are absorbed in to systemic circulation through the absorptive areas of intestine. Intestinal flora transforms aglycones in to bioactive compounds they are structurally similar to estradiol and therefore have a similar affinity towards cellular estrogen receptors. Colonic bacteria convert aglycones to inactive compounds, these are excreted through faeces. After absorption the primary and secondary aglycones are conjugated in part with sulfates and mainly with glucuronic acid. These conjugated products are freely circulate in the blood stream and produce their therapeutic benefit until they are excreted in the urine and bile as metabolites. When they are excreted in bile in to the intestine again absorbed by the intestinal mucosa and enter enterohepatic circulation or are excreted in faeces as conjugated forms and bacterial end products. After oral administration of
isoflavones the peak plasma concentration attained at 7.2 to 7.4 hours. They remain for 24 hr in circulation, with an average 6-8 hours of plasma half-life. (Pabich and Materska, 2019; Cederroth and Nef, 2009)

**Mechanism of action of isoflavones**

Isoflavones may exert their biological action interacted through estrogen receptors. There are two types of estrogen receptors such as ER$\alpha$ (Estrogen Receptor $\alpha$) and ER$\beta$ (Estrogen Receptor $\beta$) to exert estrogenic action of 17$\beta$-estradiol. Both the estrogen receptors may occupied with isoflavones but isoflavones show more affinity towards ER$\beta$ compare with ER$\alpha$. Estradiol shows similar affinity towards ER$\alpha$ and ER$\beta$ (Viggiani et al., 2019; Krizova et al., 2019).

Estrogen binds to membrane and cytoplasmic estrogen receptors produce rapid nongenomic and genomic based cellular effects respectively. ER$\alpha$ and ER$\beta$ both are shown important role in modulation of cardiovascular diseases (Desmawati and Sulastri, 2019).

In vascular endothelial cells rapid activation of endothelial nitric oxide synthase (eNOS) to release nitric oxide to produce vasodilation as shown in Figure 2, endothelial cell growth and migration, platelet inhibition and prevention of thrombus formation this mechanism happened through activation of membrane bound ER$\alpha$ receptor initiate rapid signal transduction through kinases activation such as phosphatidylinositol-3-OH kinase (PI3K), mitogen-activated protein kinase (MAPK) and eNOS. ER$\alpha$ regulates activation of superoxide dismutase 2 results in decrease in formation of reactive oxygen species (ROS) results in antioxidant activity in mitochondria and prevents mitochondrial dysfunction. ER$\beta$ maintains normal function of superoxide dismutase 2. In vascular smooth muscle cells proliferation leads to vascular related diseases, estrogen controls proliferation by activating ER$\alpha$ and ER$\beta$ associated with caveolin-1 present at plasma membrane of the vascular smooth muscle cell upon activation of ER$\alpha$ stimulates the various phosphatases reverses the phosphorylation results in a decrease of cell growth and migration. In cardiomyocytes through activation of ER$\beta$ decreases the cardiac hypertrophy. Mitochondria are the main source of energy i.e ATP and play an important role in regulation of myocardial contraction, calcium homeostasis and regulates cell death and apoptosis by forming reactive oxygen species (ROS) (Mahmoodzadeh and Dworatzek, 2019).

**Blood pressure lowering effects of isoflavones**

Prevention of cardiovascular diseases is mainly depending on maintenance of normal arterial function. Low level of NO production in blood vessel leads to vasoconstriction results in hypertension. Hypertension increases the risk of cardiovascular disease by increasing the endothelial injury through activation of proinflammatory mediators such as angiotensin II, causes vasoconstriction results in hypertension and activates lipooxygenase, the key enzyme in smooth muscles generates free radicals and formation of oxidized LDL cholesterol. Increased availability of oxidized form of LDL cholesterol in blood vessels leads to atheroma plaque formation. Isoflavones reduce the blood pressure by activating genes produces endothelial nitric oxide, interacts with estrogen receptors (Garg et al., 2016; Liu et al., 2012). Isoflavones have blood pressure lowering effect and the blood vessel endothelium rich in ER$\beta$ receptors are target for isoflavones, activation of receptors results in expression of endothelial nitric oxide synthase (eNOS) produces nitric oxide (NO) is a potent vasorelaxant decreases elevated blood pressure (Ramdath et al., 2017). Six months of isoflavone administration in postmenopausal women has been shown to increase endothelial vasodilation and reduce the number of cell adhesion molecules such as intracellular adhesion molecule 1, vascular cell adhesion protein 1, and E-selectin. Increased renal blood flow and inhibition of angiotensin converting enzyme (ACE) also occurred with isoflavones (Krizova et al., 2019; Pabich and Materska, 2019).

One of the research results reveal that attenuation of raise in blood pressure by hastening of nitric oxide (NO) production and prevention of inflammation occurred with isoflavones. A meta-analysis of 11 studies showed that soy isoflavones having blood pressure lowering activity in hypertensive patients not in normal individuals (Krizova et al., 2019).

Liu et al. meta-analysis results concluded that ingestion of 65-153 mg/day soy isoflavones along with the soy protein 1-12 months decreased blood pressure in hypertensives but this effect was not observed in normotensive subject (Liu et al., 2012).

Tao sun et al., studied vasorelaxant and antihypertensive effects of formononetin through endothelium-dependent and endothelium-independent mechanisms. Endothelium dependent pathway produce vasorelaxant effect by releasing NO and endothelium independent pathway produces vasorelaxant effect probably due to inhibition of voltage dependent Ca$^{2+}$channels and intracellular Ca$^{2+}$ release. Vasorelaxant effect of formononetin studied by using segments of rat
mesenteric arteries, renal arteries, cerebral arteries, coronary arteries and abdominal aortas precontraction induced with KCl or U46619 or phenylephrine. Formononetin produced concentration-dependent relaxation the rat artery segments (Tao et al., 2011).

**Antioxidant activity of isoflavones**

Isoflavones produce a considerable antioxidant property along with estrogenic activity due to the availability of two hydroxyl groups in the chemical structure like daidzein. Aglycones are more active than glycosides. The antioxidant effect of isoflavones was evaluated by both in vitro and in vivo experimental models. Administration of genistein in to mice and rats enhanced activation of antioxidant enzymes in epidermis and intestine was observed. (Krizova et al., 2019; Pandey and Rizvi, 2009).

Puerarin was studied for antioxidant property in pretreated neonatal murine cardiomyocytes with puerarin (50, 100 μM) decreased NADPH oxidase activity, inhibited ROS production and activated oxidative stress protective signaling pathways as shown in Figure 2. (Lim et al., 2013; Yoon and Park, 2014).

**Lipid lowering effect of isoflavones**

Epidemiological studies recommended that the ingestion of soy and soy products for longer period decreases the cardiovascular risks. In 38 research studies meta-analysis results showed that the average intake of soy protein 47g per day a reduces bad cholesterol levels and increases good cholesterol levels and protect from cardiovascular risks. (Krizova et al., 2019; Rizzo and Baroni, 2018).

Low density lipoproteins (LDL) are oxidized with free radicals present in walls of the blood vessels and accumulate then causes thrombosis, leads to cardiovascular disorders. The LDL cholesterol lowering effect of soy protein was already established. Zhan and Ho carried out meta-analysis results indicating that consumption of soy protein containing isoflavones notably reduced serum total cholesterol, LDL-cholesterol, and triglyceride, and remarkably increased high-density lipoprotein (HDL) cholesterol but it depends on duration of intake, sex, and initial plasma concentration of the individual as shown in Figure 2. (Pabich and Materska, 2019).

Puerariae radix is an edible crude drug used for different health problem. The important chemical constituents of this herb are flavonoids, coumarins and isoflavones, such as daidzein, genistein and puerarin and they are responsible to produce different pharmacological actions. P. lobata total isoflavones (PTIF), which contain the unique isoflavone, puerarin, studied on menopausal symptoms. Puerarin has shown significant protection against menopausal symptoms such as dyslipidemia and osteoporosis by producing strong estrogenic activity compared with other isoflavones (Lim et al., 2013).

**Isoflavones effect on myocardial and vascular cell proliferation**

Chunmei Liang et al., evaluated the formononetin action on cell proliferation and migration by using of human umbilical vein endothelial cells (HUVECs). Formononetin is the isoflavone one of the main active constituent of Chinese herbal medicine clover and it showed protective effect on the human umbilical vascular endothelial cells and the molecular mechanisms may correlate with insulin like growth factor 1 receptor (IGF-1R) and intercellular adhesion molecule 1 (ICAM-1). Insulin like growth factor 1 (IGF-1R) binds with insulin like growth factor receptor (IGF-1R) to inhibit endothelial cell apoptosis and promote endothelial cell proliferation, migration and angiogenesis. ICAM-1 produce adhesion between leukocytes and endothelial cells and serve as the initiating event of angiogenesis (Liang et al., 2019).

Barbara Deodato et al., evaluated phytoestrogen genistein cardioprotective action in experimental myocardial ischaemia reperfusion injury in rats. Genistein, the principal isoflavone present in soy is structurally similar to estradiol, binds to estrogen receptors and produce estrogenic activity in some tissues. Genistein administration lowered myocardial necrosis and myeloperoxidase (MPO) activity both in the area at risk and in the necrotic area, decreased serum creatinine phosphokinase activity (CPK), increased myocardial contractility, decreased occurrence of ventricular arrhythmias, reduced serum and macrophages levels of tumour necrosis factor-α (TNF-α) and blunted intracellular adhesion molecule-1 (ICAM-1) expression in the injured myocardium (Deodato et al., 1999).

Isoflavone formononetin offered protection to rat cardiomyocyte H9c2 cells against oxygen glucose deprivation and reoxygenation by inhibiting ROS formation and promoting glycogen synthase kinase -3β (GSK -3β) phosphorylation. In ischaemic reperfusion injury the major cause of cell death is opening of mitochondrial permeability transition pore (mPTP). GSK-3β stimulates the mPTP opening and there by induces mitochondrial dysfunction. Phosphorylation regulates the enzymatic activity of GSK-3β. Phosphorylation occurs at tyrosine 216 increases the activity and at serine 9 significantly decreases the enzymatic activity of GSK-3β (Cheng
CONCLUSIONS

Isoflavones are the group of chemical substances and sub class of phytoestrogens, these are present in legumes such as soybean, clover, kudzu and beverages, especially soybean rich in isoflavone content. Commonly available isoflavones are genistein, daidzein, formononetin and glycitein have nutritional and therapeutic value because of their estrogenic and antiestrogenic action. Epidemiological studies showing that increased intake of isoflavones reduce the hazard of cardiac problems, menopausal symptoms, osteoporosis and cancer. Although, the information available on protective role of isoflavones in cardiovascular diseases, still there is need to evaluate the clear mechanism responsible for protective action.

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Conflict of Interest

The authors declare no conflict of interest.

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REFERENCES

Abdelrazek, H., Mahmoud, M., Tag, H. M., Greish, S. M., Eltamany, D. A., Soliman, M. T. 2019. Soy isoflavones ameliorate metabolic and immunological alterations of ovariectomy in female Wistar rats: antioxidant and estrogen sparing potential. *Oxidative Medicine and Cellular Longevity*, 19:1–13.

Cederroth, C. R., Nef, S. 2009. Soy, phytoestrogens and metabolism: A review. *Molecular and Cellular Endocrinology*, 304(1-2):30–42.

Cheng, Y., Xia, Z., Han, Y., Rong, J. 2016. Plant natural product formononetin protects rat cardiomyocyte H9c2 cells against oxygen glucose deprivation and reoxygenation via inhibiting ROS formation and promoting GSK-3β phosphorylation. *Oxidative medicine and cellular longevity*, pages 1–15.

Deodato, B., Altavilla, D., Squadrato, G., Campo, G. M., Arlotta, M., Minutoli, L., Saitta, A., Cucinotta, D., Calapai, G., Caputi, A. P., Miano, M., Squadrato, F. 1999. Cardioprotection by the phytoestrogen genistein in experimental myocardial ischaemia-reperfusion injury. *British Journal of Pharmacology*, 128(8):1683–1690.

Desmawati, D., Sulastri, D. 2019. A Phytoestrogens and Their Health Effect. *Open Access Macedonian Journal of Medical Sciences*, 7(3):495–499.

Garg, S., Lule, V. K., Malik, R. K., Tomar, S. K. 2016. Soy Bioactive Components in Functional Perspective: A Review. *International Journal of Food Properties*, 19(11):2550–2574.

Kitajewska, W., Szela, G. W., Maslyak, Z., Sklyaroy, Z., I 2014. Chorobycywilizacyjnei ich pwewencia. *Journal of Clinical Healthcare*, 1:3–7.

Ko, K. P. 2014. Isoflavones: chemistry, analysis, functions and effects on health and cancer. *Asian Pac. J. Cancer Prev*, 15(17):7001–7011.

Krizova, L., Dadakova, K., Kasparovska, J., Kasparovsky, T. 2019. Isoflavones. *Molecules. MDPI*, 24(6).

Liang, C., Zhou, A., Sui, C., Huang, Z. 2019. The effect of formononetin on the proliferation and migration of human umbilical vein endothelial cells and its mechanism. *Biomedicine & Pharmacotherapy*, 111:86–90.

Lim, D. W., Kim, J. G., Kim, Y. T. 2013. Effects of dietary isoflavones from Puerariae radix on lipid and bone metabolism in ovariectomized rats. *Nutrients*, 5(7):2734–2746.

Liu, X. X., Li, S. H., Chen, J. Z., Sun, K., Wang, X. J., Wang, X. G., Hui, R. T. 2012. Effect of soy isoflavones on blood pressure: a meta-analysis of randomized controlled trials. *Nutrition, Metabolism and Cardiovascular Diseases*, 22:463–470.

Mahmoodzadeh, S., Dworatzek, E. 2019. The Role of 17β-Estradiol and Estrogen Receptors in Regulation of Ca2+ Channels and Mitochondrial Function in Cardiomyocytes. *Frontiers in Endocrinology*, 10.

Pabich, M., Materska, M. 2019. Biological Effect of Soy Isoflavones in the Prevention of Civilization Diseases. *Nutrients*, 11(7):1660–1660.

Pandey, K. B., Rizvi, S. I. 2009. Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. *Oxidative Medicine and Cellular Longevity*, 2(5):270–278.

Poluzzi, E., Piccinni, C., Raschi, E., Rampa, A., Recanatini, M., Ponti, F. 2013. Phytoestrogens in Postmenopause: The State of the Art from a Chemical, Pharmacological and Regulatory Perspective. *Current Medicinal Chemistry*, 21(4):417–436.

Ramdath, D., Padhi, E., Sarfaraz, S., Renwick, S., Duncan, A. 2017. Beyond the Cholesterol-Lowering
Effect of Soy Protein: A Review of the Effects of Dietary Soy and Its Constituents on Risk Factors for Cardiovascular Disease. *Nutrients*, 9(4):324–324.

Rizzo, G., Baroni, L. 2018. Soy, Soy Foods and Their Role in Vegetarian Diets. *Nutrients*, 10(1):43–43.

Sathyapalan, T., Aye, M., Rigby, A. S., Thatcher, N. J., Dargham, S. R., Kilpatrick, E. S., Atkin, S. L. 2018. Soy isoflavones improve cardiovascular disease risk markers in women during the early menopause. *Nutrition, Metabolism and Cardiovascular Diseases*, 28:691–697.

Sirotkin, A. V., Harrath, A. H. 2014. Phytoestrogens and their effects. *European Journal of Pharmacology*, 741:230–236.

Tao, S. U. N., Rui, L. I. U., Cao, Y. X. 2011. Vasorelaxant and antihypertensive effects of formononetin through endothelium-dependent and-independent mechanisms. *Acta Pharmacologica Sinica*, 32(8):1009–1018.

Viggiani, M. T., Polimeno, L., Leo, A. D., Barone, M. 2019. Phytoestrogens: dietary intake, bioavailability, and protective mechanisms against colorectal neoproliferative lesions. *Nutrients*, 11(8).

Yoon, G., Park, S. 2014. Antioxidant action of soy isoflavones on oxidative stress and antioxidant enzyme activities in exercised rats. *Nutrition research and practice*, 8(6):618–624.