Annals of Pharmacology and Pharmaceutics

Review Article
Published: 02 Sep, 2024

Application of Raloxifene in Breast Cancer and Its Effect on Other Tissues: A Review Study

Mirzapur P, Khazaei MR, Rezakhani L and Khazaei M**

Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Iran

Abstract

As a leading cause of mortality among women, breast cancer can be targeted by Estrogen Receptors (ERs), which are in turn affected by SERMs compounds. Various clinical trials have approved the effect of Raloxifene (RAL) on reducing the risk of Breast Cancer (BC) and osteoporosis in postmenopausal women. Due to its pivotal role in the health of women with a high risk of BC, there is currently a strong demand to use RAL as an alternative hormonal treatment capable of reducing the risk of invasive BC. RAL has effects on most tissues, but its use is contingent upon specific conditions.

Keywords: SERM; Raloxifene; Cancer; Estrogen receptor; Chemoprevention

Introduction

Cancer is generally recognized as a serious health problem worldwide, and the American Cancer Society categorizes Breast Cancer (BC) as the second-leading type of cancer or cause of death in women [1]. Mortality rate of this type of cancer in industrialized countries has recently decreased due to widespread screening programs and early detection [2]. BC is associated with estrogen as a risk factor, the carcinogenic mechanisms of which include estrogen genotoxic metabolism, mutagenic metabolites, and tissue growth stimulation [3]. Although several studies have been carried out on Raloxifene (RAL), it is necessary to explore it comprehensively in order to bring to light its related new findings and even side effects. This study, therefore, aims to investigate the effects of RAL on target tissues and their molecular mechanisms.

Selective Estrogen Receptor Modulators (SERM)

SERMs are non-steroidal, chemically diverse compounds with a tertiary structure that allows them to bind to the Estrogen Receptor (ER) (Figure 1) and possess a specific ER agonist/antagonist in the target tissues [4]. For example, these ER ligands act like estrogen in the bone and cardiovascular system [5], while in the reproductive system they behave as its antagonists [6]. ERs have two α and β types, to which SERMs connect and exhibit agonistic or antagonistic effects based on the type of recipient [7].

ERs act by regulating transcription activation as well as inducing non-genomic effects through cytoplasmic and membrane-dependent transmission pathways [8]. ERβ basically inhibits ERα gene-dependent transcription when it is present, whereas in the absence of ERα it can be partially replaced [9]. ERα is associated with the expression of progesterone receptors, and it is recognized as the dominant regulator of the estrogen-induced gene in breast tumors [10]. ERα also increases MCF-7 cell proliferation, while ERβ is known to inhibit cell proliferation and tumor formation [11].

One type of second-generation SERMs is RAL, identified under the brand-name of Evista. It was approved by the FDA in 1999 following research on the treatment and prevention of osteoporosis, which was later approved for reduction of Invasive Breast Cancer (IBC) risk in menopausal women [12]. The drug has been confirmed for its low side effects in the global index provided by the Women's Health Initiation (WHI) [13].

RAL is similar to Tamoxifen (TAM) in that it is effective in reducing the risk of IBC with a lower likelihood of causing complications from thromboembolism and cataracts. However, both drugs are similar regarding the risk of fractures emanating from induced osteoporosis, ischemic heart disease, and stroke [14]. Therefore, due to the effect of RAL on reducing the risk of BC and the occurrence of fewer side effects compared to TAM, RAL is currently prescribed as a BC prevention drug [15].

OPEN ACCESS

Correspondence:
Mozafar Khazaei, Fertility and Infertility Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran, Fax: +988334281563;
Pegah Mirzapur, Fertility and Infertility Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran,

Received Date: *14 Aug 2024*
Accepted Date: *27 Aug 2024*
Published Date: *02 Sep 2024*

Citation:
Mirzapur P, Khazaei MR, Rezakhani L, Khazaei M. Application of Raloxifene in Breast Cancer and Its Effect on Other Tissues: A Review Study. Ann Pharmacol Pharm. 2024; 9(1): 1213.

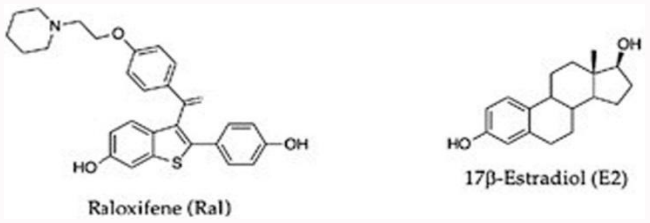

Figure 1: RAL contains Alkylaminoethoxy side chains with varying tertiary amine groups.

Mechanism of SERMs action

1. Differential ER expression in the target tissue

2. Differential ER conformation on ligand binding

3. Differential expression and binding to the ER of co-regulator proteins [4].

In postmenopausal women, SERMs are known to improve bone formation while inhibiting the growth of positive ER breast cancer cells. RAL in particular is currently used in the treatment and prevention of BC and osteoporosis. It also selectively affects diseases caused by the endocrine system [16].

Organs

Breast

Research has indicated that RAL significantly reduces the incidence of BC in postmenopausal women who have undergone treatment for osteoporosis, together with a reduction in the incidence of IBC (positive ER) during the treatment period. A decreased incidence of IBC has even been seen in postmenopausal women with osteoporosis up to 4 years after treatment [17]. Although RAL and TAM are good options for breast cancer in high-risk postmenopausal women, lower levels of toxicity induced by RAL (such as the reduced risk of thromboembolism and endometrial cancer) has turned it into a priority. Long-term use of RAL is up to 76% more effective in preventing invasive cancer compared to TAM [18]. The effect of RAL is associated with a reduced risk of IBC in postmenopausal women, regardless of the presence or absence of risk factors, and this effect is more pronounced in women with a family history of breast cancer [19]. The risks and gains of RAL treatment depend on such factors as age, race, BC risk, and a history of hysterectomy [20].

RAL has also proven effective in reducing the incidence of BC in menopausal women with high levels of serum estradiol [21]. In addition, it can decrease the expression of the vascular endothelial growth factor in a variety of positive ER breast carcinomas in postmenopausal women [22]. *In vitro* experiments on MCF7 and MDA-MB-231 BC cell lines showed that RAL increased the Bax/Bcl2 ratio; affected the expression of p53, caspase-3, and caspase-8 genes; and increased apoptosis. All these effects can be enhanced by a simultaneous administration of such compounds as resveratrol [23]. Furthermore, RAL is effective in reducing breast tissue size by inhibiting ER activity in persistent pubertal gynecomastia [24].

The NLRP3 inflammasome is a critical component of the innate immune system, whose activation is suppressed by RAL as an effective measure for inhibiting breast tumor growth. Technically speaking, RAL reduces cellular levels of Reactive Oxygen Species (ROS) through modulation of redox signaling which is mediated by the Aryl hydrocarbon Receptor (AhR), Nuclear factor erythroid 2-related factor 2 (Nrf2), Heme Oxygenase-1 (HO-1) axis or the impaired generation of mitochondrial ROS in a mitophagy-dependent manner. Furthermore, it has been shown that blocking AhR signaling or inhibiting mitophagy abrogated the tumor suppressive effect of RAL in a human BC xenograft model [25].

Uterus

A hormonal imbalance between estrogen and progesterone and a combination of morphological disorders of cells (glands and stroma) can cause endometrial hyperplasia, a condition which can further develop into endometrial cancer. Endometrial hyperplasia can be treated using SERMs as it can prevent the effects of estrogen in the uterus. RAL can trigger apoptosis of human endometrial stromal cells through the mitochondrial internal pathway and affect the Bax/Bcl-2 ratio and the caspase-3 pathway [26]. The administration of RAL eliminates the risk of endometrial cancer despite a slight increase in the thickness of the endometrium [27]. In general, RAL is associated with fewer complications in the uterus in terms of malignancy and endometrial hyperplasia [28].

The use of RAL in human endometrial cell culture does not affect progesterone receptor expression; it can however inhibit the expression of estrogen receptors together with a significant reduction in the expression of Ki67 [29], which is an indicator of cell proliferation and is expressed during tumor growth. On the other hand, Ki67 protein (pKi67) is also associated with metastasis and the clinical stage of the tumor, and its expression is more pronounced in malignant tissues [30]. Therefore, due to the inhibitory effect of RAL on the proliferation of endometrial carcinoma cells and induction of apoptosis, its application in combination with chemotherapy treatments is recommended [31]. In general, the incidence of invasive endometrial cancer in RAL recipients is significantly lower than its incidence in TAM users [18].

The efficacy of RAL in treating precancerous lesions of the mice cervix has also been demonstrated [32]. It has also been shown to prevent the proliferation of estradiol-induced CaSki cells (Cell line of cervical cancer contains several copies of the DNA of the HPV16 virus), thereby indicating an anti-estrogenic activity in cervical cells [33]. Once treatment with RAL comes to a halt, cervical cancer cells do not completely destroy and spread quickly while continuous administration of RAL in mice prevents them from recurring. Therefore, preventing the recurrence of neoplastic diseases and cancers of the reproductive tract mainly relies on its long-term use [34].

RAL demonstrates varying dose-dependent effects on endometrial tissue which results in distinctive impacts on endometriosis. A high dose of RAL presents an inhibitory effect on the growth of healthy endometrial tissue and prevents endometrial growth and angiogenesis in endometriosis. On the contrary, a lower concentration accompanies an increase in the level of angiogenesis and epithelial growth. At the same time, no relationship has been observed between endometrial hyperplasia and carcinogenesis in breast cancer patients being treated with RAL [35].

Prostate

RAL inhibits tumor metastasis in rats and induces apoptosis in androgen-dependent and independent prostate cancer cell lines, hence affecting these cells through an androgen-independent pathway [36,37]. Prostate stromal cell receptors are ERα type, and their cellular response to estrogen is cell proliferation. ERβ, on the

other hand, is mainly expressed in epithelial cells and inhibits their proliferation under estrogen influence. Epithelial estrogen receptors are involved in both hyperplasia and prostate cancer [38]. In general, RAL has little tendency to bind to ERα. It, however, binds more to ERβ, inhibits epithelial cell proliferation, and prevents the proliferation of stroma cells by acting on ERα. By doing so, it inhibits prostate cancer metastasis and also reduces the number of prostate acini and smooth muscle cells around them [39]. As human prostate cancer is an androgen-sensitive disease, RAL can inhibit migration and metastasis to or proliferation of its cells [40]. On the other hand, RAL encapsulation increases aggregation in tumor tissue and reduces the growth of Prostate Cancer Castrate-Resistant (CRPC) in the xenograft mouse model. Once-a-week treatment with RAL delays growth, which can subsequently control prostate cancer [41].

Bone

The mechanism of RAL action on bone is to inhibit the estrogen-dependent activity of osteoclasts and thereby reducing bone absorption, leading to a balance between bone formation and demineralization. It also improves bone formation by having a positive effect on the activity and proliferation of osteoblasts. In other words, not only does RAL have an anti-resorptive role, but it also leaves a stimulating effect on osteoblasts [42]. Due to those effects, RAL is now used in the prevention and treatment of postmenopausal osteoporosis. By reducing bone turnover, RAL protects it from damage and destruction and helps reduce osteoporotic fractures. RAL can modulate cytokines that affect the activity of osteoclasts during bone destruction, a mechanism similar to the reabsorption of bone mineralization and is due to the inhibitory effect of RAL on IL-6 and TNF-α production in bone [43]. RAL, on the other hand, regulates the expression of TGFβ 3, which inhibits the differentiation of osteoclasts [44].

There is another effect of RAL which is mediated by Osteoprotegerin (OPG). OPG is a protein expressed by osteoblasts and inhibits the process of osteoporosis by osteoclasts, which ultimately leads to increased bone mineral density. It has been shown that serum OPG levels increase in postmenopausal women after RAL treatment [45]. In general, RAL is an effective SERM in the treatment of osteoporosis fractures [46] and represents an effective treatment in preventing vertebral fractures in postmenopausal women [47].

The results of a meta-analysis comparing the effect of RAL and Alendronate used for the treatment and prevention of osteoporosis in postmenopausal women indicated that after two years of follow-up, taking RAL managed to reduce the risk of vertebral and other bone fractures and increased bone mineral density. Obviously, despite the effectiveness of Alendronate in the treatment of osteoporosis and regarding its gastrointestinal side effects, the choice of either of these drugs should be decided depending on the patient's condition [48].

RAL has also been shown to reduce the risk of vertebral fractures due to improved bone mineral density and reduced bone turnover in Japanese postmenopausal women with osteoporosis or osteopenia [49]. Additionally, the preventive effect of RAL on bone loss induced by GnRH agonists in premenopausal women with uterine leiomyomas has been indicated [50]. In men receiving GnRH agonists to treat prostate cancer, RAL use increases the density of the femoral and spinal cord minerals and reduces the risk of fractures [51].

The combination of alfacalcidol and RAL soft capsules in the postoperative treatment of chest fractures combined with postmenopausal osteoporosis can improve dysfunction and bone strength by increasing bone density and reducing the rate of fracture recurrence through regulating bone metabolic indicators [52].

Central nervous system

Some SERMs have demonstrated a neuroprotective effect in various neurogenic experimental models. These agents are capable of reducing the inflammatory response of glial cells, lowering anxiety and depression, helping strengthen cognition, and modulating synaptic plasticity in the rodent hippocampus. In ovariectomized rats, Estradiol, TAM, and RAL can improve cognitive performance in the prefrontal cortex [53]. As SERMs are estrogen agonists in the brain, RAL protects the nervous system by affecting the concentration of glutamate-dependent calcium ions, an effect which is however reversible [6]. RAL therapy activates areas of the brain that are related to cognition and ultimately enhances such brain functions as memory, executive function, verbal skills, and episodic memory [54]. Furthermore, at a dose of 120 mg/day, RAL reduces the risk of cognitive impairment and Alzheimer's disease in postmenopausal women [55].

RAL treatment enhances nerves in the prefrontal and parietal cortex areas following bilateral cerebral cortex damage and leads to increased cognition, proving effective in improving memory in the Morris Water Maze model (MWM) but with no reported effect on reference memory in MWM [56]. Taking RAL 60 mg/day in postmenopausal women along with the usual antipsychotic therapies improves all the symptoms of schizophrenia including negative, positive, and general psychopathological symptoms [57].

Studies on RAL in orchiectomized male rats reveal an improved cognitive function and enhanced hippocampus-dependent memory [58]. It has been demonstrated that RAL in combination with risperidone can improve the negative and psychological symptoms of chronic schizophrenia in men [59], suggesting that estrogen-based therapies may be helpful in schizophrenia-related reverse cognitive impairment [60]. RAL treatment in menopausal women with schizophrenia is also associated with improved cognitive function [61].

It has been found that RAL increases activity in the parahippocampus area, improves learning, and reforms learning disabilities in women and men with schizophrenia [62]. RAL administration of 120 mg/day in women with schizophrenia reduces the disease severity and increases the possibility of treatment [63]. In addition to having beneficial effects on increasing the attention and processing speed and memory of men and women with schizophrenia, RAL is also used as an adjunct therapy for cognitive deficits related to schizophrenia [64].

RAL can express proteins and mRNAs astrocytic glutamate transporters (EAAT1 and EAAT2) *via* the NF-κB pathway. EAAT, as excitatory amino acid transporters, control the absorption of excess glutamate from the synaptic space. RAL also plays a role in raising glutamate uptake by increasing EAAT2 expression and activates several intracellular signaling pathways that are effective in the up-regulation of GLT-1-induced RAL. Impaired astrocytic glutamate transport is associated with various neurological diseases such as Parkinson's disease, Alzheimer's disease, and amyotrophic sclerosis. RAL increases GLT-1 and GLST protein levels and glutamate uptake into astrocytes, an increase which is due to the expression of GLT-1 by both receptors (ERα and ERβ) and G protein with ER GPR30

that functions by activating several signaling proteins such as MAPK, EGFR, PKA, CREB, and NF-κB [65].

The effect of RAL in the brain is similar to that of conjugated estrogen on dopamine and serotonin. RAL may, however, be an even better option as it does not have negative estrogenic effects on breast and uterine tissues while at the same time it has a beneficial effect on some verbal memory processes compared to the effects observed in women receiving antipsychotic drugs [66]. RAL treatment may have preventive and protective effects on the rat brain against oxidative stress and lipid peroxidation [67], and it is also used to treat and prevent cerebral vasospasm and subsequent cerebral ischemia [68].

Adjuvant therapies have recently been used in the treatment of schizophrenia due to the recognition of the therapeutic effects of SERMs in this case, with RAL appearing to be more effective [69]. A meta-analysis study revealed the effective role of RAL as an adjunct therapy in improving the symptoms of schizophrenia [70]. Compared to other estrogenic drugs such as tibolone, RAL has even proven effective in improving verbal memory and health status [71]. Experimentally, it performs a strong neuroprotective function against autoimmune encephalomyelitis, which takes place through inhibiting CCL20 expression and the NF-κB pathway in reactive astrocytes [72].

Cardiovascular system

Estrogen and RAL inhibit the growth of vascular smooth muscle cells by apoptosis through the p38 cascade, which are also activated by a non-genomic ERα mechanism [73]. RAL, *via* the ERα, can also have an anti-proliferative effect on vascular smooth muscle cells treated with PDGF (Platelet-Derived Growth Factor) by inhibiting pRb (Retinoblastoma Protein) phosphorylation [74]. By targeting calcium channels and inhibiting cellular contraction, RAL can modify cardiac function [75]. It can also have a protective effect on the function of endothelial vessels in the postmenopausal period [76].

Another positive role of RAL is the rise in the NO production in the endothelium. RAL treatment helps regenerate the L-arginine/NO pathway and modulate endothelial dysfunction. Even its long-term use brings about a positive effect on blood pressure in older men and ovariectomized women, which in turn reduces the release of free radicals and improves eNOS/NO function, ultimately improving endothelial dysfunction [77].

Due to the increase in calcium ions, RAL is capable of preventing myogenic contraction in endothelial cells through the NO-dependent mechanism as the result of the activation of eNOS. Resistant arteries in female rats dilate further, indicating a significant gender-related action on the activity of endothelial cells in capillary microcirculation [78].

The advantageous effects of RAL on the cardiovascular system go on even further. RAL dilates coronary arteries by increasing the release of endogenous nitric oxide, which can subsequently improve collateral circulation. In addition, it can hyperpolarize the cell membrane by opening Ca^{+2} -activated K^{+} channels, which ultimately leads to a reduction in the additional Ca^{+2} load during ischemic injury and reperfusion [79]. Another ability of RAL is the improvement of Flow-Mediated Dilatation (FMD), which is disrupted following surgical menopause, making it a treatment of choice for improving and maintaining endothelial function in premenopausal women undergoing ovariectomy [80].

As vascular thrombosis develops following ovariectomy in mice, the use of estradiol and RAL for four months reduces intravascular thrombosis due to their antithrombotic effects, a process which is followed by increased expression of Cyclooxygenase 2 and inhibition of platelet adhesion [81]. Administration of RAL to postmenopausal women yields a positive effect on the endothelial function of the brachial artery and the thickness of the carotid artery wall [82], and increases the endothelial function of coronary arteries by elevating *in vitro* phosphorylation of the eNOS enzyme [83]. RAL treatment is beneficial in reducing postmenopausal heart diseases and preventing heart failure-induced hypertrophy while ruling out the negative effects of estrogen. Symptoms of hypertrophy such as increased heart weight, cardiac wall thickness, and increased myocyte diameter in response to estrogen depletion are all reversible with RAL [84].

Long-term treatment with RAL (1 mg/kg) has been found to reduce the severity of MI-induced injuries and arrhythmias in ovariectomized rats (cases with increased neutrophilic myeloperoxidase following a myocardial infarction). Lactate dehydrogenase levels and plasma creatine kinase have also been shown to decline. These protective effects may be due to the inhibition of neutrophil infiltration and suppression of Nuclear Factor-κB activity [85].

However, due to elevated HDL, RAL should be used with caution for women with hypertriglyceridemia [86]. Furthermore, a treatment regimen with RAL raises glutathione levels significantly in cardiac tissue, presumably one of the most effective barriers to oxidative stress in the heart [87]. RAL gets involved in regulating blood pressure (especially in the case of systemic arterial hypertension) by increasing the renal excretion of sodium and water and indirectly raising plasma NO [88].

Considering postmenopausal vascular problems, a daily intake of 60 mg RAL by women at risk for coronary heart disease may contribute to the risk of stroke and venous thromboembolism in smokers. Therefore, when using RAL for the treatment of osteoporosis or inhibiting invasive breast cancer metastasis in postmenopausal women, stroke and venous thromboembolic risks should be taken into account based on individual history of smoking, thromboembolism, or inactivity [89]. Additionally, according to the results of a meta-analysis, RAL increases the risk of DVT and pulmonary embolism in postmenopausal women, hence necessitating caution in its administration for high-risk patients [90]. Another mechanism of action of RAL on blood vessels is that it can relax rat cerebral arteries without affecting the endothelium of renal, pulmonary, or porcine coronary arteries through inhibition of Ca^{+2} release from calcium channels [91-94].

Thyroid gland

The effect of RAL on thyroid gland morphology is similar to that of estrogen in ovariectomized rats [95]. As estrogen stimulates the growth of thyroid cells, RAL can regulate VEGF and NOS III (Nitric Oxide Synthase -3) due to its estrogen-like effect, thus leaving a beneficial effect on the thyroid of ovariectomized rats [96]. In postmenopausal women, RAL may also increase the production of serum TBG (Thyroxine-Binding Globulin) while the levels of serum TSH and free T4 do not change significantly [97]. However, in the case of hypothyroidism, RAL may cause levothyroxine malabsorption in the patients, a temporary effect to be resolved by avoiding concomitant administration of these two drugs at intervals of 12 h. Nevertheless, RAL administration should be done with caution in postmenopausal women with hypothyroidism [98].

Table 1: A selection of researches about raloxifene effects on body tissues.

Study/Year	Population	Treatment/Dose	Outcome	Tissue
Mirzapur, *et al*. 2018	MCF7, MDA231 Cell lines	1 mg	Increase in apoptosis and gene expression	Breast
Nikolic, *et al*. 2017	T hESC cell line (human endometrial stromal cell)	From 10–5 M to 10–10 M	Increase in the Bax/Bcl-2 ratio and activation of caspase 3.	Uterus
Yang, *et al*. 2010	Cell Culture	0.1 or 1 μm/L	WPMY-1 and BPH-1 cells investigated, antagonized the effect of estradiol in promoting the proliferation of the two cells	Prostate
	Male Wistar Rats	0.075, 0.15, 0.3 mg for 17 days	The number of acini decreased	
Gianni, *et al*. 2004	Postmenopausal women with osteoporosis	60 mg/day for 6 months	Modulate circulating levels of cytokines involved in osteoclastogenesis and bone resorption	Bone
Velazquez-Zamora, *et al*. 2012	Ovariectomized Rats	1 mg/kg Single dose	Increase in the numerical density of dendritic spines in the prelimbic prefrontal cortex	Nervous System
Liew R, *et al*. 2004	Myocytes Culture	1 μm	Shortened action potential durations at 50 and 90% repolarisation and decreased peak L-type Ca^{+2}	Heart (contraction)
LFB deAraujo, *et al*. 2010	Rats	2.5 mg/kg for 50 consecutive days	Upregulate VEGF and NOS III on the thyroid microvasculature of the Ovx rats.	Thyroid
Nishi, *et al*. 2013	Mice	5 mg/kg/day, gavage) for 4 weeks	Improved tubulointerstitial fibrosis, the tubular damage score was lower in the RAL group than in the OVX group	Kidney
	Cell Culture	1 μmol/l		
Azevedo, *et al*. 2005	Postmenopausal Women	60 mg/day (oral)	Factor VIII, Factor XI and XII activities increased, APC sensitivity ratio decreased	Coagulative System
Oztas, *et al*. 2011	Healthy postmenopausal women	60 mg/day	Reduction in serum CRP concentrations, TC and LDL levels	Serum Lipid Profiles

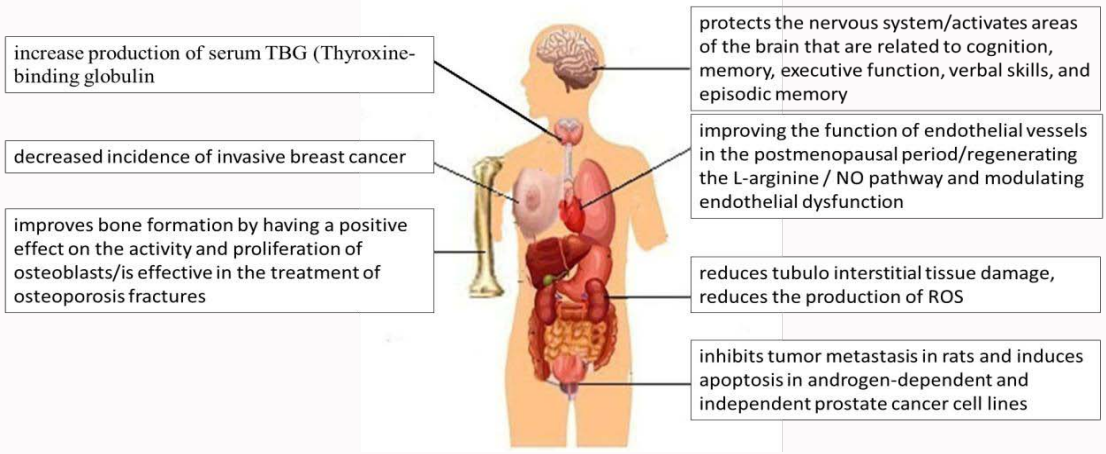

Figure 2: RAL effect on body organs.

Kidney

Oral administration of RAL (60 mg/day for 3 months) in postmenopausal women undergoing hemodialysis due to chronic renal failure can reduce serum MDA, NO, and LDL levels while increasing HDL levels at the same time [99]. RAL reduces tubulointerstitial tissue damage by decreasing the production of Reactive Oxygen Species (ROS) and improves mitochondrial respiratory function, mechanisms that lower mitochondrial oxidative stress induced by proteinuria in proximal tubular cells and changes such as fibrosis by RAL effect. This indicates that stimulating the estrogen receptor in ovariectomized mice can protect the kidney against damage through anti-fibrotic and anti-inflammatory mechanisms. Therefore, RAL not only prevents the progression of osteoporosis due to the absence of ovaries but also hinders the development of chronic kidney disease [100].

Blood coagulation parameters

RAL exerts a prothrombotic effect on the coagulation system [101] and, according to the results of MORE and CORE trials, increases the risk of venous thromboembolism similar to that occurring with TAM and estrogen in postmenopausal women. After RAL treatment, increased plasma levels of factors VIII, XI, and XII and decreased sensitivity to Activated Protein C (APC) are observed in postmenopausal women [102]. Furthermore, six months of RAL treatment can also lead to an increase in the blood's procoagulant parameters, but 12 months later the anticoagulant parameters will decline. Similarly, it also increases fibrinogen in the first 6 months of treatment; but 12-month treatment can reduce the activity of protein C and antithrombin [103].

Serum lipid profiles

RAL improves serum lipid levels and increases HDL in women with type 2 diabetes, especially in the absence of statin therapy [104]. It also reduces the concentration of cholesterol and beta lipoprotein, which are even seen in high-risk women with heart disease and hypertriglyceridemia [105]. In postmenopausal women, taking 60 mg of RAL daily for six months leads to a decrease in LDL, cholesterol, and CRP levels, and can exert a preventive effect on the development of cardiovascular diseases in healthy postmenopausal women [106]. RAL, in general, lowers cholesterol and Low-Density Lipoprotein (LDL) [98]. It should be noted that serum triglyceride levels should be monitored after initiating RAL treatment in people with a history of oral estrogen use and high blood triglyceride levels [86]. Table 1 and Figure 2 summarize the effects of RAL on some organs, and Figure

3 illustrates some molecular roles of RAL on various organs of the body.

Oxidative stress

Oxidative stress is involved in the pathogenesis of BC by inducing nuclear DNA damage through mutations in tumor suppressor genes such as p53 [107]. RAL plays an antioxidant role by increasing the activity of antioxidant enzymes and reducing the production of lipid peroxidation products. As catalase activity is low in postmenopausal women, RAL consumption improves catalase activity and reduces oxidative stress [108].

Nitric oxide

Compared with estrogen, RAL can improve mesenteric vascular endothelial dysfunction in terms of restoring NO production in ovarian hormone-deficient mice and induce endothelial adaptation, quite similar to what estrogen does in the case of NO availability and the production of inflammatory biomarkers [76]. RAL has anti-inflammatory effects on RAW264.7 cells of murine macrophages which are induced and activated by lipopolysaccharide. Due to the inhibition of the PI 3-kinase-Akt-NF-κB signaling pathway, RAL decreases expression of inflammatory protein genes. What follows is the decreased expression of iNOS inflammatory protein genes and the consequent reduction in NO production [109].

SERMs can regulate cellular signaling events by interacting with targets other than ER. RAL is a potent inducer of an anti-inflammatory enzyme, *i.e.* Heme Oxygenase-1 (HO-1), and can interfere with anti-inflammatory responses in RAW264.7 macrophages [110]. Heme oxygenase is a microsomal enzyme that contains both heme oxygenase and NADPH-cytochrome P450 reductase. In addition, it plays an important role in physiological catabolism's, the final products of which are biliverdin, carbon monoxide, and iron [111]. RAL also inhibits iNOS expression and NO production by inducing the expression of HO-1 through stimulating xanthine oxidase and ROS production, meaning that HO-1 mediates the anti-inflammatory properties of RAL. SERMs, and especially RAL, are synthetic estrogen ligands and are strong stimulants of HO-1 [110].

The anti-inflammatory properties of HO-1 include the inhibition of adhesion-molecule expression and reduction of oxidative stress [112]. RAL induces vasorelaxation in rat aorta through estrogen and NF-κB receptors. NF-κB is a compound that is also involved in iNOS activation, an agent that triggers a cGMP-dependent signaling pathway leading to aortic relaxation and ultimately increasing cGMP, nitrite, and iNOS protein levels in the microvascular system. Calcium release from Ca^{+2} channels in rat aortic smooth muscle is also inhibited in this case. Therefore, this relaxation is not due to the direct effect on endothelial function [113].

Ocular system

Blasts often damage the retina and optic nerves, and the pro-inflammatory activities of microglia exacerbate this damage. Cannabinoid type 2 (CB2) receptor inverses agonists, target activated microglia in particular, and turns them off. RAL is a CB2 inverse agonist, and treatment results have indicated improvements in photosensitivity and optic nerve axons [114]. It needs to be noted that RAL consumption does not increase the incidence of cataracts [115].

COVID-19

RAL has been used as a molecule for the treatment of COVID-19 due to its modulation of Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) and as an immunomodulator to reduce pro-inflammatory cytokines capable of shortening the time of virus clearance. Another possible effect of the drug is the modulation of estrogen-regulated signaling, which provides a mechanism in the host for the body to maintain its activity against emerging virus types. It therefore provides a favorable option for preventing or slowing down COVID-19 progression and its related complications. This research has been implemented until phase 2 so far [116].

Another application of RAL concerns the management of inflammatory diseases associated with "cytokine storm". Human C5a, one of the pro-inflammatory glycoproteins of the complement system, responds to stress and infection and is associated with the pathogenesis of many chronic and acute diseases through creating a "cytokine storm" by binding to the C5aR receptor. Therefore, it is optimal to neutralize the harmful effect of C5a in the case of diseases. Human C5a (hC5a) can sequentially accept more than one molecule of RAL on its surface, an effective measure in managing inflammatory, cytokine-storm-related diseases such as COVID-19. The role of hC5a in causing "cytokine storm" and respiratory distress caused by inflammation has been proven, and finding an effective vaccine for COVID-19 and the synergy of a combination of drugs in its treatment is worth consideration [117].

In the case of the new coronavirus SARS-CoV-2, RAL was used as a selective estrogen receptor modulator for the treatment of patients with mild to moderate COVID-19. The beneficial effect of RAL against viral infection is due to its ability to interact with viral proteins and activate estrogen receptor protective mechanisms in host cells. Studies have shown that RAL has a significant affinity for the Spike protein, and drug treatment does not directly affect Spike/ACE2 interaction or viral internalization in infected cell lines. RAL can counteract spike-induced ADAM17 activation in human lung cells, hence a viable agent in the future management of COVID-19 patients [118].

Other cancers

RAL is effective in the treatment of pancreatic cancer by interrupting the cell cycle in the G2-M phase and causing apoptosis [119]. The use of RAL in the form of liposomes improves the effectiveness of lung cancer treatment [120]. SERMs as ERβ agonists may help suppress cancer progression not only in the liver but also in other tissues such as the breast. RAL suppresses TGF-α-induced hepatocellular carcinoma HCC cell migration through inhibition of the ERβ-mediated AKT signaling pathway. ERβ activation inhibits the migration activity of HCC cells and is important in preventing HCC metastasis [121].

Others

RAL improves the effectiveness of the current standard of care in the treatment of postmenopausal women with chronic hepatitis C and can be used as an adjunct in the standard antiviral therapy of these patients [122]. RAL also reduces IGF-I levels in women with mild to moderate acromegaly, returning the disease condition to normal in many patients [123]. RAL nanoemulsion gel has been found to cause re-epithelialization wound healing, neovascularization, fibroblast proliferation, and collagen deposition in oophorectomized rats, developments which are also important in menopausal skin wound healing [124].

RAL Molecular Mechanisms in Cancers

The anti-tumor and anti-metastatic effects of RAL [125] are

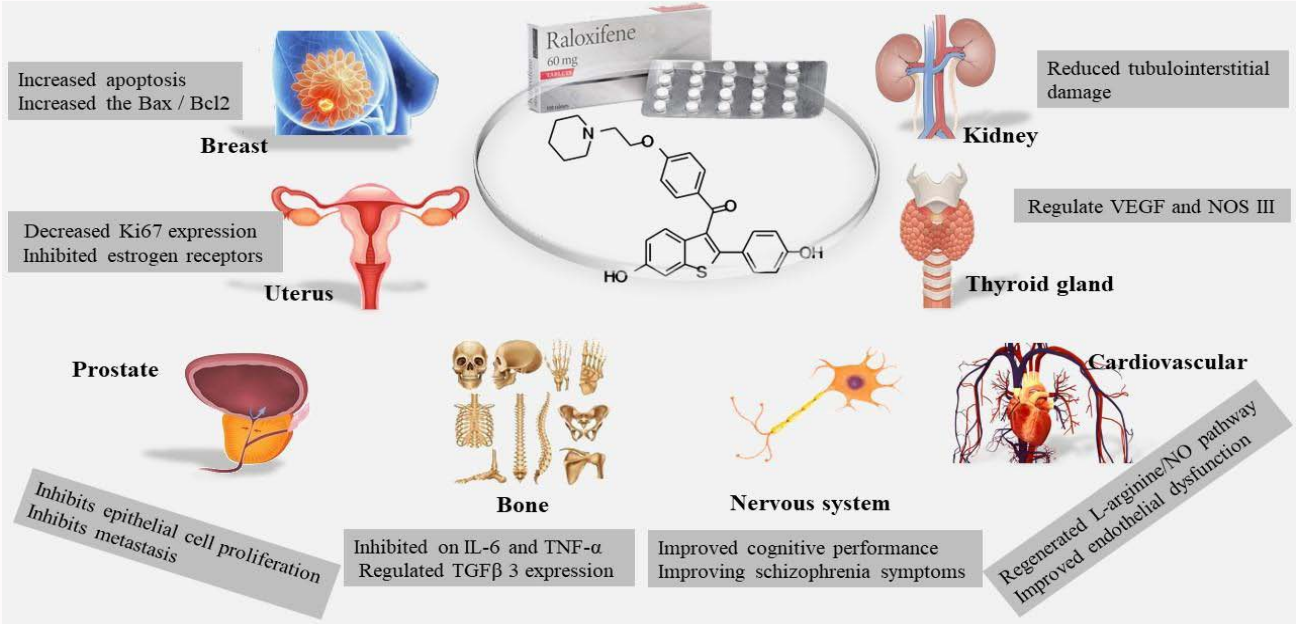

Figure 3: The molecular role of RAL on various body organs.

significant in reducing the risk of IBC and estrogen-induced metastasis in postmenopausal women. By weakening the restructuring and remodeling of cytoskeletal actin through the formation of special structures associated with cell membranes, RAL reduces the movement and remodeling of estrogen-affected cancer cells. This effect of RAL is on cell migration due to its interference with an extra-nuclear signaling cascade involving G proteins, RhoA-associated kinase, ROCK-2, and associated with myosin (Cytoskeletal Controller). Thus, it can be said that RAL acts as an ER antagonist in the presence of estradiol [126].

In most human cancers, activation of the IL-6/STAT3 signaling pathway, including BC and colorectal cancer as well as multiple myeloma, is observed. It is promising to pay attention to the IL-6/STAT3 pathway in cancer prevention and treatment. RAL is capable of inhibiting IL-6 STAT3 phosphorylation in breast cancer cell lines. Inhibition of IL-6 induced STAT3 phosphorylation can therefore be used as a chemotherapeutic drug in BC and colorectal cancer or myeloma [127].

RAL can accelerate the progression of clinical treatments in cancers associated with IL6/GP130/STAT3 pathways [128]. An interesting feature of STAT proteins in cancer treatment is that blocking STAT3 function is sufficient to inhibit tumor cell growth and induction of apoptosis. STAT3 is directly involved in oncogenesis by increasing cell proliferation and preventing apoptosis while STATs in general participate in oncogenesis by modulating genes that upregulate apoptosis and cell cycle inhibitors [129]. In the treatment of cancers, targeting STAT3 can prevent the progression of cancer, which is a promising factor for molecular chemotherapy against tumors [130].

The oral dose of daily RAL reduces EGFR expression up to 27-fold, decreases cell proliferation and apoptosis, and suppresses tumor growth in two xenograft mouse model of Triple Negative BC (TNBC). RAL is effective in reducing migration and invasion of BC cell lines *in vitro*, in which case treatment is independent of ERα. Therefore, it can be considered a valuable cure for Triple-Negative BC (TNBC), a condition with a poor prognosis in response to chemotherapy [131].

Another RAL effect on molecular pathways is activating AMPK, which causes autophagy by reducing ATP and cell death, thus resulting in anti-cancer effects of RAL on BC cells [132].

The response of prostate cells to RAL treatment *in vitro* depends on the expression of ERβ or ERα. RAL consumption affects transcriptional regulation and non-genomic signals and ultimately affects the signaling pathway of apoptosis and the progression of the cell cycle [133]. It also affects the growth of MCF7 cancer cells *in vitro* and *in vivo* by inhibiting the cell cycle in the G2/M phase, reducing NF-κB activity, and increasing apoptosis by increasing FAS expression [134]. Despite the fact that the predominant receptor expressed in bladder cancer cell lines is ERβ and their number increases with increasing tumor grading, the positive effects of antiestrogens on them have been seen [135]. RAL seems to induce apoptosis in the human bladder cancer cell line (TSU-Pr1). This line, like other bladder cancer cell lines, has a large number of ERβs and can indicate the potential role of the beta receptor in the effectiveness of RAL [136].

RAL in Combination with Other Drugs

The combined effect of RAL with natural-derived drugs such as resveratrol on cancer cells in the breast is enhanced and includes such changes as decreased viability and increased apoptosis in estrogen receptor-positive and negative BC cells [23]. Concomitant use of RAL/fluoxetine in rats is a new method in the treatment of BC, and their combination has a better effect on breast cancer than their individual effects separately [137]. Fluoxetine is a Selective Serotonin Reuptake Inhibitor (SSRIs) and an anti-depressant drug with anti-cancer properties [138].

RAL combined with risperidone is helpful in the adjuvant treatment of schizophrenia and improvement in positive symptoms although it is not effective in the treatment of negative and general psychiatric symptoms [139]. Continuous administration of RAL in combination with 5-FU/MTX has an anti-cancer effect with simultaneous protective effects on the bone marrow, proving useful

Table 2: Review of breast cancer chemoprevention trials of selective estrogen receptor modulators.

	Raloxifene	Number of patients	Duration of follow-up (years)	Population
CORE	60, 120 mg daily	4011	4	Postmenopausal women
RUTH	60 mg daily	10101	5.6	CHD and postmenopausal women
STAR	60 mg daily/tamoxifene 20 mg daily	19747	5	High risk Gail score ≥ 1.66% and postmenopausal
MORE	60, 120 mg daily	7705	4	Postmenopausal women with osteoporosis

for patients at risk of osteoporosis [140]. It was found that in women over 70, RAL and tibolone significantly increased body mass density but did not alter muscle strength [71].

RAL in Clinical Trials

Various experiments have been performed to evaluate the effect of RAL. The most important of these studies which are briefly reviewed below include MORE, CORE, RUTH, and STAR trials. In these studies, the incidence of BC decreased with RAL.

Multiple Outcomes of Raloxifene Evaluation (MORE) trial

This study was designed over a period of 3 years to investigate the use of RAL in the treatment of fracture risk in postmenopausal women with osteoporosis. The results showed that the risk of vertebral fractures, Bone Mineral Density (BMD) in the femoral neck and spine was reduced by up to 30% in women treated with RAL compared with placebo recipients. In this study, 7,705 postmenopausal women 31 to 80 years old were evaluated based on osteoporosis or a history of fractures and were randomly divided into three groups receiving placebo, 60 mg/day RAL, and 120 mg/day RAL. The groups were followed up and studied for 3 years [141]. MORE evaluation results showed that 4 years of RAL treatment had reduced the incidence of invasive breast cancer in postmenopausal women by 72% compared with placebo [17]. After 40 months of follow-up, breast cancer was reported in 40 patients. 13 out of 5,129 RAL recipients and 27 out of 2,576 women in the placebo group were diagnosed with BC. It was found that RAL reduced the risk of estrogen receptor-positive breast cancer by up to 90% but had no effect on estrogen receptor-negative invasive cancer. It also increased the risk of venous thromboembolic disease but did not increase the risk of endometrial cancer. Among postmenopausal women with osteoporosis, the risk of invasive breast cancer was reduced by 76% during RAL treatment [27].

Continuing Outcomes Relevant to Evista (CORE) trial

Following the initial findings from the MORE study on the risk of BC using RAL, research called the Continuing Outcomes Relevant to Evista trial (CORE) continued. This study was performed to evaluate RAL in the incidence of IBC in postmenopausal women with osteoporosis for 4 years. The results were presented in 2004 and showed that RAL reduced the incidence of positive ER IBC by 66% compared with placebo whereas no negative effects on ER-negative IBC were detected. Furthermore, the incidence of IBC was reduced by 59% in the RAL group over a 4-year period compared with the placebo group [17].

RAL Use for the Heart (RUTH) trial

RUTH was the first large-scale global clinical trial conducted in 26 countries to evaluate the efficacy and long-term safety of RAL in the prevention of chronic heart disease and BC among women at risk for cardiovascular disorders [142]. In this trial, 10,101 postmenopausal women with cardiovascular disease or several risk factors for coronary artery disease were randomly assigned to two groups of RAL or placebo. According to the Gail model, only about 40% of the participants were at high risk of BC. The average time of the study including the treatment and follow-up periods was 5.6 years. It was found that RAL did not reduce the risk of cardiovascular disease, but the incidence of IBC was reduced by 44% and the rate of positive receptor BC was reduced by 55% [143,144]. The final conclusion was that RAL did not significantly affect the risk of primary chronic heart disease, and the benefits of using it in reducing the risk of IBC and bone composition and vertebral fractures should be considered against the increased risks of venous thromboembolism and stroke [143]. The Gail model examines the following risk factors: Age of the menarche, age of the first live birth, number of previous breast biopsies, and number of first-degree relatives with BC. This model reveals that the relative risks associated with previous breast biopsies were lower for women aged 50 years or more compared to young women [145].

The NSABP Study of Tamoxifen and Raloxifene (STAR Trial)

In a five-year study, 19,747 postmenopausal women with a mean age of 58.5 years who were at high risk of BC were compared based on TAM or RAL use. Patients in the study were divided into two groups (20 mg/day TAM and 60 mg/day RAL) based on either studying the Gail model or taking the personal history of the disease and the history of Lobular Carcinoma *in situ* (LCIS) [14]. There was no significant difference between the two groups in terms of IBC, but in the TAM group there were fewer cases of non-invasive cancer and more cases of uterine cancer. Cases of thromboembolic events and cataracts were also lower among the women in the RAL group. Finally, the results showed that RAL was as effective as TAM in reducing the risk of IBC and had fewer side effects [15].

The updated results of STAR study showed that the incidence of invasive endometrial and uterine cancers in the RAL group was lower than the TAM group, and the mean annual incidence of Uterine Hyperplasia (mostly atypical hyperplasia) and the number of hysterectomies were also lower in this group. Furthermore, the main STAR report indicated a significant reduction in the number of cases of cataracts in the RAL group [18]. Table 2 summarizes the SERMs trials.

Side Effects and Contraindications

From the beginning of RAL consumption, prevalence of high hot flashes and leg cramps are two common complications among consumers, but they do not prevent continued use [146]. RAL is contraindicated with a history of deep-vein thrombosis, pulmonary embolism, thrombotic stroke, transient ischemic attack, and pregnancy [147].

Conclusion

RAL, a type of SERM, is an effective treatment for preventing osteoporosis and vertebral fractures and maintaining the skeletal

system in postmenopausal women. It prevents BC without increasing the risk of endometrial cancer and venous thromboembolism without inducing hot flashes. Administration of this drug for the prevention of BC needs to be done in light of its benefits, problems, and side effects. Therefore, despite being less effective in reducing the risk of recurrence or metastasis than TAM, it offers a more acceptable role in healthy but high-risk women who decide not to undergo surgery. Once measures are taken to monitor its risk of thromboembolism in patients, RAL has proven to be a value drug.

Acknowledgment

We extend our thanks to the staffs in Fertility and Infertility Research Center, Health Technology Institute, Kermanshah University of Medical Sciences.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA: Cancer J Clin. 2016;66(1):7-30.
2. Marmot MG, Altman DG, Cameron DA, Dewar JA, Thompson SG, Wilcox M. The benefits and harms of breast cancer screening: An independent review. Br J Cancer. 2013;108(11):2205-40.
3. Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. N Engl J Med. 2006;354(3):270-82.
4. Riggs BL, Hartmann LC. Selective estrogen-receptor modulators -- mechanisms of action and application to clinical practice. N Engl J Med. 2003;348(7):618-29.
5. Lewis JS, Jordan VC. Selective Estrogen Receptor Modulators (SERMs): Mechanisms of anticarcinogenesis and drug resistance. Mutat Res. 2005;591(1-2):247-63.
6. Huang Y, Huang YL, Lai B, Zheng P, Zhu YC, Yao T. Raloxifene acutely reduces glutamate-induced intracellular calcium increase in cultured rat cortical neurons via inhibition of high-voltage-activated calcium current. Neuroscience. 2007;147(2):334-41.
7. Sundararajan V, Chen S, Rosengren R. Raloxifene: Promises and challenges as a drug treatment for castrate resistant prostate cancer. Enliven: Toxicol Allied Clin Pharmacol. 2017;4(1):001.
8. Levin ER, Pietras RJ. Estrogen receptors outside the nucleus in breast cancer. Breast Cancer Res Treat. 2008;108(3):351-61.
9. Lindberg MK, Moverare S, Skrtic S, Gao H, Dahlman-Wright K, Gustafsson JA, et al. Estrogen Receptor (ER)-beta reduces ERalpha-regulated gene transcription, supporting a "ying yang" relationship between ERalpha and ERbeta in mice. Mol Endocrinol. 2003;17(2):203-8.
10. Fuqua SA, Schiff R, Parra I, Moore JT, Mohsin SK, Osborne CK, et al. Estrogen receptor beta protein in human breast cancer: correlation with clinical tumor parameters. Cancer Res. 2003;63(10):2434-9.
11. Paruthiyil S, Parmar H, Kerekatte V, Cunha GR, Firestone GL, Leitman DC. Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. Cancer Res. 2004;64(1):423-8.
12. Visvanathan K, Chlebowski RT, Hurley P, Col NF, Ropka M, Collyar D, et al. American society of clinical oncology clinical practice guideline update on the use of pharmacologic interventions including tamoxifen, raloxifene, and aromatase inhibition for breast cancer risk reduction. J Clin Oncol. 2009;27(19):3235-58.
13. Lee WL, Chao HT, Cheng MH, Wang PH. Rationale for using raloxifene to prevent both osteoporosis and breast cancer in postmenopausal women. Maturitas. 2008;60(2):92-107.
14. Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, et al. Effects of tamoxifen *vs.* raloxifene on the risk of developing invasive breast cancer and other disease outcomes: the NSABP Study of Tamoxifen and Raloxifene (STAR) P-2 trial. JAMA. 2006;295(23):2727-41.
15. Vogel VG. The NSABP Study of Tamoxifen and Raloxifene (STAR) trial. Expert Rev Anticancer Ther. 2009;9(1):51-60.
16. Jordan VC. Beyond raloxifene for the prevention of osteoporosis and breast cancer. Br J Pharmacol. 2007;150(1):3-4.
17. Martino S, Cauley JA, Barrett-Connor E, Powles TJ, Mershon J, Disch D, et al. Continuing outcomes relevant to Evista: Breast cancer incidence in postmenopausal osteoporotic women in a randomized trial of raloxifene. J Natl Cancer Inst. 2004;96(23):1751-61.
18. Vogel VG, Costantino JP, Wickerham DL, Cronin WM, Cecchini RS, Atkins JN, et al. Update of the national surgical adjuvant breast and bowel project Study of Tamoxifen and Raloxifene (STAR) P-2 trial: Preventing breast cancer. Cancer Prev Res (Phila). 2010;3(6):696-706.
19. Lippman ME, Cummings SR, Disch DP, Mershon JL, Dowsett SA, Cauley JA, et al. Effect of raloxifene on the incidence of invasive breast cancer in postmenopausal women with osteoporosis categorized by breast cancer risk. Clin Cancer Res. 2006;12(17):5242-7.
20. Freedman AN, Yu B, Gail MH, Costantino JP, Graubard BI, Vogel VG, et al. Benefit/risk assessment for breast cancer chemoprevention with raloxifene or tamoxifen for women age 50 years or older. J Clin Oncol. 2011;29(17):2327-33.
21. Cummings SR, Duong T, Kenyon E, Cauley JA, Whitehead M, Krueger KA, et al. Serum estradiol level and risk of breast cancer during treatment with raloxifene. JAMA. 2002;287(2):216-20.
22. da Silva BB, dos Santos AR, Pires CG, Lopes-Costa PV. Effect of raloxifene on vascular endothelial growth factor expression in breast carcinomas of postmenopausal women. Cell Prolif. 2009;42(4):506-10.
23. Mirzapur P, Khazaei MR, Moradi MT, Khazaei M. Apoptosis induction in human breast cancer cell lines by synergic effect of raloxifene and resveratrol through increasing proapoptotic genes. Life Sci. 2018;205:45-53.
24. Lawrence SE, Faught KA, Vethamuthu J, Lawson ML. Beneficial effects of raloxifene and tamoxifen in the treatment of pubertal gynecomastia. J Pediatr. 2004;145(1):71-6.
25. Tran VTH, Pham DV, Choi DY, Park PH. Mitophagy induction and aryl hydrocarbon receptor-mediated redox signaling contribute to the suppression of breast cancer cell growth by raloxifene via regulation of inflammasomes activation. Antioxid Redox Signal. 2022;37(13-15):1030-50.
26. Nikolic I, Andjelkovic M, Zaric M, Zelen I, Canovic P, Milosavljevic Z, et al. Induction of mitochondrial apoptotic pathway by raloxifene and estrogen in human endometrial stromal ThESC cell line. Arch Med Sci. 2017;13(2):293-301.
27. Cummings SR, Eckert S, Krueger KA, Grady D, Powles TJ, Cauley JA, et al. The effect of raloxifene on risk of breast cancer in postmenopausal women: Results from the MORE randomized trial. Multiple Outcomes of Raloxifene Evaluation. JAMA. 1999;281(23):2189-97.
28. Komm BS, Mirkin S. An overview of current and emerging SERMs. J Steroid Biochem Mol Biol. 2014;143:207-22.
29. Blauer M, Heinonen PK, Rovio P, Ylikomi T. Effects of tamoxifen and raloxifene on normal human endometrial cells in an organotypic in vitro model. Eur J Pharmacol. 2008;592(1-3):13-8.
30. Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep. 2015;11(3):1566-72.
31. Morishima S, Shibata MA, Ohmichi M, Otsuki Y. Raloxifene, a selective estrogen receptor modulator, induces mitochondria-mediated apoptosis in human endometrial carcinoma cells. Med Mol Morphol.

2008;41(3):132-8.

32. Chung SH, Lambert PF. Prevention and treatment of cervical cancer in mice using estrogen receptor antagonists. Proc Natl Acad Sci U S A. 2009;106(46):19467-72.

33. Ma JQ, Wang XH, Tang LP, Chen XW, Lou G. Raloxifene suppress proliferation-promoting function of estrogen in CaSKi cervical cells. Int J Clin Exp Med. 2015;8(4):5571-5.

34. Spurgeon ME, Chung SH, Lambert PF. Recurrence of cervical cancer in mice after selective estrogen receptor modulator therapy. Am J Pathol. 2014;184(2):530-40.

35. Ranjbarvan P, Khazaei F, Chobsaz F, Khazaei M. Comparison of raloxifene effect on the growth and angiogenesis of human endometrium of healthy and endometriosis subjects: An *in vitro* three-dimensional tissue culture model. J Endometr Pelvic Pain Disord. 2021;13(3):178-84.

36. Kim IY, Seong DH, Kim BC, Lee DK, Remaley AT, Leach F, et al. Raloxifene, a selective estrogen receptor modulator, induces apoptosis in androgen-responsive human prostate cancer cell line LNCaP through an androgen-independent pathway. Cancer Res. 2002;62(13):3649-53.

37. Kim IY, Kim BC, Seong DH, Lee DK, Seo JM, Hong YJ, et al. Raloxifene, a mixed estrogen agonist/antagonist, induces apoptosis in androgen-independent human prostate cancer cell lines. Cancer Res. 2002;62(18):5365-9.

38. Royuela M, de Miguel MP, Bethencourt FR, Sanchez-Chapado M, Fraile B, Arenas MI, et al. Estrogen receptors alpha and beta in the normal, hyperplastic and carcinomatous human prostate. J Endocrinol. 2001;168(3):447-54.

39. Yang R, Ma YX, Chen LF, Zhou Y, Yang ZP, Zhu Y, et al. Antagonism of estrogen-mediated cell proliferation by raloxifene in prevention of ageing-related prostatic hyperplasia. Asian J Androl. 2010;12(5):735-43.

40. Piccolella M, Crippa V, Messi E, Tetel MJ, Poletti A. Modulators of estrogen receptor inhibit proliferation and migration of prostate cancer cells. Pharmacol Res. 2014;79:13-20.

41. Pritchard T, Rosengren RJ, Greish K, Taurin S. Raloxifene nanomicelles reduce the growth of castrate-resistant prostate cancer. J Drug Target. 2016;24(5):441-9.

42. Taranta A, Brama M, Teti A, De luca V, Scandurra R, Spera G, et al. The selective estrogen receptor modulator raloxifene regulates osteoclast and osteoblast activity *in vitro*. Bone. 2002;30(2):368-76.

43. Gianni W, Ricci A, Gazzaniga P, Brama M, Pietropaolo M, Votano S, et al. Raloxifene modulates interleukin-6 and tumor necrosis factor-alpha synthesis *in vivo*: Results from a pilot clinical study. J Clin Endocrinol Metab. 2004;89(12):6097-9.

44. Yang NN, Bryant HU, Hardikar S, Sato M, Galvin RJ, Glasebrook AL, et al. Estrogen and raloxifene stimulate transforming growth factor-beta 3 gene expression in rat bone: A potential mechanism for estrogen- or raloxifene-mediated bone maintenance. Endocrinol. 1996;137(5):2075-84.

45. Messalli EM, Mainini G, Scaffa C, Cafiero A, Salzillo PL, Ragucci A, et al. Raloxifene therapy interacts with serum osteoprotegerin in postmenopausal women. Maturitas. 2007;56(1):38-44.

46. Vogelvang TE, van der Mooren MJ, Mijatovic V, Kenemans P. Emerging selective estrogen receptor modulators: special focus on effects on coronary heart disease in postmenopausal women. Drugs. 2006;66(2):191-221.

47. Hansdottir H. Raloxifene for older women: A review of the literature. Clin Interv Aging. 2008;3(1):45-50.

48. Lin T, Yan SG, Cai XZ, Ying ZM, Yuan FZ, Zuo X. Alendronate versus Raloxifene for postmenopausal women: A meta-analysis of seven head-to-head randomized controlled trials. Int J Endocrinol. 2014;2014:796510.

49. Fujiwara S, Hamaya E, Sato M, Graham-Clarke P, Flynn JA, Burge R. Systematic review of raloxifene in postmenopausal Japanese women with osteoporosis or low bone mass (osteopenia). Clin Interv Aging. 2014;9:1879-93.

50. Palomba S, Orio F, Jr., Morelli M, Russo T, Pellicano M, Nappi C, et al. Raloxifene administration in women treated with gonadotropin-releasing hormone agonist for uterine leiomyomas: Effects on bone metabolism. J Clin Endocrinol Metab. 2002;87(10):4476-81.

51. Smith MR, Fallon MA, Lee H, Finkelstein JS. Raloxifene to prevent gonadotropin-releasing hormone agonist-induced bone loss in men with prostate cancer: A randomized controlled trial. J Clin Endocrinol Metab. 2004;89(8):3841-6.

52. Xiang Y, Feng C, Tang J, Xiao Y, Chen J. Study on application of alfacalcidol soft capsule combined with raloxifene in the postoperative treatment of postmenopausal osteoporotic thoracolumbar fractures. Indian J Pharm Sci. 2022:79-83.

53. Velazquez-Zamora DA, Garcia-Segura LM, Gonzalez-Burgos I. Effects of selective estrogen receptor modulators on allocentric working memory performance and on dendritic spines in medial prefrontal cortex pyramidal neurons of ovariectomized rats. Horm Behav. 2012;61(4):512-7.

54. Goekoop R, Duschek EJ, Knol DL, Barkhof F, Netelenbos C, Scheltens P, et al. Raloxifene exposure enhances brain activation during memory performance in healthy elderly males; it's possible relevance to behavior. Neuroimage. 2005;25(1):63-75.

55. Yaffe K, Krueger K, Cummings SR, Blackwell T, Henderson VW, Sarkar S, et al. Effect of raloxifene on prevention of dementia and cognitive impairment in older women: The Multiple Outcomes of Raloxifene Evaluation (MORE) randomized trial. Am J Psychiatry. 2005;162(4):683-90.

56. Kokiko ON, Murashov AK, Hoane MR. Administration of raloxifene reduces sensorimotor and working memory deficits following traumatic brain injury. Behav Brain Res. 2006;170(2):233-40.

57. Usall J, Huerta-Ramos E, Iniesta R, Cobo J, Araya S, Roca M, et al. Raloxifene as an adjunctive treatment for postmenopausal women with schizophrenia: A double-blind, randomized, placebo-controlled trial. J Clin Psychiatry. 2011;72(11):1552-7.

58. Lagunas N, Calmarza-Font I, Grassi D, Garcia-Segura LM. Estrogen receptor ligands counteract cognitive deficits caused by androgen deprivation in male rats. Horm Behav. 2011;59(4):581-4.

59. Khodaie-Ardakani MR, Khosravi M, Zarinfard R, Nejati S, Mohsenian A, Tabrizi M, et al. A placebo-controlled study of raloxifene added to risperidone in men with chronic schizophrenia. Acta Med Iran. 2015;53(6):337-45.

60. Weickert TW, Allen KM, Weickert CS. Potential role of oestrogen modulation in the treatment of neurocognitive deficits in schizophrenia. CNS Drugs. 2016;30(2):125-33.

61. Kulkarni J, Gurvich C, Gilbert H, Mehmedbegovic F, Mu L, Marston N, et al. Hormone modulation: A novel therapeutic approach for women with severe mental illness. Aust N Z J Psychiatry. 2008;42(1):83-8.

62. Kindler J, Weickert CS, Skilleter AJ, Catts SV, Lenroot R, Weickert TW. Selective estrogen receptor modulation increases hippocampal activity during probabilistic association learning in schizophrenia. Neuropsychopharmacology. 2015;40(10):2388-97.

63. Kulkarni J, Gavrilidis E, Gwini SM, Worsley R, Grigg J, Warren A, et al. Effect of adjunctive raloxifene therapy on severity of refractory schizophrenia in women: A randomized clinical trial. JAMA Psychiatry. 2016;73(9):947-54.

64. Weickert TW, Weinberg D, Lenroot R, Catts SV, Wells R, Vercammen A, et al. Adjunctive raloxifene treatment improves attention and memory in men and women with schizophrenia. Mol Psychiatry. 2015;20(6):685-94.

65. Karki P, Webb A, Zerguine A, Choi J, Son DS, Lee E. Mechanism of raloxifene-induced upregulation of glutamate transporters in rat primary astrocytes. Glia. 2014;62(8):1270-83.

66. Huerta-Ramos E, Iniesta R, Ochoa S, Cobo J, Miquel E, Roca M, et al. Effects of raloxifene on cognition in postmenopausal women with schizophrenia: A double-blind, randomized, placebo-controlled trial. Euro Neuropsychopharmacology. 2014;24(2):223-31.

67. Osmanova S, Sezer E, Turan V, Zeybek B, Cosan Terek M, Kanit L. The effects of raloxifene treatment on oxidative status in brain tissues and learning process of ovariectomized rats. Iran J Reprod Med. 2011;9(4):295-300.

68. Gurses L, Seckin H, Simsek S, Senel OO, Yigitkanli K, Ozturk E, et al. Effects of raloxifene on cerebral vasospasm after experimental subarachnoid hemorrhage in rabbits. Surg Neuro. 2009;72(5):490-4; discussion 4-5.

69. Tharoor H, Goyal A. Raloxifene trial in postmenopausal woman with treatment-resistant schizophrenia. Arch Womens Ment Health. 2015;18(5):741-2.

70. Wang Q, Dong X, Wang Y, Li X. Raloxifene as an adjunctive treatment for postmenopausal women with schizophrenia: A meta-analysis of randomized controlled trials. Arch Womens Ment Health. 2018;21(1):31-41.

71. Jacobsen DE, Melis RJ, Verhaar HJ, Olde Rikkert MG. Raloxifene and tibolone in elderly women: A randomized, double-blind, double-dummy, placebo-controlled trial. J Am Med Dir Assoc. 2012;13(2):189 e1-7.

72. Li R, Xu W, Chen Y, Qiu W, Shu Y, Wu A, et al. Raloxifene suppresses experimental autoimmune encephalomyelitis and NF-kappaB-dependent CCL20 expression in reactive astrocytes. PloS One. 2014;9(4):e94320.

73. Mori-Abe A, Tsutsumi S, Takahashi K, Toya M, Yoshida M, Du B, et al. Estrogen and raloxifene induce apoptosis by activating p38 mitogen-activated protein kinase cascade in synthetic vascular smooth muscle cells. J Endocrinol. 2003;178(3):417-26.

74. Takahashi K, Ohmichi M, Yoshida M, Hisamoto K, Mabuchi S, Arimoto-Ishida E, et al. Both estrogen and raloxifene cause G1 arrest of vascular smooth muscle cells. J Endocrinol. 2003;178(2):319-29.

75. Liew R, Stagg MA, MacLeod KT, Collins P. Raloxifene acutely suppresses ventricular myocyte contractility through inhibition of the L-type calcium current. Br J Pharmacol. 2004;142(1):89-96.

76. Lamas AZ, Caliman IF, Dalpiaz PL, de Melo AF, Jr., Abreu GR, Lemos EM, et al. Comparative effects of estrogen, raloxifene and tamoxifen on endothelial dysfunction, inflammatory markers and oxidative stress in ovariectomized rats. Life Sci. 2015;124:101-9.

77. Leitzbach D, Weckler N, Madajka M, Malinski T, Wiemer G, Linz W. Restoration of endothelial function via enhanced nitric oxide synthesis after long-term treatment of raloxifene in adult hypertensive rats. Arzneimittelforschung. 2005;55(2):86-92.

78. Chan YC, Leung FP, Wong WT, Tian XY, Yung LM, Lau CW, et al. Therapeutically relevant concentrations of raloxifene dilate pressurized rat resistance arteries via calcium-dependent endothelial nitric oxide synthase activation. Arterioscler Thromb Vasc Biol. 2010;30(5):992-9.

79. Ogita H, Node K, Asanuma H, Sanada S, Liao Y, Takashima S, et al. Amelioration of ischemia- and reperfusion-induced myocardial injury by the selective estrogen receptor modulator, raloxifene, in the canine heart. J Am Coll Cardiol. 2002;40(5):998-1005.

80. Takahashi K, Mori-Abe A, Takata K, Ohta T, Kawagoe J, Tsutsumi S, et al. Raloxifene improves the ovariectomy-induced impairment in endothelium-dependent vasodilation. Menopause. 2007;14(4):656-61.

81. Abu-Fanne R, Brzezinski A, Golomb M, Grad E, Foldes AJ, Shufaro Y, et al. Effects of estradiol and raloxifene on arterial thrombosis in ovariectomized mice. Menopause. 2008;15(1):98-104.

82. Sumino H, Ichikawa S, Kasama S, Takahashi T, Sakamoto H, Koizumi A, et al. Effects of raloxifene on brachial arterial endothelial function, carotid wall thickness, and arterial stiffness in osteoporotic postmenopausal women. Int Heart J. 2010;51(1):60-7.

83. Leung FP, Yung LM, Leung HS, Au CL, Yao X, Vanhoutte PM, et al. Therapeutic concentrations of raloxifene augment nitric oxide-dependent coronary artery dilatation *in vitro*. Br J Pharmacol. 2007;152(2):223-9.

84. Selvaraj S, Sehgal R, Mehra RD, Dhar P, Rajamanickam G. Prevention of cardiac myocyte changes by raloxifene, a Selective Estrogen Receptor Modulator (SERM) in rat model of surgically induced estrogen depleted state. Int J Med Res Health Sci. 2017;6(9):139-50.

85. Chung MT, Cheng PY, Lam KK, Chen SY, Ting YF, Yen MH, et al. Cardioprotective effects of long-term treatment with raloxifene, a selective estrogen receptor modulator, on myocardial ischemia/reperfusion injury in ovariectomized rats. Menopause. 2010;17(1):127-34.

86. Carr MC, Knopp RH, Brunzell JD, Wheeler BS, Zhu X, Lakshmanan M, et al. Effect of raloxifene on serum triglycerides in women with a history of hypertriglyceridemia while on oral estrogen therapy. Diabetes Care. 2005;28(7):1555-61.

87. Konyalioglu S, Durmaz G, Yalcin A. The potential antioxidant effect of raloxifene treatment: a study on heart, liver and brain cortex of ovariectomized female rats. Cell Biochem Funct. 2007;25(3):259-66.

88. Moraes AN, Gouvea SA, Goncalves WL, Romero WG, Moyses MR, Bissoli NS, et al. Raloxifene reduces blood pressure in hypertensive animals after ovarian hormone deprivation. Basic Clin Pharmacol Toxicol. 2011;109(5):334-8.

89. Mosca L, Grady D, Barrett-Connor E, Collins P, Wenger N, Abramson BL, et al. Effect of raloxifene on stroke and venous thromboembolism according to subgroups in postmenopausal women at increased risk of coronary heart disease. Stroke. 2009;40(1):147-55.

90. Adomaityte J, Farooq M, Qayyum R. Effect of raloxifene therapy on venous thromboembolism in postmenopausal women. A meta-analysis. Thromb Haemost. 2008;99(2):338-42.

91. Tsang SY, Yao X, Essin K, Wong CM, Chan FL, Gollasch M, et al. Raloxifene relaxes rat cerebral arteries *in vitro* and inhibits L-type voltage-sensitive Ca2+ channels. Stroke. 2004;35(7):1709-14.

92. Leung FP, Yao X, Lau CW, Ko WH, Lu L, Huang Y. Raloxifene relaxes rat intrarenal arteries by inhibiting Ca2+ influx. Am J Physiol Renal Physiol. 2005;289(1):F137-44.

93. Chan YC, Leung FP, Yao X, Lau CW, Vanhoutte PM, Huang Y. Raloxifene relaxes rat pulmonary arteries and veins: Roles of gender, endothelium, and antagonism of Ca2+ influx. J Pharmacol Exp Ther. 2005;312(3):1266-71.

94. Leung HS, Seto SW, Kwan YW, Leung FP, Au AL, Yung LM, et al. Endothelium-independent relaxation to raloxifene in porcine coronary artery. Eur J Pharmacol. 2007;555(2-3):178-84.

95. de Araujo LF, Grozovsky R, de Campos Pinheiro M, de Carvalho JJ, Vaisman M, Carvalho DP. Raloxifene effects on thyroid gland morphology in ovariectomized rats. Fertil Steril. 2008;90(4):1211-4.

96. de Araujo LF, Grozovsky R, dos Santos Pereira MJ, de Carvalho JJ, Vaisman M, Carvalho DP. Expressions of vascular endothelial growth factor and nitric oxide synthase III in the thyroid gland of ovariectomized rats are upregulated by estrogen and selective estrogen receptor modulators. Thyroid. 2010;20(1):85-92.

97. Ceresini G, Morganti S, Rebecchi I, Bertone L, Ceda GP, Bacchi-Modena A, et al. A one-year follow-up on the effects of raloxifene on thyroid function in postmenopausal women. Menopause. 2004;11(2):176-9.

98. Garwood CL, Van Schepen KA, McDonough RP, Sullivan AL. Increased thyroid-stimulating hormone levels associated with concomitant administration of levothyroxine and raloxifene. Pharmacotherapy.

2006;26(6):881-5.

99. Ozbasar D, Toros U, Ozkaya O, Sezik M, Uzun H, Genc H, et al. Raloxifene decreases serum malondialdehyde and nitric oxide levels in postmenopausal women with end-stage renal disease under chronic hemodialysis therapy. J Obstet Gynaecol Res. 2010;36(1):133-7.

100. Nishi Y, Satoh M, Nagasu H, Kadoya H, Ihoriya C, Kidokoro K, et al. Selective estrogen receptor modulation attenuates proteinuria-induced renal tubular damage by modulating mitochondrial oxidative status. Kidney Int. 2013;83(4):662-73.

101. Gizzo S, Saccardi C, Patrelli TS, Berretta R, Capobianco G, Di Gangi S, et al. Update on raloxifene: Mechanism of action, clinical efficacy, adverse effects, and contraindications. Obstet Gynecol Surv. 2013;68(6):467-81.

102. Azevedo GD, Franco RF, Baggio MS, Maranhao TM, Sa MF. Procoagulant state after raloxifene therapy in postmenopausal women. Fertil Steril. 2005;84(6):1680-4.

103. Sgarabotto M, Baldini M, Dei Cas A, Manotti C, Luciana Barilli A, Rinaldi M, et al. Effects of raloxifene and continuous combined hormone therapy on haemostasis variables: A multicenter, randomized, double-blind study. Thromb Res. 2007;119(1):85-91.

104. Matsumura M, Monden T, Nakatani Y, Shimizu H, Domeki N, Yanagi K, et al. Effect of raloxifene on serum lipids for type 2 diabetic menopausal women with or without statin treatment. Med Princ Pract. 2010;19(1):68-72.

105. Dayspring T, Qu Y, Keech C. Effects of raloxifene on lipid and lipoprotein levels in postmenopausal osteoporotic women with and without hypertriglyceridemia. Metabolism. 2006;55(7):972-9.

106. Oztas E, Kurtay G. Randomized, controlled study of the effects of raloxifene on high sensitivity C-reactive protein and serum lipids. Arch Gynecol Obstet. 2011;283(1):71-7.

107. Kang DH. Oxidative stress, DNA damage, and breast cancer. AACN Clin Issue. 2002;13(4):540-9.

108. Ozgocmen S, Kaya H, Fadillioglu E, Yilmaz Z. Effects of calcitonin, risedronate, and raloxifene on erythrocyte antioxidant enzyme activity, lipid peroxidation, and nitric oxide in postmenopausal osteoporosis. Arch Med Res. 2007;38(2):196-205.

109. Lee SA, Park SH, Kim BC. Raloxifene, a selective estrogen receptor modulator, inhibits lipopolysaccharide-induced nitric oxide production by inhibiting the phosphatidylinositol 3-kinase/Akt/nuclear factor-kappa B pathway in RAW264.7 macrophage cells. Mol Cells. 2008;26(1):48-52.

110. Lee SA, Kim EY, Jeon WK, Woo CH, Choe J, Han S, et al. The inhibitory effect of raloxifene on lipopolysaccharide-induced nitric oxide production in RAW264.7 cells is mediated through a ROS/p38 MAPK/CREB pathway to the up-regulation of heme oxygenase-1 independent of estrogen receptor. Biochimie. 2011;93(2):168-74.

111. Kikuchi G, Yoshida T, Noguchi M. Heme oxygenase and heme degradation. Biochem Biophys Res Commun. 2005;338(1):558-67.

112. Sawle P, Foresti R, Mann BE, Johnson TR, Green CJ, Motterlini R. Carbon monoxide-Releasing Molecules (CO-RMs) attenuate the inflammatory response elicited by lipopolysaccharide in RAW264.7 murine macrophages. Br J Pharmacol. 2005;145(6):800-10.

113. Wong CM, Au CL, Tsang SY, Lau CW, Yao X, Cai Z, et al. Role of inducible nitric oxide synthase in endothelium-independent relaxation to raloxifene in rat aorta. Br J Pharmacol. 2017;174(8):718-33.

114. Honig MG, Del Mar NA, Henderson DL, O'Neal D, Yammanur M, Cox R, et al. Raloxifene, a cannabinoid type-2 receptor inverse agonist, mitigates visual deficits and pathology and modulates microglia after ocular blast. Exp Eye Res. 2022;218:108966.

115. Grady D, Ettinger B, Moscarelli E, Plouffe L, Jr., Sarkar S, Ciaccia A, et al. Safety and adverse effects associated with raloxifene: Multiple outcomes of raloxifene evaluation. Obstet Gynecol. 2004;104(4):837-44.

116. Nicastri E, Marinangeli F, Pivetta E, Torri E, Reggiani F, Fiorentino G, et al. A phase 2 randomized, double-blinded, placebo-controlled, multicenter trial evaluating the efficacy and safety of raloxifene for patients with mild to moderate COVID-19. EClinicalMedicine. 2022;48:101450.

117. Mishra R, Behera LM, Rana S. Binding of raloxifene to human complement fragment 5a (hC5a): A perspective on cytokine storm and COVID-19. J Biomol Struct Dyn. 2022;40(3):982-94.

118. Iaconis D, Bordi L, Matusali G, Talarico C, Manelfi C, Cesta MC, et al. Characterization of raloxifene as a potential pharmacological agent against SARS-CoV-2 and its variants. Cell Death Dis. 2022;13(5):1-9.

119. Fahmy UA, Badr-Eldin SM, Aldawsari HM, Alhakamy NA, Ahmed OAA, Radwan MF, et al. Potentiality of raloxifene loaded melittin functionalized lipidic nanovesicles against pancreatic cancer cells. Drug Deliv. 2022;29(1):1863-77.

120. Altyar AE, Fahmy O. Preparation of liposomal raloxifene-graphene nanosheet and evaluation of its *in vitro* anticancer effects. Dose Response. 2022;20(1):15593258211063983.

121. Matsushima-Nishiwaki R, Yamada N, Hattori Y, Hosokawa Y, Tachi J, Hori T, et al. SERMs (selective estrogen receptor modulator), acting as estrogen receptor β agonists in hepatocellular carcinoma cells, inhibit the transforming growth factor-α-induced migration *via* specific inhibition of AKT signaling pathway. PloS One. 2022;17(1):e0262485.

122. Furusyo N, Ogawa E, Sudoh M, Murata M, Ihara T, Hayashi T, et al. Raloxifene hydrochloride is an adjuvant antiviral treatment of postmenopausal women with chronic hepatitis C: A randomized trial. J Hepatol. 2012;57(6):1186-92.

123. Attanasio R, Barausse M, Cozzi R. Raloxifene lowers IGF-I levels in acromegalic women. Eur J Endocrinol. 2003;148(4):443-8.

124. Singh TP, Verma N, Ahmad FJ, Chandra P, Jain GK. *In vivo* wound healing potential of raloxifene nanoemulsion gel for the management of postmenopausal cutaneous wounds. Indian J Pharm Educ Res. 2022;56(2):589-99.

125. Palmer H, Nimick M, Mazumder A, Taurin S, Rana Z, Rosengren RJ. Raloxifene suppresses tumor growth and metastasis in an orthotopic model of castration-resistant prostate cancer. Biomedicines. 2022;10(4):853.

126. Flamini MI, Fu XD, Sanchez AM, Giretti MS, Garibaldi S, Goglia L, et al. Effects of raloxifene on breast cancer cell migration and invasion through the actin cytoskeleton. J Cell Mol Med. 2009;13(8B):2396-407.

127. Shi W, Yan D, Zhao C, Xiao M, Wang Y, Ma H, et al. Inhibition of IL-6/STAT3 signaling in human cancer cells using Evista. Biochem Biophys Res Commun. 2017;491(1):159-65.

128. Li H, Xiao H, Lin L, Jou D, Kumari V, Lin J, et al. Drug design targeting Protein-Protein Interactions (PPIs) using Multiple Ligand Simultaneous Docking (MLSD) and drug repositioning: Discovery of raloxifene and bazedoxifene as novel inhibitors of IL-6/GP130 interface. J Med Chem. 2014;57(3):632-41.

129. Buettner R, Mora LB, Jove R. Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. Clin Cancer Res. 2002;8(4):945-54.

130. Frank DA. STAT3 as a central mediator of neoplastic cellular transformation. Cancer Lett. 2007;251(2):199-210.

131. Taurin S, Allen KM, Scandlyn MJ, Rosengren RJ. Raloxifene reduces triple-negative breast cancer tumor growth and decreases EGFR expression. Int J Oncol. 2013;43(3):785-92.

132. Kim DE, Kim Y, Cho DH, Jeong SY, Kim SB, Suh N, et al. Raloxifene induces autophagy-dependent cell death in breast cancer cells *via* the activation of AMP-activated protein kinase. Mol Cells. 2015;38(2):138-44.

133. Rossi V, Bellastella G, De Rosa C, Abbondanza C, Visconti D, Maione

L, et al. Raloxifene induces cell death and inhibits proliferation through multiple signaling pathways in prostate cancer cells expressing different levels of estrogen receptor alpha and beta. J Cell Physiol. 2011;226(5):1334-9.

134. Liu H, Lee ES, Gajdos C, Pearce ST, Chen B, Osipo C, et al. Apoptotic action of 17beta-estradiol in raloxifene-resistant MCF-7 cells *in vitro* and *in vivo*. J Natl Cancer Inst. 2003;95(21):1586-97.

135. Shen SS, Smith CL, Hsieh JT, Yu J, Kim IY, Jian W, et al. Expression of estrogen receptors-alpha and -beta in bladder cancer cell lines and human bladder tumor tissue. Cancer. 2006;106(12):2610-6.

136. Kim HT, Kim BC, Kim IY, Mamura M, Seong DH, Jang JJ, et al. Raloxifene, a mixed estrogen agonist/antagonist, induces apoptosis through cleavage of BAD in TSU-PR1 human cancer cells. J Biol Chem. 2002;277(36):32510-5.

137. Kabel AM, Elkhoely AA. Ameliorative potential of fluoxetine/raloxifene combination on experimentally induced breast cancer. Tissue Cell. 2016;48(2):89-95.

138. Stopper H, Garcia SB, Waaga-Gasser AM, Kannen V. Antidepressant fluoxetine and its potential against colon tumors. World J Gastrointest Oncol. 2014;6(1):11-21.

139. Kianimehr G, Fatehi F, Hashempoor S, Khodaei-Ardakani MR, Rezaei F, Nazari A, et al. Raloxifene adjunctive therapy for postmenopausal women suffering from chronic schizophrenia: A randomized double-blind and placebo-controlled trial. Daru. 2014;22(1):55.

140. Das JR, Fryar EB, Epie NN, Southerland WM, Bowen D. Raloxifene attenuation of methotrexate cytotoxicity in human bone marrow by sequence-dependent administration of raloxifene, 5-FU/methotrexate. Anticancer Res. 2006;26(3A):1877-83.

141. Ettinger B, Black DM, Mitlak BH, Knickerbocker RK, Nickelsen T, Genant HK, et al. Reduction of vertebral fracture risk in postmenopausal women with osteoporosis treated with raloxifene: Results from a 3-year randomized clinical trial. Multiple Outcomes of Raloxifene Evaluation (MORE) Investigators. JAMA. 1999;282(7):637-45.

142. Mosca L. Rationale and overview of the Raloxifene Use for the Heart (RUTH) trial. Ann N Y Acad Sci. 2001;949:181-5.

143. Barrett-Connor E, Mosca L, Collins P, Geiger MJ, Grady D, Kornitzer M, et al. Effects of raloxifene on cardiovascular events and breast cancer in postmenopausal women. N Engl J Med. 2006;355(2):125-37.

144. Grady D, Cauley JA, Geiger MJ, Kornitzer M, Mosca L, Collins P, et al. Reduced incidence of invasive breast cancer with raloxifene among women at increased coronary risk. J Natl Cancer Inst. 2008;100(12):854-61.

145. Gail MH, Brinton LA, Byar DP, Corle DK, Green SB, Schairer C, et al. Projecting individualized probabilities of developing breast cancer for white females who are being examined annually. J Natl Cancer Inst. 1989;81(24):1879-86.

146. Davies GC, Huster WJ, Lu Y, Plouffe L, Jr., Lakshmanan M. Adverse events reported by postmenopausal women in controlled trials with raloxifene. Obstet Gynecol. 1999;93(4):558-65.

147. Coopey SB, Mazzola E, Buckley JM, Sharko J, Belli AK, Kim EM, et al. The role of chemoprevention in modifying the risk of breast cancer in women with atypical breast lesions. Breast Cancer Res Treat. 2012;136(3):627-33.