

ORIGINAL
INVESTIGATION

Estrogen-like Activity of Quercetin in Female Rats

Semra Yiğitaslan¹, Kevser Erol¹, Fikriye Yasemin Özatik², Orhan Özatik³, Sabiha Şahin⁴, Çiğdem Çengelli¹

ABSTRACT

Objective: Quercetin is a phytoestrogen that exerts both *in vitro* agonistic and antagonistic activities on estrogen receptors. The present study evaluated the *in vivo* estrogen-like activity of quercetin on the reproductive organs of female rats. For this purpose, a partial estrogen agonist tamoxifen (TMX) and an estrogen antagonist fulvestrant (FLV) were used to mimic and antagonize the effects of estrogen on uterine tissue, respectively. 4-Vinylcyclohexene dioxide (VCD) was used to induce primary ovarian failure in rats.

Materials and Methods: In experiment 1, immature female rats (21–22 days old) were treated with a vehicle (control), quercetin (10, 30, and 90 mg/kg), 10 mg/kg of quercetin (Q10)+TMX, Q10+ FLV, 17 β -estradiol (17 β E), 17 β E+TMX, or 17 β E+FLV. In experiment 2, prepubertal female rats (28–29 days old) were treated with a vehicle (dimethyl sulfoxide), VCD-alone, VCD+Q10, or VCD+17 β E. A uterotrophic assay and histological analysis of uteri were performed. The partial estrogen agonist TMX and the estrogen antagonist FLV were used to mimic and antagonize the effects of estrogen on uterine tissue, respectively. VCD was used to induce primary ovarian failure in rats.

Results: In immature female rats, the uterine weight was significantly higher in animals treated with Q10 compared to those treated with the vehicle. Although TMX did not result in a significant change, FLV significantly decreased the uterine weight in Q10-treated rats. In prepubertal female rats, the uterine weight significantly decreased in VCD \pm Q10- or 17 β E-treated animals compared that in VCD-treated animals. Although the endometrial thickness was unchanged in Q10-treated animals, it was significantly decreased in the Q10+FLV-treated animals. VCD significantly decreased the endometrial thickness, which was prevented by Q10.

Conclusion: Quercetin may have a dose-dependent and biphasic effect on the uterus by modulating estrogen receptors.

Keywords: Quercetin, estrogen, 4-vinylcyclohexene dioxide, uterus, 17 β -estradiol

INTRODUCTION

Estrogens are steroid hormones that influence the growth, differentiation, and functioning of many target organs including the male and female reproductive organs. Estrogen is implicated in the development of breast, ovarian, endometrial, and prostate cancer, while estrogen deficiency has been associated with osteoporosis, neurodegenerative diseases, cardiovascular disease, and obesity (1). It induces several cellular changes on binding to an intranuclear binding protein called the estrogen receptor (ER). There are two main forms of ERs, ER α and ER β , with distinct tissue expression patterns (2).

Quercetin is a phytoestrogen belonging to the flavonol subclass of flavonoid compounds, and it exerts many potential beneficial effects on human health. It is found in a variety of fruits and vegetables, particularly in onions (3). Several *in vivo* and *in vitro* studies have suggested a wide range of biological effects of quercetin including antioxidant (4, 5), anticancer (6, 7), antihypertensive (8), anti-inflammatory (9), and antimicrobial (10) effects. Several phytoestrogens have been suggested to bind to and activate ER α and ER β . However, there are inconsistent results in the literature regarding the effects of quercetin on ERs. Quercetin has shown agonistic or antagonistic activity on these receptors. Initial studies on the estrogenic activity of quercetin have reported only anti-estrogenic effects on an estrogen-sensitive breast cancer cell line (MCF-7) (11), while subsequent studies have found that quercetin exerts both estrogenic and anti-estrogenic effects in a dose-dependent manner (12, 13).

Because there are at least two receptors for estrogen in the target tissues and as they have different functions, selective ER modulators (SERMs), including tamoxifen (TMX), have been developed. TMX exerts estrogen agonistic (at the bone and uterus) or antagonistic effects (at the breast) depending on the tissue and receptor subtype (14).

¹Department of Pharmacology, Eskişehir Osmangazi University School of Medicine, Eskişehir, Turkey

²Department of Medical Pharmacology, Ahi Evran University School of Medicine, Kırşehir, Turkey

³Department of Histology and Embriology, Ahi Evran University School of Medicine, Kırşehir, Turkey

⁴Department of Pediatrics, Eskişehir Osmangazi University School of Medicine, Eskişehir, Turkey

Submitted
08.01.2016

Accepted
15.04.2016

Correspondence

Semra Yiğitaslan, MD
Eskişehir Osmangazi Üniversitesi
Tıp Fakültesi Farmakoloji
Anabilim Dalı, Eskişehir, Türkiye
Phone: +90 222 239 29 79
e-mail: scelebi@ogu.edu.tr

Fulvestrant (FLV) is a complete ER antagonist with no agonistic effects. The co-administration of FLV with estradiol or TMX has been shown to prevent the maximum and partial uterotrophic activity induced by estradiol and TMX (15, 16).

Vinylcyclohexene diepoxide (VCD) is an occupational chemical causing the selective destruction of ovarian primordial and primary follicles in rats and mice by accelerating natural atresia (17).

The present study evaluated the *in vivo* estrogen-like activity of quercetin on the reproductive organs of female rats by a uterotrophic assay and histological analysis.

MATERIALS and METHODS

Study animals and treatments

Sprague-Dawley female rats were supplied by the Medical and Surgical Experimental Research Center of Eskisehir Osmangazi University. All animals were housed in a room at 22°C in a 12-h light/dark cycle. Tap water and a pelleted commercial diet were available *ad libitum* throughout the study. The experiments were conducted in accordance with the Animal Care and Use Committee of our institution, and approval was obtained from the local ethical committee (date: 04/26/2011; protocol no: 190-1).

Experiment 1: Immature female rats

Immature female rats (21–22 days old) weighing approximately 35–40 g were randomly divided into nine groups comprising six animals each. The control group (C) received a vehicle [20% dimethyl sulfoxide (DMSO)] (Sigma- Aldrich, St. Louis, MO, USA)/water, and quercetin groups (Q10, Q30, and Q90) were treated with quercetin (Sigma- Aldrich, St. Louis, MO, USA) at doses of 10, 30, or 90 mg/kg/day, respectively. Quercetin was used at a dose of 10 mg/kg/day for the remaining experiments because it was the minimum quercetin dose that induced a significant uterine weight increase. An additional two groups of animals, Q10+TMX and Q10+FLV, received either TMX (Sigma-Aldrich, St. Louis, MO, USA) (2 µg/kg/day) or FLV (Sigma-Aldrich, St. Louis, MO, USA) (5 mg/kg/day), respectively, in combination with 10 mg/kg of quercetin. TMX is a partial estrogen agonist and FLV is a complete estrogen antagonist; these were used to mimic and antagonize the effects of estrogen on uterine tissue, respectively. The remaining three groups of animals, i.e., 17β-estradiol (17βE), 17βE+TMX, and 17βE+FLV, were treated with 4 mg/kg/day of 17βE alone or in combination with either TMX or FLV. All test substances were perorally administered for 3 days using an oral gavage tube.

Experiment II: Prepubertal female rats with primary ovarian failure

Prepubertal female rats (28–29 days old) weighing approximately 50–60 g were randomly divided into four groups comprising six animals each. Intraperitoneally, 2–3 mL of DMSO was injected for 15 days to the control animals (DMSO group). VCD (Fluka, Buchs, Switzerland) dissolved in DMSO was injected to the second group of animals (VCD group) at a dose of 60 mg/kg/day for 15 days. For rats, VCD injection at a dose range of 40–80 mg/kg has been shown to cause ovotoxicity by selectively targeting primordial and primary follicles (18, 19). Twenty-four hours following the last VCD injection, the third (VCD+Q10) and fourth (VCD+17βE) groups of animals were treated with 10 mg/kg/day of quercetin and 4 mg/kg/day of 17βE, respectively, by oral gavage for 4 days.

Uterotrophic assay

Twenty-four hours following the last dose in each experiment, the overnight-fasted animals were weighed, and 1 mL of venous blood was withdrawn by cardiac puncture under ether anesthesia. Blood samples were collected in dry tubes, and serum samples obtained by centrifugation were stored at –20°C until analysis. The animals were sacrificed by an overdose of anesthetics, and the uteri were quickly removed, trimmed of fat and connective tissue, and weighed. Then, the uteri were cut into two pieces: one of the pieces was used for a histological examination and the other for the determination of the ratio of uterine dry weight to uterine wet weight (UDW/UWW). Uterine dry weights were measured after drying the uterine piece in an oven at 60°C for 12 h.

The ratio of uterine wet weight to body weight (UW/BW) in mg/g and UDW/UWW in mg/mg were determined, and the percentage of body weight increase (BW%) were calculated using the following formula:

$$\text{BW}\% = \frac{(\text{post-experiment body weight} - \text{pre-experiment body weight}) \times 100}{\text{pre-experiment BW}}$$

Uterine and Ovarian Histology

The uterus and ovary were fixed in 10% neutral buffered formalin immediately after weighing. They were dehydrated at room temperature for 24 h and embedded in paraffin. The tissues were then sectioned to a thickness of 5 µm and mounted on slides. These sections were stained with hematoxylin and eosin (H&E), and histopathological changes were examined under a light microscope (Olympus U-PM-TVC).

Increased endometrial epithelium thickness, hypertrophy in the uterus, increased number of mitotic cells, spindle-shaped cells underlying the basal lamina, and cells with a dark nuclei were considered as findings of increased estrogenic activity. On the other hand, decreased endometrial epithelium thickness, cubic shape of epithelial cells, round-shaped cells underlying the basal lamina, cells with a round nuclei, and atrophic appearance of the uterus were considered as findings of the antagonistic characteristics of the test substances. In ovarian tissue, a decreased number of primordial follicles compared to the number in the control group was considered as ovarian failure. The number of primordial follicles was determined in every 40th section of the ovaries.

Statistical analysis

Data are expressed as mean±SD. The results were analyzed on SPSS 17.0 (SPSS Inc.; Chicago, IL, USA) and one-Way ANOVA and multiple comparison test of Tukey. The significance level was set at $p < 0.05$.

RESULTS

Uterotrophic assay

In 21-day old immature female rats, initial body weights were not different among the groups. BW%, UW/BW, and UDW/UWW are shown in Table 1.

BW% was significantly lesser in the Q10, Q90, Q10+TMX, Q10+FLV ($p < 0.01$, in each), and 17βE+FLV ($p < 0.05$) group than in the control group. UW/BW on day 4 was significantly higher

Table 1. Morphological and histological effects of the compounds on the uterus in immature female rats

	BW% Mean±SD	UW/BW (mg/g) Mean±SD	UDW/UWW (mg/mg) Mean±SD	Endometrial thickness (µm) Mean±SD
Control	30.80±6.4	2.82±0.39	0.23±0.04	19.58±1.02
Q10	17.91±6.21**	3.85±0.50**	0.15±0.01**	18.40±1.74
Q30	25.10±2.86	3.80±0.76*	0.26±0.04	19.57±1.93
Q90	18.34±2.76**	2.38±0.51	0.37±0.08	13.38±0.78***
Q10+TMX	18.13±4.06**	3.84±0.51**	0.24±0.04	19.83±0.46
Q10+FLV	16.82±4.08**	1.22±0.26***+++	0.41±0.08	12.30±0.15+++
17βE	24.20±4.40	3.16±0.63*	0.20±0.04	26.38±1.84***
17βE+TMX	24.65±2.63	2.57±0.27	0.25±0.03	23.94±2.08
17βE+FLV	21.45±1.40*	1.25±0.25***	0.38±0.18	11.93±0.19xxx

*:p<0.05, **:p<0.01, ***:p <0.001 as compared to the control group
 +++: p<0.01 as compared to the Q10 group
 xxx: p <0.001 as compared to the 17βE group
 BW%: percentage of body weight increase; UW/BW: ratio of uterine wet weight to body weight; UDW/UWW: ratio of uterine dry weight to uterine wet weight; Q: quercetin; TMX: tamoxifen; FLV: fulvestrant; 17βE: 17β-estradiol; SD: standard deviation

Table 2. Morphological and histological effects of the compounds on uterus in prepubertal female rats

	BW% Mean±SD	UW/BW (mg/g) Mean±SD	UDW/UWW (mg/mg) Mean±SD	Endometrial thickness (µm) Mean±SD
DMSO Control	94.86±8.37	3.30±0.78	0.33±0.07	21.0±0.63
VCD	42.84±17.19***	1.83±0.66*	0.27±0.11	11.0±0.89***
VCD+Q10	58.17±19.87*	1.22±0.26*	0.27±0.11	19.1±1.37+++
VCD+17βE	40.40±3.05***	2.41±0.86+	0.27±0.10	28.7±2.49+++

*: p<0.05, ***: p<0.001 as compared to the control group
 +: p<0.05, +++: p<0.001 as compared to the VCD group
 BW%: percentage of body weight increase; UW/BW: ratio of uterine wet weight to body weight; UDW/UWW: ratio of uterine dry weight to uterine wet weight; VCD: 4-vinylcyclohexene dioxide; Q: quercetin; 17βE: 17β-estradiol

in the Q10 (p<0.01), Q30 (p<0.05), Q10+TMX (p<0.01), and 17βE (p<0.05) groups and significantly lower in the Q10+FLV (p<0.001) and 17βE+FLV (p<0.001) groups than in the control group. Of note, UW/BW was significantly lower in the Q10+FLV group than in the Q10 group (p<0.001). UDW/UWW was significantly lower in the Q10 (p<0.01) group than in the control group. The endometrial thickness was found to be significantly lower in the Q90 (p<0.001) group and significantly higher in the 17βE (p<0.001) group than in the control group. While the endometrial thickness did not change in the Q10 and Q10+TMX groups compared to that in the control group, the thickness was significantly decreased in Q10+FLV (p<0.001) group compared to that in the Q10 group. Treatment with 17βE resulted in an increased endometrial thickness (p<0.001) compared to control rats, which was significantly decreased in rats treated with 17βE+FLV (p<0.001).

In 28-day old prepubertal female rats, initial body weights were not different among the groups. BW%, UW/BW, and UDW/UWW are shown in Table 2.

BW% was significantly lower in the VCD (p<0.001), VCD+Q10 (p<0.05), and VCD+17βE (p<0.001) groups than in the control group. UW/BW on day 20 was significantly lower in the VCD and VCD+Q10 (p<0.05, in each) group than in the control group. In the animals treated with 17βE after the completion of VCD injections, UW/BW was significantly higher than those treated with 10 mg/kg of quercetin (p<0.05). UDW/UWW was not different among these four groups.

Histological findings

In 21-day old immature female control rats, the uteri were immature, endometrial epithelial cells were cuboidal-shaped and short in length, and connective tissue cells were few and had a round nucleus. The histological appearance of the uteri was not different in 21-day old rats treated with Q10, Q10+TMX, Q10+FLV and 17βE+FLV compared to that of control animals. However, the uteri were hypertrophic, cells in the lamina propria of the endometrium were spindle-shaped and had a dark nucleus, and there were numerous mitotic cells in the animals treated with 17βE

and 17 β E+TMX (Figure 1). The mean endometrial thickness was $19.58 \pm 1.02 \mu\text{m}$ in control animals. Although it was not significantly different in animals treated with Q10, a significant decrease was observed in animals treated with Q90 ($p < 0.001$) and in those treated with Q10+FLV ($p < 0.001$). As expected, the mean thickness of the endometrium was significantly increased with the administration of 17 β E ($p < 0.001$) and significantly decreased with the administration of 17 β E+FLV ($p < 0.001$).

In 28-day old prepubertal female control rats (DMSO group), the uteri were immature, and endometrial epithelial cells were cuboidal-shaped and short, and connective tissue cells were few and had a round nucleus. Although the histological appearance of the uteri was not different in VCD-treated animals, the uteri of animals treated with VCD+ Q10 and VCD+17 β E were hypertrophic, cells in the lamina propria of the endometrium were spindle-shaped and had a dark nucleus, and there were numerous mitotic cells (Figure 2). The mean endometrial thickness was $21.0 \pm 0.63 \mu\text{m}$ in the DMSO-treated control animals. The endometrial thickness was significantly decreased in VCD-treated animals ($p < 0.001$); treatment of animals with Q10 after repeated injections of VCD for 15 days significantly increased the endometrial thickness to a level close to that observed in control animals ($p < 0.001$). On the other hand, the VCD+17 β E ($p < 0.001$) group showed a significantly increased endometrial thickness compared to the control group.

The mean primordial follicle number in the ovaries of DMSO-treated 28-day old control rats was 8.71 ± 0.76 , and it was significantly decreased to 1.66 ± 0.52 in VCD-treated animals ($p < 0.001$). However, primordial follicle number was not different in 28-day old rats treated with VCD+Q10 and VCD+17 β E compared to animals in the DMSO group.

DISCUSSION

In the present study, we evaluated the effects of quercetin on the reproductive organs of 21-day old immature as well as in 28-day old prepubertal female rats with ovarian failure induced by VCD and found estrogen-like effects at relatively low doses (10 and 30 mg/kg/day) of quercetin but no effects at higher doses (90 mg/kg/day).

Quercetin at doses of 10 and 30 mg/kg, but not at the dose of 90 mg/kg, increased UW/BW to the same level as that with 17 β E in 21-day old immature rats. While the co-administration of the partial estrogen agonist TMX and 10 mg/kg of quercetin did not cause

any significant change, the addition of the estrogen antagonist FLV to 10 mg/kg of quercetin significantly decreased UW/BW. Therefore, it seems that quercetin exerts estrogen-like activity that does not change with TMX but that is inhibited by FLV. On the other hand, VCD treatment resulted in significantly decreased UW/BW and quercetin did not antagonize this effect of VCD in 28-day old prepubertal rats, suggesting that quercetin has estrogenic or anti-estrogenic effects depending on the presence of estrogen. Both estrogenic and anti-estrogenic effects have been suggested for quercetin in a dose-dependent manner (12, 13). In line with the results of previous studies (12, 20, 21), we found a dose-dependent effect of quercetin on the uterine weight.

In histological sections from immature rats, 10 mg/kg of quercetin resulted in no difference in the endometrial thickness; however, 90 mg/kg of quercetin resulted in a significantly decreased endometrial thickness, suggesting that quercetin exerts no proliferative and antiproliferative effects on the uterus when used in low and high doses, respectively. In a recent study, Shahzad et al. (20) reported an antiestrogenic effect for 10 mg/kg of quercetin via binding type-2 ER with no proliferative effect on the uterus. Although we found an antiproliferative effect for 90 mg/kg of quercetin in our study, the authors of that study found an estrogenic activity for

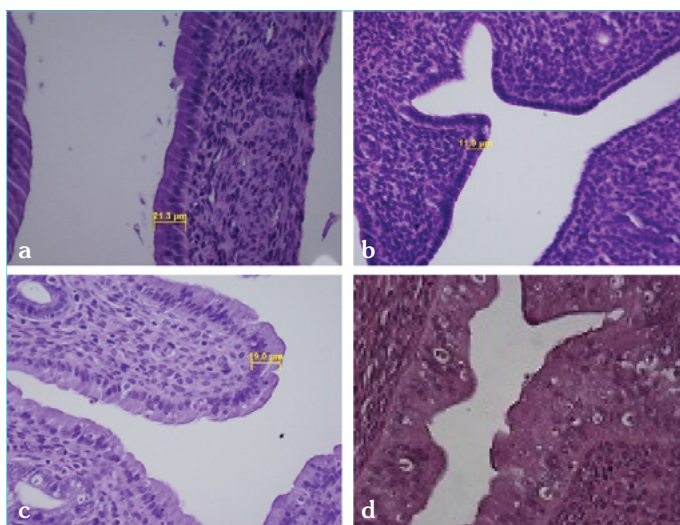


Figure 2. The histological appearance of the uteri and endometrial thickness of prepubertal rats from the control (a), VCD (b), VCD+Q10 (c), and VCD+17 β E (d) groups (40 \times magnification)

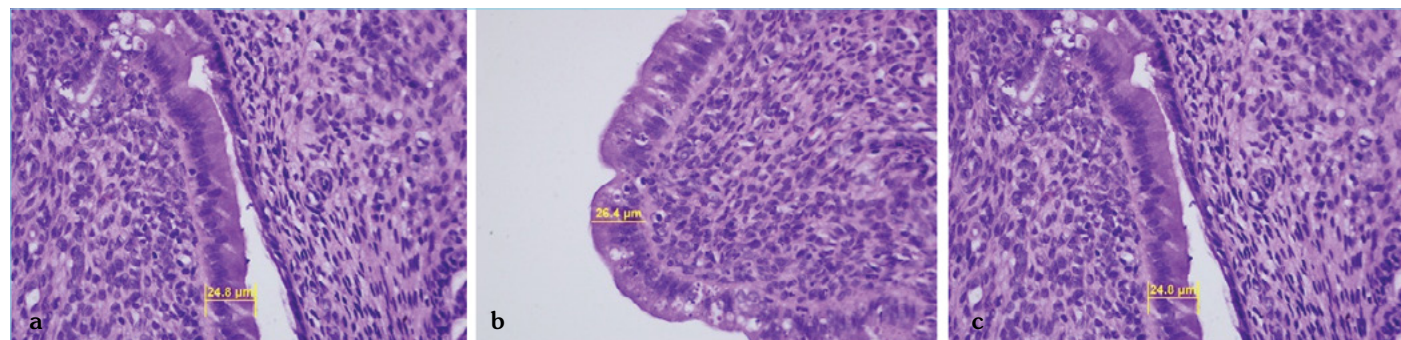


Figure 1. The histological appearance of the uteri and endometrial thickness of immature rats from the control (a), 17 β E (b), and 17 β E+TMX (c) groups (40 \times magnification)

100 mg/kg of quercetin, which can trigger estrogen-dependent cancer in humans (20, 21). Furthermore, the outer scale of onions known to contain quercetin has been found to induce morphological changes in the uteri of mice (22). However, these studies used adult animals and evaluated the effects of quercetin under the influence of estrogen and progesterone, which may have resulted in findings different from those in our study. In contrast, we used immature and prepubertal rats, which are free of the influence of the menstrual cycle.

Phytoestrogens has been suggested to be SERMs, and they may be used in postmenopausal women for helping to reduce some health risks associated with the lack of estrogen (23, 24). Quercetin, which is a phytoestrogen, has also been found to stimulate both ER α and ER β (11), with a higher capacity for stimulating ER β (25).

In the present study, VCD was used to induce a condition resembling primary ovarian failure in prepubertal rats and to evaluate the possible beneficial effects of quercetin treatment on the uterus. VCD is an industrial chemical that selectively destroys primordial and primary ovarian follicles following 15 days of intraperitoneal (i.p) injection (18, 26). Moreover, VCD is known to induce apoptotic processes similar to processes associated with atresia (26). We observed decreased uterine weight and decreased primordial follicle number in the ovaries with repeated i.p. injection of VCD for 15 days, all of which were prevented by 17 β E but not by quercetin. The endometrial thickness was significantly decreased in VCD-treated animals, which was prevented by both 17 β E and, to a lesser extent, by quercetin. These results also imply that the estrogen-like activity of quercetin depends on the presence of estrogen.

Quercetin has been generally studied by *in vitro* test systems. However, it should be emphasized that unlike *in vivo* systems, *in vitro* test systems do not include absorption or other metabolic transformation processes, which can result in false-negative or false-positive results. Moreover, *in vivo* studies have usually tested the estrogenicity of quercetin given in a mixture of phytoestrogens, which can result in additive, synergistic, or antagonistic interactions.

CONCLUSION

In conclusion, the results of the present study suggest that quercetin has a dose-dependent effect on the uterus. However, because the biological activity of this compound depends on many other factors, such as the route of administration, chemical form administered, endogen estrogenic status, and compound's concentration, the use of quercetin as a dietary supplement may not always be beneficial and may even be harmful and increase disease risk in some situations. Therefore, further studies are needed to investigate the possible clinical benefits of quercetin in individuals with estrogen deficiency, such as in menopausal women, before giving dietary recommendations for quercetin.

Ethics Committee Approval: Ethics committee approval was received for this study from Eskişehir Osmangazi University. (date: 26/04/2011; protocol no: 190-1).

Informed Consent: The informed consent was not required because the study was performed on animals.

Peer-review: Externally peer-reviewed.

Authors' Contributions: Conceived and designed the experiments or case: SY. Performed the experiments or case: SY, FYÖ, OÖ, SŞ, ÇÜ. Analyzed the data: SY, KE. Wrote the paper: SY. All authors have read and approved the final manuscript.

Acknowledgement: The authors are alone responsible for the content and writing of the paper.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

REFERENCES

1. Deroo BJ, Korach KS. Estrogen receptors and human disease. *J Clin Invest* 2006; 116(3): 561-70. [\[CrossRef\]](#)
2. Mueller SO, Korach KS. Estrogen receptors and endocrine diseases: lessons from estrogen receptor knockout mice. *Curr Opin Pharmacol* 2001; 1(6): 613-9. [\[CrossRef\]](#)
3. Bischoff, SC. Quercetin: potentials in the prevention and therapy of disease. *Curr Opin Clin Nutr Metab Care* 2008; 11(6): 733-40. [\[CrossRef\]](#)
4. Meyers KJ, Rudolf JL, Mitchell AE. Influence of dietary quercetin on glutathione redox status in mice. *J Agric Food Chem* 2008; 56(3): 830-6. [\[CrossRef\]](#)
5. Huk I, Brovkovich V, Nanobash Vili J, Weigel G, Neumayer C, Partyka L, et al. Bioflavonoid quercetin scavenges superoxide and increases nitric oxide concentration in ischaemia-reperfusion injury: an experimental study. *Br J Surg* 1998; 85(8): 1080-5. [\[CrossRef\]](#)
6. Aalinkeel R, Bindukumar B, Reynolds JL, Sykes DE, Mahajan SD, Chadha KC et al. The dietary bioflavonoid, quercetin, selectively induces apoptosis of prostate cancer cells by down-regulating the expression of heat shock protein 90. *Prostate* 2008; 68(16): 1773-89. [\[CrossRef\]](#)
7. Jeong JH, An JY, Kwon YT, Rhee JG, Lee YJ. Effects of low dose quercetin: cancer cell-specific inhibition of cell cycle progression. *J Cell Biochem* 2009; 106(1): 73-82. [\[CrossRef\]](#)
8. Mackraj I, Govender T, Ramesar S. The antihypertensive effects of quercetin in a salt-sensitive model of hypertension. *J Cardiovasc Pharmacol* 2008; 51(3): 239-45. [\[CrossRef\]](#)
9. Kumazawa Y, Kawaguchi K, Takimoto H. Immunomodulating effects of flavonoids on acute and chronic inflammatory responses caused by tumor necrosis factor alpha. *Curr Pharm Des* 2006; 12(32): 4271-9. [\[CrossRef\]](#)
10. Davis JM, Murphy EA, McClellan JL, Carmichael MD, Gangemi JD. Quercetin reduces susceptibility to influenza infection following stressful exercise. *Am J Physiol Regul Integr Comp Physiol* 2008; 295(2): R505-9. [\[CrossRef\]](#)
11. Miodini P, Fioravanti L, Di Fronzo G, Cappelletti V. The two phytoestrogens genistein and quercetin exert different effects on oestrogen receptor function. *Br J Cancer* 1999; 80(8): 1150-5. [\[CrossRef\]](#)
12. Harris DM, Besselink E, Henning SM, Go VL, Heber D. Phytoestrogens induce differential estrogen receptor alpha- or Beta-mediated responses in transfected breast cancer cells. *Exp Biol Med (Maywood)* 2005; 230(8): 558-68.
13. Han DH, Denison MS, Tachibana H, Yamada K. Relationship between estrogen receptor-binding and estrogenic activities of environmental estrogens and suppression by flavonoids. *Biosci Biotechnol Biochem* 2002; 66(7): 1479-87. [\[CrossRef\]](#)
14. Moutsatsou P. The Spectrum of phytoestrogens in nature: our knowledge is expanding. *Hormones* 2007; 6(3): 173-93.
15. C K Osborne, A Wakeling, R I Nicholson. Fulvestrant: an oestrogen receptor antagonist with a novel mechanism of action. *Br J Cancer* 2004; 90(Suppl 1): S2-S6. [\[CrossRef\]](#)

16. Wakeling AE, Dukes M, Bowler J. A potent specific pure antiestrogen with clinical potential. *Cancer Res* 1991; 51(15): 3867-73.
17. Kappeler CJ, Hoyer PB. 4-vinylcyclohexene diepoxide: a model chemical for ovotoxicity. *Syst Biol Reprod Med* 2012; 58(1): 57-62. [\[CrossRef\]](#)
18. Hoyer PB, Sipes IG. Development of an animal model for ovotoxicity using 4-vinylcyclohexene: a case study. *Birth Defects Res B Dev Reprod Toxicol* 2007; 80(2): 113-25. [\[CrossRef\]](#)
19. Muhammad FS, Goode AK, Kock ND, Arifin EA, Cline JM, Adams MR, et al. Effects of 4-vinylcyclohexene diepoxide on peripubertal and adult Sprague-Dawley rats: ovarian, clinical, and pathologic outcomes. *Comp Med* 2009; 59(1): 46-59.
20. Shahzad H, Giribabu N, Muniandy S, Salleh N. Quercetin induces morphological and proliferative changes of rat's uteri under estrogen and progesterone influences. *Int J Clin Exp Pathol* 2014; 7(9): 5484-94.
21. Shahzad H, Giribabu N, Sekaran M, Salleh N. Quercetin Induces Dose-Dependent Differential Morphological and Proliferative Changes in Rat Uteri in the Presence and in the Absence of Estrogen. *J Med Food* 2015; 18(12): 1307-16. [\[CrossRef\]](#)
22. Alrefaie ZA, Amin HA, Elgayed SH. Estrogenicity of outer scales of onion on uteri of immature mice. *Can J Physiol Pharmacol* 2011; 89(11): 829-35.
23. Moutsatsou P. The spectrum of phytoestrogens in nature: our knowledge is expanding. *Hormones (Athens)* 2007; 6(3): 173-93.
24. Cassidy A. Potential risks and benefits of phytoestrogen-rich diets. *Int J Vitam Nutr Res* 2003; 73(2): 120-6. [\[CrossRef\]](#)
25. van der Woude H, Ter Veld MG, Jacobs N, van der Saag PT, Murk AJ, Rietjens IM. The stimulation of cell proliferation by quercetin is mediated by the estrogen receptor. *Mol Nutr Food Res* 2005; 49(8): 763-71. [\[CrossRef\]](#)
26. Mayer LP, Devine PJ, Dyer CA, Hoyer PB. The follicle-deplete mouse ovary produces androgen. *Biol Reprod* 2004; 71(1): 130-8. [\[CrossRef\]](#)