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Anti-cancer activity of quercetin via apoptosis induction pathways in human breast cancer cell lines-a systematic review and meta-analysis

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ABSTRACT

A systematic review and meta-analysis were conducted to summarize and review the current literature surrounding quercetin in breast cancer and evaluate its efficacy as an anticancer agent in human breast cancer cell lines. Electronic databases, PUBMED, WEB OF SCIENCE and MEDLINE were systematically searched in December 2020 using the key terms, Quercetin AND Breast cancer AND apoptosis OR cell cycle. Fourteen papers surrounding effects of quercetin on cell viability were obtained. Risk of bias assessment revealed that most papers were reliable. Three meta-analyses were conducted to confirm the efficacy of quercetin at the concentrations of 40-50 μM, 100μM, and >100μM (120- 200 μM). Two subgroup analyses on incubation time and cell type were performed to ascertain whether these factors affect mechanisms of quercetin. Results showed that the decrease in percentage cell viability is directly proportional to increasing concentration. The greatest decrease in cell viability was observed in 100 μM (Risk Ratio (RR) = 0.56; $p<0.00001$), followed by 100 μM (RR= 0.74) and then 40-50 μM (RR=0.79). However, effects of quercetin may be determined by other factors such as incubation time or a particular cell type signaling cascade. Subgroup analysis revealed that significant differences between 24 and 48 hours were not observed. MCF7 cells showed to be most sensitive to actions of quercetin ($p< 0.0001$). It was concluded that there is enough information surrounding the cytotoxic effects of quercetin to be progressed forward, however optimal doses that are physiologically relevant and safe in humans should be elucidated.

Keywords: quercetin, breast cancer cells, apoptosis, meta-analysis, systematic review, cell viability

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Introduction

Breast cancer is the most prevalent cancer worldwide and is the leading cause of cancer related death in women [2], in which its most common treatment plans; chemotherapy and radiotherapy, exert side effects that are often unbearable to the patient and are highly susceptible to resistance [82] [90].

The usage of natural products as anticancer agents has received great attention in recent times given the reported chemo preventive and chemotherapeutic qualities they possess [18]. Plant-derived anticancer drugs such as taxanes and vinca alkaloids already exist and are widely used in clinical settings which highlights the potential of plants as anti-cancer drugs [29]. These natural products would be highly beneficial as they are expected to be widely accessible, less processed, and less susceptible to resistance [18]. Flavonoids from plants are largely distributed across the common diet and have been reported to possess a broad range of health benefits including antimicrobial, anti-inflammatory, chemoprevention, and chemotherapeutic effects [6][41] [42][61][70]. A major flavonoid, part of the flavanol subclass, quercetin has been shown to induce anticancer effects through, loss of cancer cell viability, apoptosis, and reduced cell proliferation which is determined via the role of quercetin in cytotoxic pathways [71].

A crucial hallmark of cancer, evasion of apoptosis is an attractive cancer therapy target, as it decelerates and halts the progression of cancer by re-sensitizing the cancer cells to undergo apoptosis [31][36][63]. Additionally, resistance to chemotherapy and radiotherapy has been attributed to this apoptotic defect, hence scrutinizing novel therapies in rectifying dysregulated apoptosis is important to solve the prominent aspect of resistance to treatment [36][63]. Quercetin has been widely reported to induce dose and time-dependent apoptosis and inhibit proliferation in various cancers including lung, colon, breast, prostate, and ovarian cancers without affecting the tissue's normal counterparts [7][33][44][45].

Nonetheless, reports regarding quercetin and its roles in apoptotic pathways and cell cycle arrest have been inconsistent. Many studies report the induction of apoptosis by quercetin via the mitochondrial pathway [80] but others mention that quercetin upregulates the expression of members of the class O of forkhead box transcription factors (FOXO), which induces both mitochondrial dependent and independent apoptosis, in an MDA-MB-231 cell line [89][22]. Furthermore, other studies involving non-cancer cells such as damaged rat neurons demonstrated that low doses quercetin induced anti-apoptotic effects instead [28]. Additionally, there have been inconsistencies in the literature regarding the concentration at which quercetin is the most effective where some report statistical significance in concentrations as low as 10μM [21] whereas others demonstrate an effect seen only after 50μM [13]. Interestingly there has been some reports insinuating that quercetin promotes cancer cell growth at low concentrations [87].

Hence, these findings warrant a need to explore the activity of quercetin in breast cancer to determine whether there is a particular signaling cascade induced by quercetin and if this may be influenced by other factors such as dosage and cell type.

Moreover, despite the abundance of studies demonstrating the significant cytotoxic effects of quercetin in various human breast cancer cell lines the progression to human clinical trials is scarce and even animal *in vivo* studies are rather limited, to further consolidate these promising findings clinical application is required. This may be due to the lack of confidence in the pharmacokinetics of flavonoids, specifically quercetin where many reports its lack of bioavailability due to poor absorption and very rapid metabolism [11]. However, many have demonstrated that this challenge can be overcome via the manipulation of the delivery of quercetin, where successful administration is

seen through nanoparticles [11][72]. In spite of this, a phase 1 clinical trial has demonstrated that quercetin was able to achieve plasma levels which can inhibit tyrosine kinase activity [26]. This is further reinforced by the fact that more emerging studies have demonstrated a considerable level of diet- derived quercetin can be absorbed through the digestive tract [57].

It may be more economical, time-efficient, and safer to use quercetin conventionally by finding its optimal dosage in humans, as reports have shown increased risks of toxicities using novel drug delivery systems such as incomplete capsule degradation and organ accumulation [11]. This makes it important to review the optimal dose in cellular studies and possibly inform future human clinical trials.

Consequently, a systematic review and meta-analysis was conducted to summarise and review the current literature surrounding quercetin in breast cancer and determine its efficacy as an anticancer agent in various human breast cancer cell lines. In all, this was done with the aim to conclude whether it is worth further investigation in clinical settings and if this flavonoid can be implemented into future breast cancer treatment regimes.

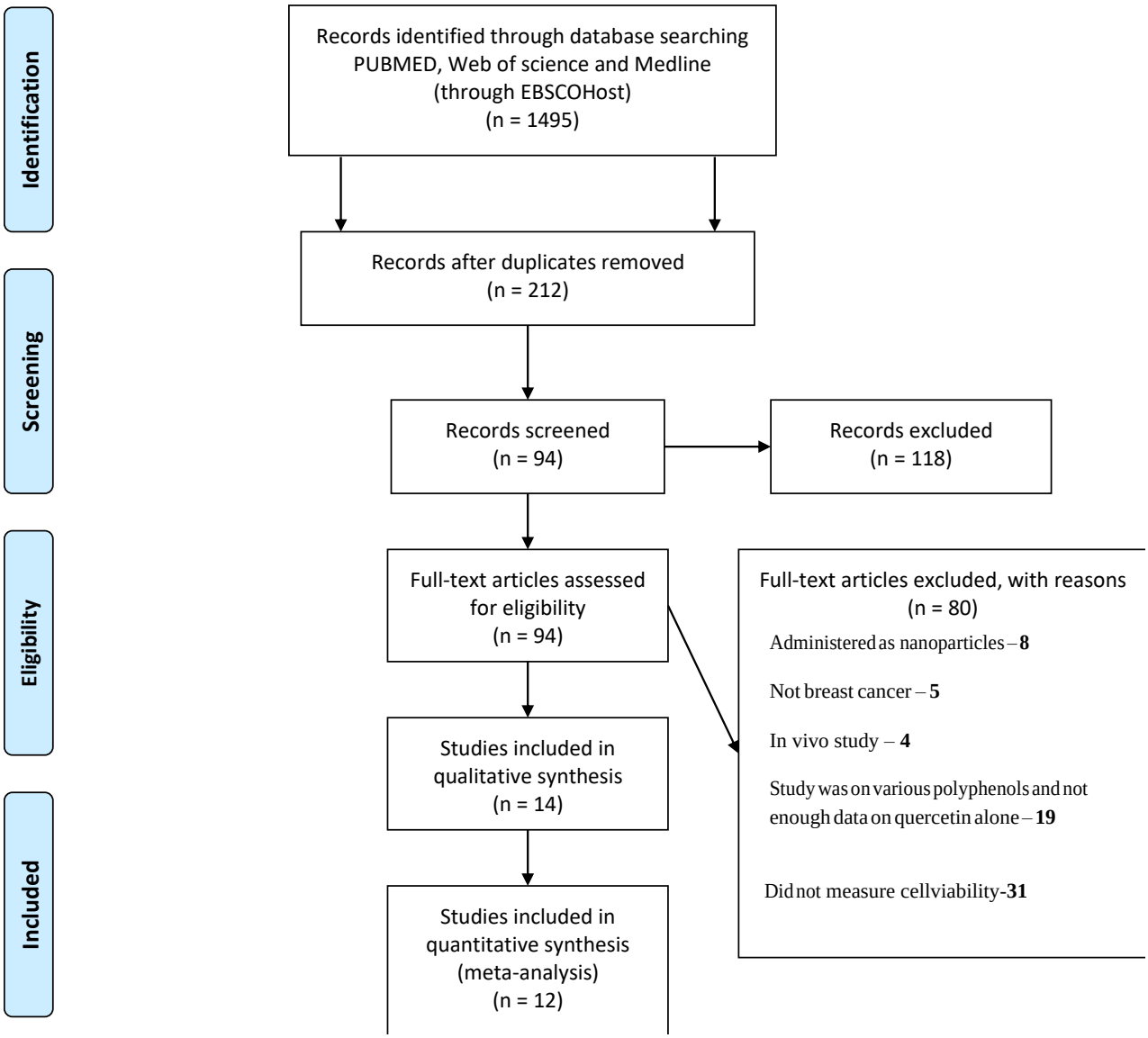

Figure 1: Flow chart of study selection process

Materials and methods

Search Strategy:

The population, intervention, controls, and outcomes (PICO) search strategy was employed for this systematic review. Papers based on human breast cancer cell lines (P) treated with quercetin (I) compared to a control (C) reporting an outcome on cell viability upon quercetin treatment which in turn was used as an indicator of apoptosis (O) were obtained (Figure 1).

The electronic databases: PUBMED, WEB OF SCIENCE and MEDLINE were searched in December 2020, using advanced search Booleans. Search terms were inputted as, Quercetin AND Breast cancer AND apoptosis OR cell cycle. MESH terms in PUBMED were also used and inputted as; (("Apoptosis"[Mesh]) AND "Breast Neoplasms"[Mesh]) AND "Quercetin"[Mesh] AND (flavanol)). All collected papers were saved in a Microsoft Excel spreadsheet where duplicates were then removed.

Data Extraction:

Data was extracted from each paper and relevant information was inputted into a table (table 1). Data to be inputted into RevMan 5 was extracted from each study using the software WebplotDigitizer, where screenshots of graphs and bar plots were taken and loaded into this software.

Statistical Analysis:

Quantitative analysis was undertaken via the completion of meta-analyses using the statistical software RevMan 5. This meta-analysis aimed to measure the significance of the effects of quercetin treatment on cell viability. Percentage cell viability was treated as dichotomous data where a risk ratio and confidence intervals were calculated. A random effects model was employed as variations between studies were bound to occur, therefore this model calculated the mean of distribution of effects, hence giving a more accurate representation of the actual impact of quercetin. Heterogeneity was calculated through the I^2 index. To determine sources of heterogeneity two subgroup analyses were conducted on variables suspected to affect the functional outcomes of quercetin on cell viability. The first being a subgroup analysis on incubation time, the second was cell type.

Risk of Bias

The evaluation of study methodologies was employed through answering the eligibility criteria of the in vitro section in the ToxRtool and categorizing studies based on reliability (Table 1).

Results

In all, 14 papers were evaluated, in which 12 were included in the quantitative analysis to evaluate the impact of quercetin on percentage cell viability. Although 12 papers were included, some did test more than one cell type, concentration and incubation time and were therefore considered as separate studies.

Table 1: Risk of bias assessment of each study using *In Vitro* section of ToxRTool

Study	1 [93]	2 [13]	3 [47]	4 [94]	5 [64]	6 [78]	7 [87]	8 [49]	9 [16]	10 [20]	11 [21]	12 [33]	13 [43]	14 [58]
Study Criteria														
Is test Substance identified?	1	1	1	1	1	1	1	2	1	1	1	1	1	1
Is origin of test Substance described?	1	1	1	1	1	3	1	1	1	1	1	1	3	1
Is test system sufficiently	1	1	1	2	1	1	1	1	1	1	1	1	1	1

described?														
Is the method of test system given?	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Was a reference on the test system method given or is method generally known?	1	1	1	1	1	1	1	2	1	1	1	1	1	1
Is Necessary information regarding test system properties and conditions given?	1	1	1	1	1	1	1	1	1	1	1	1	2	1
Are concentrations administered given?	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Is duration and time of exposure described?	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Were variables which may impose secondary effects and influence results given ex: solubility/ temperature?	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Was a negative control included?	1	1	3	3	1	1	1	1	4	1	1	4	1	1
Was a positive control included?	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Is number of replicates given?	1	1	1	1	1	1	1	4	1	1	1	1	3	1
Is description of results complete and have all variables described in methods been described in results?	1	1	1	1	1	1	1	1	1	1	1	1	3	1
Has statistical analysis been described completely?	1	1	1	1	1	1	1	1	1	1	1	1	2	1
Total Average	1.07	1.07	1.21	1.21	1.07	1.21	1.07	1.21	1.21	1.07	1.07	1.28	1.64	1.07
Category	1	1	1	1	1	1	1	1	1	1	1	1	2	1

Category 1 => Reliable without restriction, **Category 2** => Reliable with Restriction**, Category 3** => Not reliable, **Category 4** => Not assignable

Table 2 - Summarised characteristics of each included study

Author	Location	Cell Type	Detection Method	Cell Number	Treatment	Findings on cell Viability	Findings on cytotoxicity
Chien *et al*., 2009	China	MDA-MB-231 cells	viable cells were determined by MTT	(2 x 10^5 cells/well; 12- well plates)	0, 50, 100, 150, 200, 250 and 300 µM for 24h or 48h	Quercetin significantly decreased the percentage of viable cells – effects were dose dependent - (50% inhibition of cell growth, 24 h) was 278 µM	Apoptosis induced by the mitochondrial pathway
Chou *et* al., 2010	Taiwan	MCF-7 cells	Trypan blue exclusion and PI-exclusion	(2 x 10^5 cells/well; 12- well plates)	0, 10, 50, 100, 150 and 175 µM quercetin and was incubated for 24 and 48 h	Quercetin decreased the percentage of viable cells - effects were dose and time dependent - (50% inhibition of cell growth, 48 h) was 92.4 µM	Apoptosis induced by the mitochondrial pathway
Deng *et al*., 2013	China	MCF 7 Cells	viable cells were determined by MTT	(5×10^3/well) were plated in 96-well plates	0, 2.5, 5, 10, 20 and 40 mg/ml after 24 or 48 h	highest inhibition rate was 58.72% and the rate of inhibition was concentration- and time- dependent.	The apoptosis rate of the quercetin 40 mg/ml group was 37.81%, which was higher than the apoptosis rates in the low concentration (20 mg/ml) and control groups. surviving mRNA levels were reduced when the concentration of quercetin increased.
Devipria *et al*., 2015	India	MCF 7	viable cells were determined by MTT	(1 x 10^6) were plated in 96- well plates	0, 10, 20, 30, 40, 50, 60 ug/ml quercetin and incubated for 24, 48 and 72 h	quercetin decreased the percentage of viable cells in dose and time dependent manner. Incubation for 72 h resulted in complete loss of cells	Dose and time dependent decrease in cytosolic calcium
Dhumale *et al*.,	India	MCF 7	Trypan Blue	(1 x 10^6) were	0,10,25, 50 µM	Dose and time dependent cell	MCF-7 cells treated with 50 µM of
2015			exclusion	plated in 96-well plates	quercetin incubated for 6, 12, 24, 48 h	viability decrease	quercetin showed massive cell death at 6 h onward
emzaei *et al*., 2017	Iran	MCF7	Viable cells were determined by MTT	(1 x 10^6) were plated in 96- well plates	10, 20, 40, 80 and 120 µM incubated for following 24, 48 and 72 h	Cancer cell growth inhibition was dose and time dependent (50% inhibition, 24 hours) was 105.4uM + (52.5uM for 48 h)	
Jeong *et al.*, 2010	USA	SK-BR3	Trypan Blue Exclusion	Not stated	100 µM incubated for 48h		level ofHer-2/neu protein began to decrease after 8 h of quercetin treatment + quercetin also dephosphorylated PI3K and Akt

Khorsandi *et al.*, 2017	Iran	MCF7 cells	Viable cells were determined by MTT	number not stated - MCF-7 left culture media for 48 h in 24 well plates	50 µM/ml for 48 h	Percentage of viable cells significantly decreased	The proliferation of MCF-7cells was significantly decreased. Apoptosis induced by mitochondrial pathway
Kiyga *et al.*, 2020	Turkey	MCF7 cells + MDAMB 231	Viable cells were determined by MTT	1 × 10^4 and 1.5 × 10^4 cells/ well	10, 25, 100 µM for 48 h.	Increase in cell death in a dose and time dependent manner + MCF-7 cells were shown to be more sensitive than MDA-MB-231 cells	Apoptosis was induced in treated cells with 25 and 100 µM. Higher level of caspase-activity was detected in MCF-7 cells - pro-caspase-3 was expressed in the MCF-7 cell lines
Lee *et al*., 2009	Korea	MCF7	Viable cells were determined by MTT	seeded on 96-well microplate s at 4,000 cells/well	25, 50, 100, 200, or 400 µM for 6 h	Decrease in cell viability was in a dose dependent manner	Apoptotic cell death was increased with 100 µM quercetin treatment, as shown with chromatin condensation + Quercetin strongly activated AMPK and increased P53 + P51
Nguyen *et al*., 2017	Korea	MDAMB2 31	Viable cells were determined by MTT	1×10^4 in 96 well-plates	2.5-80 µM for 24 h, 48 h and 72 h	Decrease in cell viability was in a dose dependent manner	quercetin caused cell cycle arrest at S and G2/M phase + of Foxo3a activity + JNK inhibition
Prandhan *et al*., 2015	India	MCF7 cells	Viable cells were determined by MTT	1×10^4 in 96 well-plates	25, 50, 75, or 100 µm for 24h	quercetin inhibited cell viability after 24h of treatment	Apoptosis induced by mitochondrial pathway Cells became elongated, losing their characteristic morphology
Sultan *et al*., 2017	Egypt	MDA-MB-157 and MDA-MB-231	Viable cells were determined by MTT	stock cell suspensio n containing 20,000 cells/ml. 100 µl were seeded per well of 96-well plate	0-550 µM for 48 h	significant dose-dependent cytotoxic effects on cells when compared to control cells + MDAMB231 => more sensitive than MDAMB157	Quercetin induced apoptosis of both treated cell lines in a caspase- dependent manner + downregulation of FASN + B catenin
Xu *et al.*, 2020	China	MCF-7 cells MDA-MB-231	CCK-8 assay and Realtime cell impedance analyser (RTCA) assay	96-well microplate s (1 × 10^4 cells/well)	0.1-500 µM with for 24-72 h	inhibited cell viability at higher concentrations (≥50 µM)	inhibited COX-2 protein expression + p300 HAT-mediated acetylation of NF-κB p50, resulting in a marked reduction in acetyl-p50 protein levels

Figure 2 Illustration demonstrating the reported apoptotic pathways induced by Quercetin

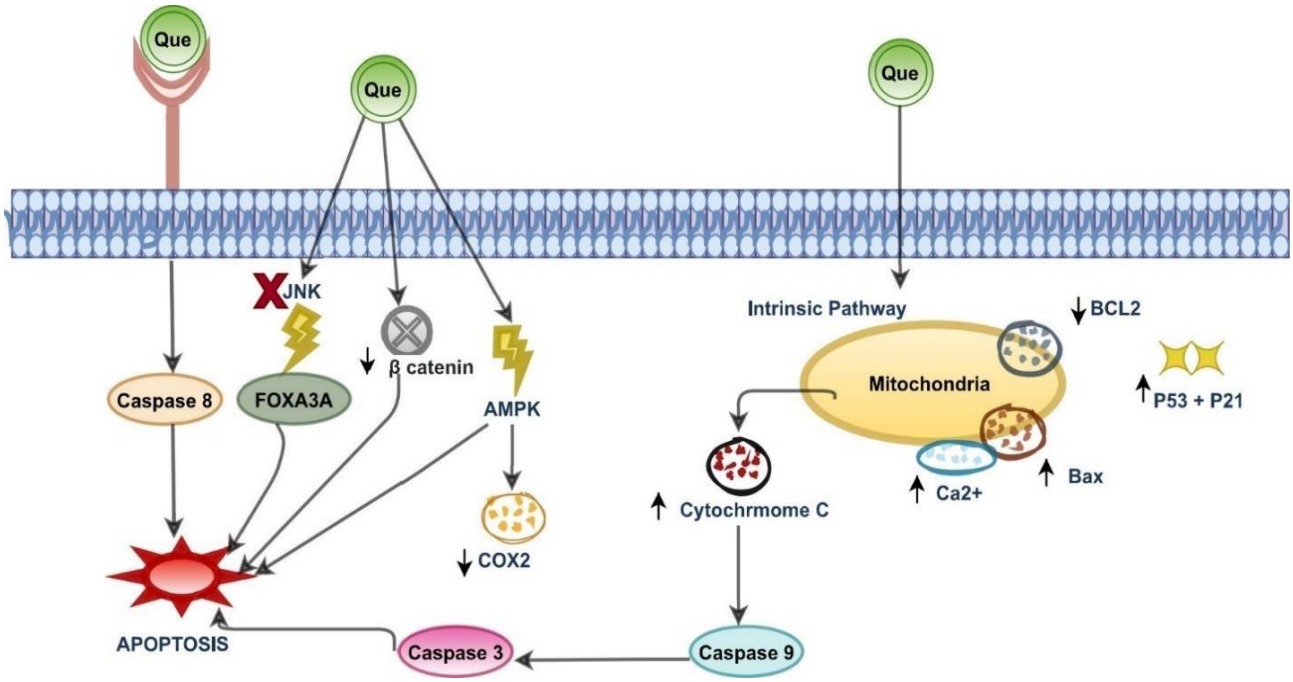

Fig 2 - Illustration showing apoptotic pathways upon quercetin treatment. Apoptosis has been shown to be induced via the mitochondrial pathway where levels of pro-survival proteins BCL2 have been seen to decrease whereas pro apoptotic proteins increase when quercetin (Que) is administered [93][13][21][43][47][64][[78] (fig 9). Others have demonstrated apoptosis through the extrinsic pathway [58]. Quercetin increases in Forkhead box O3 (FOX3a) proteins through c-Jun N-terminal kinase (JNK)pathway inhibition which have also been reported to induce apoptosis [58]. Another report suggests decrease in B catenin proteins inhibits in wnt signaling pathway and results in apoptosis [87]. The 5' AMP-activated protein kinase (AMPK) pathway has also been shown to be essential for apoptosis induction by quercetin [49].

Meta-Analysis Results:

Table 1: Summarised Meta-Analysis Results Using Various Concentrations of Quercetin

Concentration	Risk Ratio (RR)	P value	I^2 index (heterogeneity)
50 µM	0.77 [0.71,0.85]	P<0.0001	91%
100 µM	0.74 [0.66,0.83]	P<0.0001	95%
>100 µM (120-200 µM)	0.56 [0.44, 0.71]	P<0.0001	95%

The concentrations of 40-50 µM were chosen to initially determine the effects of quercetin as these were the lowest common doses across studies. Twelve studies were included in this analysis [13][21][47][47][33][58][49][64][78][87]. Cell viability in this section ranged from 46-93 %. Meta-analysis of the pooled results showed an RR value of 0.77 [0.71- 0.85] with a P value of P< 0.00001; demonstrating a significant decrease in cell viability. Heterogeneity for this analysis had an I^2 value of 91% (additional data are given in Online resource 1). To determine most effective dosage quercetin at higher concentrations of 100µM (additional data are given in Online

resource 1) and >100µM were chosen. Results showed concentrations >100 µM had the greatest effect on cell viability RR value being, 0.56, p<0.0001(additional data are given in Online resource 1).

Subgroup Analysis on Incubation Time:

Sub-group analyses were performed on potential factors which may have impacted the function of quercetin. A sub- group analysis was conducted on the various incubation times; 6, 24 and 48 hours (additional data are given in Online resource 1). Seven studies were in the 24- hour subgroup [93][13][47][64][87] and nine [103][13][94][47][78][87] in the 48- hour group; only one study was in the 6-hour subgroup [49]. Results obtained from the meta-analysis of the subgroups demonstrated that 48 hours resulted in an RR value of 0.67 [0.56,0.79]; p<0.00001 and this had the greatest effects on percentage cell viability compared to 24 hour and 6-hour incubation (additional data are given in Online resource 1).

Table 2: Summarized Subgroup Meta -analysis Results – Incubation Time = Subgroup

Incubation Time	Risk Ratio (RR)	P value	I^2 index (heterogeneity)
6 hours	0.79	P<0.0001	(only one study)
24 hours	0.69 [0.56,0.85]	P<0.0001	96%
48 hours	0.67 [0.56, 0.79]	P<0.0001	95%

Sub-group Analysis on Cell type:

The second Sub-group analysis was performed on the different cell lines (additional data are given in Online resource 1). Eleven studies were evaluated in this analysis. The breast cancer cell lines consisted of; MCF7 [13][21][49][64][87], MDAMB231[93][47][78][87], MDAMB157[78] and SKBR3[94]. Results of the meta- analysis revealed that MCF7 cells with an RR value of 0.65 [0.53,0.81] had the greatest percentage decrease compared to the other cell lines. The MDAMB157 cell line resulted in an RR value of 0.90 [0.84,0.96] showing the least effects of quercetin on percentage cell viability (additional data are given in Online resource 1).

Table 3: Summarized Subgroup Meta-Analysis Results – Cell Type = Subgroup

Cell Type	Risk Ratio	P value	I^2 index (Heterogeneity)
MCF 7	0.65 [0.53,0.81]	P<00001	92%
MDAMB231	0.78 [0.63, 0.96]	P<00001	94%
MDAMB157	0.90 [0.84,0.96]	P<0.02	Only one study
SKBR3	0.74 [0.66,0.83]	P<0.03	Only one study

Figure 3 Illustration demonstrating the reported cell cycle points that quercetin induces arrest in

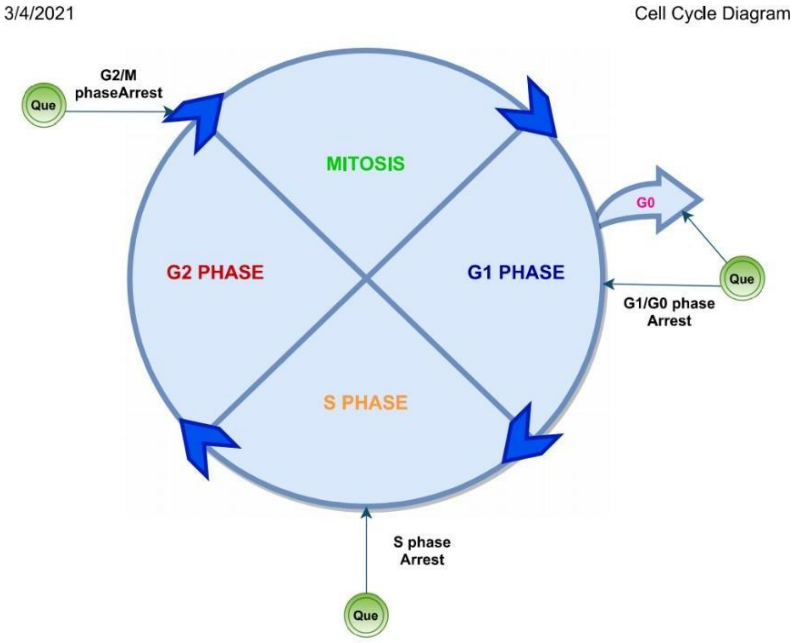

Fig 3: Illustration demonstrating the different cell cycle checkpoints quercetin induces arrest at. One report shows cell cycle arrest at S phase [13]; whereas another described cell cycle arrest at G2/M phase [93]. There have also been reports that quercetin induced cell cycle arrest at both phases [58]. Xu et al., 2020 showed G1/G0 arrest.

Discussion

Quercetin has been reported to possess cytotoxic abilities in various cancers [22][44][45][46][59]. Previous literature reviews have been published regarding the anti-cancer effects of quercetin in breast cancer [25][71] however majority have been qualitative and lack the rigorous systematic approach that allows for evidence-based conclusions [17]. More recently there has been a systematic review of the effect of quercetin on MCF-7 and MDA-MB-231cells. This systematic review and meta-analysis evaluate the efficacy of the cytotoxic effects induced by quercetin in more breast cancer cells lines with the aim to further knowledge surrounding its functional features as an anti-cancer agent and to progress to more *in vivo* studies and clinical trials. Fourteen articles were reviewed. Crucially, all studies reported a reduction in cell growth, proliferation, and apoptosis induction, however these effects are dependent on concentration, incubation time and cell type studied.

Cell viability was chosen as an indicator of cytotoxicity as the consistent method observed across the majority of papers was the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. This was chosen in consideration of the quantitative aspect of the meta-analysis in order to reduce variations across studies. Among the selected articles, cell viability was evaluated using; MTT assay by ten studies [93][16][20][33][43][47][49][58][64][78], a Cell counting Kit-8 (CCK-8) assay [87] and the Trypan blue exclusion assay [13][21][104] (table 1). It should be noted that the trypan blue assay is the only one that measures the number of viable cells whereas the other two methods measure mitochondrial metabolism [76].

Seven studies reported a dose and time dependency [93][13][16][20][33][47][49][58] on the effects of quercetin whereas three reported a dose dependent [78][64][43] response. Seven studies tested concentrations below 50 µM; 0-25µM [13][21][33][47][49][58][87] and six observed a reduction in cell viability [13][21][33][47][49][58] where two

reported statistical significance [13][21]. Interestingly, one study [87] reported a statistically significant increase in percentage cell viability at the concentrations of 5-10 µM. Other studies have also demonstrated biphasic behaviour of quercetin depending on dosage in MCF7 cells [85]. Quercetin concentrations as low as 10µM have been demonstrated to possess pharmacokinetic relevance in humans [30] thus further *in vitro* research investigating the anticancer effects of quercetin should consider understanding its mechanisms at lower concentrations.

Interestingly, quercetin has shown variations in inducing cell cycle arrest (fig 3). Hence, there is scope to say that the mechanism of action of quercetin may vary according to certain doses and time of incubation. Xu *et al.,2020* demonstrated that at high concentrations quercetin promoted G0/G1 arrest whereas lower concentrations induced transition of G1 to S phase. Seven studies reported apoptosis induction through the intrinsic mitochondrial pathway [93][13][21][43][47][64][78] (fig 2). However other apoptotic pathways have also been proposed [87][58][49][944] (fig 2).

Thus, a meta-analysis was conducted to determine the most effective dosage of quercetin for reducing cancer cell viability. Results revealed that increasing doses of quercetin did result in greater decrease cell viability therefore supporting the notion of dose dependency reported by the studies (Table A4). However, contrary to expectations, the differences in RR values of 50µM and 100µM were not substantial; 0.79 and 0.74 respectively. In contrast the concentrations greater than 100µM (120-200 µM) demonstrated a greater difference in RR value being 0.56. This suggests that the dose range of 50-100 µM may not induce clinically significant results [5]. Nonetheless, this conclusion is limited by the fact that data had to be rounded when inputted into RevMan and this may have affected the statistical interpretation [83]. Furthermore, data obtained from Chou *et al.,* 2010 was also drastically anomalous, being low value of only 9% cell viability [13] at 24 hours incubation which requires further looking into.

Based on the literature it was evident that time was also a factor that affected the actions of quercetin [93][13][16][20][33][47][49][58]. Hence, a subgroup meta- analysis was performed on the different incubation times presented in the studies. Although the highest incubation time showed the greatest decrease in percentage cell viability; it was relatively similar to the 24-hour subgroup (additional data are given in Online resource 1). This suggests that perhaps incubation beyond 24 hours does not lead to substantial differences and quercetin may have reached saturation in its effects. However concrete conclusions cannot be drawn based on these results alone due to the small range of incubation times included. For instance, Chou *et al.,*2010 reported apoptosis induction during the 48- hour time point. Nguyen *et al.*, 2017 also noted steady reduction in percentage viability between the times of 24 and 72 hours (additional data are given in Online resource 1).

Given the considerable I^2 index scores and previous reports [95][78][87], it was also speculated that cell type may have been the source of the heterogeneity. A subgroup analysis was performed on all the cell types presented across the studies. The most common cell types used were the MCF7 and the MDAMB231 cell lines which were seen in nine [13][16][21][33][43][47][49][64][87] and six [93][20][47][58][78][87] of the studies, respectively. These 2 cell lines are the most used cell types for laboratory breast cancer research as the MCF7 cell line is typically used as a hormone dependent model whereas the MDAMB231 is typically representative of triple negative [14][37][81]. Furthermore, the majority of studies were Eastern; particularly China [93][16][87], India [20][21][64] and Iran [33][43] that mostly used MCF7 cells whereas the only western study [94] used the SKBR3 cell line therefore it maybe speculated that perhaps certain cell types were more accessible in a particular location. Subgroup meta-analysis results revealed that

quercetin did induce its greatest effects in the MCF7 cell line (additional data are given in Online resource 1). In accordance with this other reported that quercetin acts as an oestrogen receptor antagonist [8][95][51][55]. These findings suggest that perhaps the role of quercetin in breast cancer would be best as an endocrine therapy.

Given the *in Vitro* nature of the studies, a risk of bias assessment using the Cochrane risk of bias reporting could not be used as this is targeted towards clinical studies [38]. Hence the *in vitro* section of the ToxRTool was used to assess reliability of studies used. From the risk of bias assessment (table 2), it can be seen that all of the papers were reliable, however those who had aspects that were classified as unreliable were because a negative control used was not named [20][94][58[49]. Majority of papers used a 0.1% Dimethylsulfoxide (DMSO) solution as a control [13][16][21][4][78][87] whereas others used untreated control cells [43][33]. Comparison of the effects of quercetin on cell viability was done with caution, given the wide variation in concentration ranges across studies. Dilution series of quercetin were conducted from different stock concentrations therefore the same doses may have still differed slightly (either being more diluted or more concentrated) [4].

Implications of this research are that there is enough information surrounding the cytotoxic effects of quercetin to be progressed forward. Although limited, previous *in vivo* studies have confirmed that quercetin does induce cytotoxicity in mice xenografted with breast cancer tumours [33][15]. However, progression to human clinical trials is scarce. Findings of this research postulate that concentrations of quercetin greater than 100µM will lead to clinical significance, therefore this concentration should be transitioned and further investigated in a more dynamic and physiological environment to determine the effects in humans and safety. A potential avenue to explore is the usage of 3D cellular models which will better mimic the tumour anatomy in a human body and therefore a better insight into the mechanisms of quercetin at different concentrations will be obtained [12][69]. However, animals should be used to observe the safety of quercetin using various doses as carcinogenic effects upon quercetin administration in animal studies have been controversial [32].

Conclusion:

This systematic review and meta-analysis postulate that quercetin has promising potential as an agent for breast cancer treatment. Although further research is warranted in elucidating an optimal dosage that is physiologically relevant; the current literature on cellular studies suggests that quercetin induces cytotoxic effects on human breast cancer cells at high doses and these are determined by incubation time and specific cell type signaling pathways.

Declarations

Ethical approval and consent to participate

The faculty research ethics committee waived the need for ethics approval and the need to obtain consent for the collection, analysis and publication of this systematic review and meta-analysis

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests

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Authors' contribution

RB contributed to the literature search, data extraction, analysis of the results and writing of

the manuscript. BI designed and directed the systematic review, confirmed accuracy of the literature searches and data interpretation, and revised the manuscript critically for intellectual content. All authors approved the version of the manuscript to be published.

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Not applicable

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