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**SHORT COMMUNICATION**

# *IN VITRO* ANTI-CANCER EFFECTS OF QUERCETIN ON TRIPLE NEGATIVE BREAST CANCER CELL LINE

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## ABSTRACT

**Phytochemicals are widely being explored for their cancer preventive properties in today's oncotherapy. Quercetin is a major constituent of various dietary products and its anti-cancer potential was explored on different cancer cell lines. The present study was conducted to find out the anti-proliferative effects of quercetin on human triple negative breast cancer cell line MDA-MB-231. The anti-cancer potential of quercetin, nano-quercetin and doxorubicin at various concentrations were assessed by cellular cytotoxicity (MTT) assay. The $IC_{50}$ values of quercetin, nano-quercetin and doxorubicin on MDA-MB-231 cell line were 394.8, 204.6 and 17.17 µM, respectively. *In vitro* anti-cancer effects of these drugs were manifested as apoptosis and cellular toxicity. The cytotoxicity was moderate in quercetin, remarkable in nano-quercetin and strong in doxorubicin treatments. The *in vitro* anti-cancer effect of quercetin was comparable with the standard drug doxorubicin. Considering the potential side effects of doxorubicin, the quercetin might be explored with further studies in the treatment of mammary tumours in animals and humans.**

**Keywords:** Doxorubicin, MDA-MB-231, MTT assay, quercetin, TNBC

## INTRODUCTION

Breast cancer is one of the most commonly occurring cancers in animals and humans. It is classified into several intrinsic subtypes such as luminal, HER2 over-expressive and basal-like breast cancer including triple negative breast cancer (TNBC). The first two subtypes have favourable outcome for the treatments based on the target specific receptors like ER and HER2. The last subtype TNBC is associated with poor clinical prognosis due to the lack of specific targeted therapies. It accounts for 10-17% among overall breast cancers and frequently occurs in younger patients (Podo *et al.,* 2010). It shows more aggressive and metastatic behaviours than other two subtypes (Carey *et al.,* 2007). The TNBC patients are usually treated by chemotherapy and radiotherapy; both of them are associated with potential side effects (Carey *et al.,* 2007). So, the current oncotherapy programmes shifted towards finding the safe drugs without toxicities. MDA-MB-231 is a specific cell line for TNBC used in early screening of phytochemicals against human breast cancers (Rahman and and Jameel, 2014).

Phytochemicals have widely been explored for their anti-cancer properties without toxic potentials. Quercetin is a flavonoid that possesses multiple biological actions including anti-oxidant, anti-inflammatory and anti-cancer potentials (Morel *et al.,* 1993; Guardia *et al.,* 2001; Gupta *et al.,* 2010). Quercetin enhances the anti-tumour effects of radiotherapy and chemotherapy in animal models (van Rijn and van den Berg 1997). The anti-cancer effects of quercetin have earlier been

investigated which depicted their role in preventing the growth, proliferation and progression of cancer through cellular signalling pathways (Sharmila *et al.,* 2014; Asgharian *et al.,* 2022). The available literatures on anti-cancer effects of quercetin on TNBC patients are meagre (Nguyen *et al.,* 2017; Asgharian *et al.,* 2022). Hence, the present study was aimed to find out the effects of quercetin on MDA-MB-231 cell line.

## MATERIALS AND METHODS

***Preparation of nano-quercetin***

Quercetin and doxorubicin were purchased from TCI Chemicals, Chennai, India. The nano-particle was prepared from quercetin using ethanol and polyvinyl alcohol (PVA) by nano-precipitation technique (Wu *et al.,* 2008). PVA (2 g) was added to 500 mL distilled water and then stirred in a magnetic stirrer overnight undisturbed. Quercetin (2 mg) was dissolved in 20 mL ethanol and the mixture was added to PVA drop by drop. This whole mixture was stirred for 2 h in a magnetic stirrer. The ethanol was completely removed by vacuum suction and the resultant material was air-dried to get the purified nano-quercetin. The formation of nano-particles appeared as reduced-sized crystals and was characterized under field emission scanning electron microscope (Tescan, MAIA3, Korea).

***Cell line and experimental design***

Triple negative breast cancer cell line (MDA-MB-231) was purchased from National Centre for Cell Sciences (NCCS), Pune, India. It was cultured in Dulbecco's modified eagle medium (DMEM) and supplemented with 10% fetal bovine serum (FBS), antibiotic-antimycotic solution and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (Himedia, Mumbai). The cells were maintained in $CO_2$ (5%) incubator at 37ºC and treated with varying concentrations of quercetin (10, 30, 100, 300, 600, 1000, 3000 and 6000 µM), nano-quercertin (10, 100, 300, 600, 1000, 3000 and 6000 µM) and doxorubicin (10, 15, 30, 100, 300, 600, 1000, and 3000 µM). Dimethyl sulfoxide (DMSO) was used as vehicle to dissolve these drugs and also added to the cultures to serve as control. Three replicates for each ingredient was used to obtain appropriate value to fit in sigmoid dose-response non-linear curve in GrsphPad Prism 5.02.

***Cell cytotoxicity assay***

Cell cytotoxicity was analysed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) dye (Ghasemi *et al.,* 2021). MDA-MB-231 cells were cultured, harvested and resuspended in DMEM (Wen *et al.,* 2019). The cells were seeded in a 96 well plate and treated with different concentrations of drugs. The DMSO without the addition of any drugs served as control. The culture was incubated for 48 h at 37ºC in 5% $CO_2$ to reach a state of confluence. The medium was removed and the cells washed twice with phosphate buffered saline (PBS). Then 10 µL of 5 mg $mL^{-1}$ MTT was added to each well and incubated for 4 h at 37ºC in 5% $CO_2$. After incubation, the absorbance was read on iMax microtitre plate reader (M/s Biorad Medisystems, Mumbai) at a wavelength of 595 nm. The $IC_{50}$ was calculated based on the OD value obtained by using GraphPad Prism 5.02. The experiment was repeated twice.

## RESULTS AND DISCUSSION

Quercetin is a polyphenolic flavonoid with several biological properties *viz*., anti-oxidant, anti-tumour, anti-bacterial and anti-proliferative effects. The limitation of quercetin in clinical application is the administration of high dose due to its poor bioavailability. It is a water-soluble derivative but its

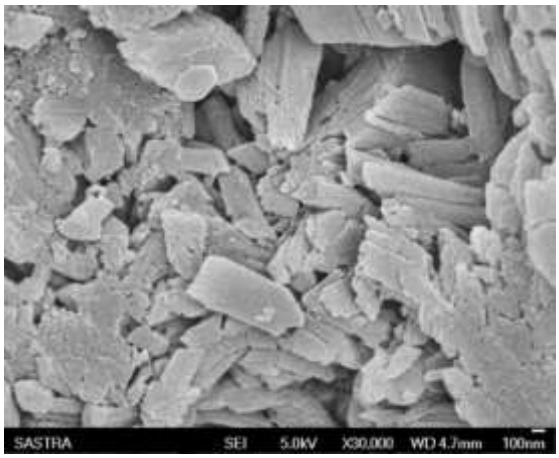


**Fig. 1: Small sized crystals of nano-quercetin after conversion from quercetin SEM x 30000**

bioavailability is only 20% (Wu *et al.*, 2008; Rahmanand and Jameel, 2014). In present study, quercetin was converted into nano-particles with reduced particle size which appeared as crystals (Fig. 1). The surface area of quercetin was 10 to 15 times greater than nano-quercetin. It might be due to the penetration of hydrophobic portion of PVA during nanoprecipitation. PVA enhances the formation of interconnected network with quercetin molecules and thus increases the efficiency (Wu *et al.*, 2008).

The confluences of MDA-MB-231 cell lines before and after treatments are depicted in Fig. 2-3. The MDA-MB-231 cells were treated with various concentrations of quercetin and nano-quercetin against a standard drug doxorubicin. The cellular cytotoxicity was determined by MTT

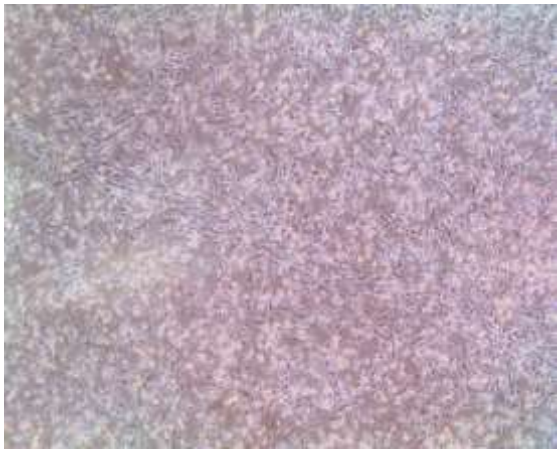

**Fig. 2: Confluence of MDA-MB-231 cell line in DMEM medium before treatment (40X)**

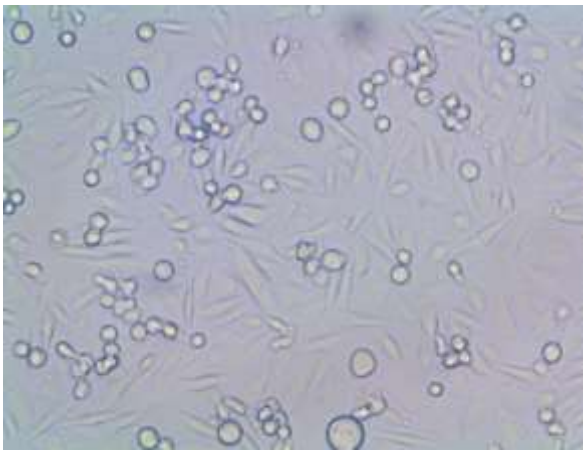

**Fig. 3: Cytotoxic effect of MDA-MB-231 cell line in DMEM medium after nano-quercetin treatment (400X)**

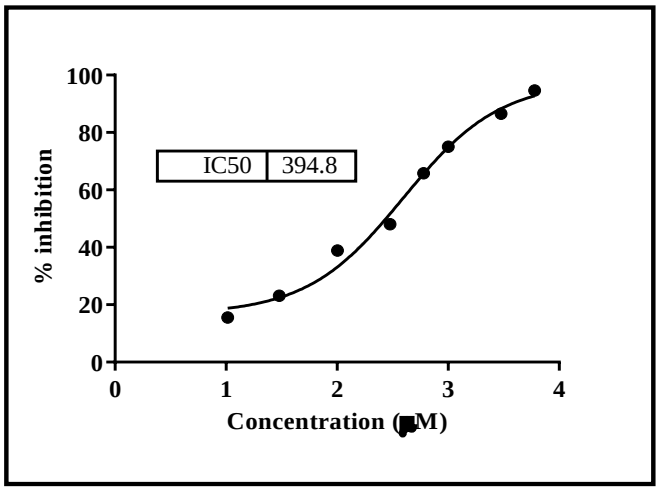


**Fig. 4: $IC_{50}$ value of quercetin on MDA-MB-231 cell line**

assay after 48 h incubation. The $IC_{50}$ values of quercetin, nano-quercetin and doxorubicin were 394.8, 204.6 and 17.17 µM, respectively. The dose dependent inhibition of quercetin, nano-quercetin and doxorubicin in MDA-MB-231 cell line are depict in Fig. 4-6.

The reported $IC_{50}$ value of quercetin on MDA-MB-231 cell lines is 230 µM (Sultan *et al.*, 2017). The cell viability was reduced to 20 and 35% after treatment with 40 and 80 µM quercetin, respectively, while lower concentration (2.5~20 µM) of quercetin caused 5-10% reduction of cell viability (Nguyen *et al.*, 2017). The

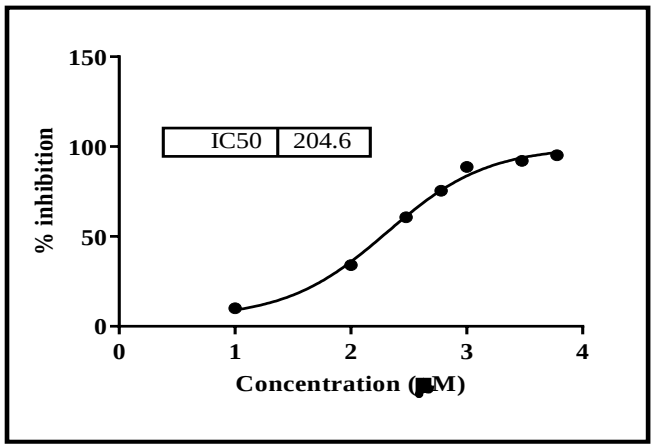


**Fig. 5: $IC_{50}$ value of nano-quercetin on MDA-MB-231 cell line**

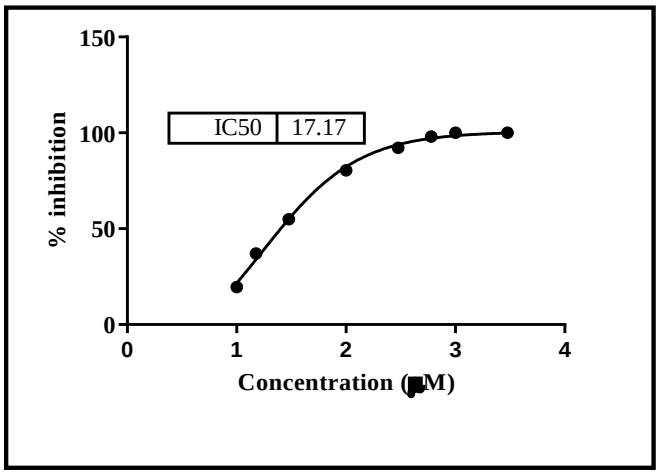


**Fig. 6: $IC_{50}$ value of doxorubicin on MDA-MB-231 cell line**

cytotoxicity of 10 µM quercetin in MDA-MB-231 cell lines was comparable with 100 nM doxorubicin. The cytotoxic effects of 10 nM doxorubicin and 10 µM quercetin combinations were equal to 100 nM doxorubicin effects.

Quercetin alone dose dependently inhibits DNA synthesis in MDA-MB-231 cells and in combination with doxorubicin enhanced the effects. Quercetin dose dependently inhibits protein synthesis and in combination enhanced the doxorubicin effects in tumour cells (Staedler *et al.,* 2011). The $IC_{50}$ value of doxorubicin in MDA-MB-231 cell lines is 6.602 µM. The percentage of cell death increased in dose-dependent manner in MDA-MB-231 cells. The apoptosis was increased by two-fold in MDA-MB-231 cells compared to control cells (Oncul and Ercan, 2017).

Quercetin possesses anti-apoptotic activity in several non-tumorigenic cells. It inhibits hydrogen peroxide, and induces apoptosis in mesangial and epithelial cells. Quercetin often promotes apoptosis in cancer cells without affecting normal cells (Yang *et al.,* 2014; Hu *et al.,* 2015). This study proved that nano-quercetin has better cytotoxicity in MDA-MB-231 cells devoid of toxicity to normal cells. The solubility and bioavailability of quercetin may be improved by the conversion of nanoparticles (Asgharian *et al.,* 2022). The side effects of doxorubicin may be evaded by using nano-quercetin in TNBC patients either alone or in combination with lesser dose of doxorubicin. Further studies are required to understand its anti-cancer effect and safety properties.

**Author's contribution:** Dr. S. Shahana, M.V.Sc. student carried out the study and prepared the draft manuscript and Dr. R. Madheswaran supervised the overall work and reviewed the drafted manuscript.

**Conflict of interest:** The authors declare to have no conflict of interest of any type for this work.

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