Research Article

Cytototoxic Activity of Liposomal Formulations of Quercetin on Ovarian Cancer cells and Normal Human Embryonic Kidney cells

Shreya Banerjee[1, 2], Debasis Dutta[2], Shila Elizabeth Besra[1*]

[1.]Central Instrumentation Facility and Cancer Biology & Inflammatory Disorder Division, CSIR- Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Kolkata, West Bengal, India.

[2.]Pharmaceutics Division, Department of Pharmacy, School of Health Sciences, NSHM Knowledge Campus, Kolkata-Group of Institutions, 124 (60), B. L. Saha Road, Tara Park, Behala, Kolkata, West Bengal, India.

***Corresponding author's E-mail:** shilabesra@iicb.res.in

Received: 10-09-2020; **Revised:** 23-11-2020; **Accepted:** 30-11-2020; **Published on:** 15-12-2020.

ABSTRACT

Quercetin is a flavonoid of natural origin known to have anti-cancer and antioxidant properties. We evaluated effect of liposomal formulations of quercetin on pro-monocytic human myeloid leukemia cell line in our previous work. However, effect of liposomal formulations of Quercetin (QTLF) on human ovarian cancer SKOV-3 and normal human embryonic kidney cells (HEK293T) has not been reported. Therefore, we investigated the cytotoxic effect of QTLF in SKOV-3 as well as Hek293T cell lines. The cytotoxic effect of QTLF was observed by MTT assay. The data reveal that QTLF significantly inhibited the growth of metabolically active cells in a time and concentration dependent manner, whereas insignificantly inhibited the growth of normal cells in a time and concentration dependent manner which reveals the toxic effect of QTLF towards cancer cells only and not in the normal human kidney cells. Thus, it can be considered safe for administration into the human body. Furthermore, mechanistic work needed to prove that the solubility enhancement of drug produces a more potent form of the Novel Drug Delivery System that can be used to modify the pharmacokinetic and pharmacodynamics properties of an insoluble compound.

Keywords: Liposome, Quercetin, Cancer, Cytotoxicity, Novel Drug Delivery.

QUICK RESPONSE CODE →

DOI: 10.47583/ijpsrr.2020.v65i02.013

DOI link: http://dx.doi.org/10.47583/ijpsrr.2020.v65i02.013

INTRODUCTION

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one) is a natural flavonoid compound with nutritional values and potential anti-proliferative effect. In spite of being a compound of notable anti-cancer properties, quercetin has limited gastrointestinal solubility and bioavailability which reduces its pharmacological potential. To overcome these shortcomings, novel carriers can be employed. [1] Nanomedicines are small systems with high surface-volume ratio and involve pharmaceutical compounds with modified permeability and an approach of targeted therapy. Hydrophobic drugs have shown to have better stability and longer release when administered via novel delivery methods. Some nanotechnology-based systems are polymeric micelles, liposomes, dendrimers, and nanoparticles. [2,3] Liposomes are sphere shaped phospholipid bilayer membrane carriers which is capable of incorporating wide ranges of drugs and also ensures biocompatibility, selectivity and stability in human body. Low particle size of liposomes ensures its accumulation in tumor tissues, phenomenon known as enhanced permeability retention (EPR), making it the perfect carrier for cytotoxic drugs. [4] Cancer is a well-known and serious pathological condition occurring mostly as a result of genetic abnormalities which leads to an unstoppable growth of cells. Lumps of genetically similar cells form tumors which can be benign or malignant. In cancerous cells, loss of normal functions such as accurate DNA replication, control over the cell cycle, orientation and adhesion within tissues, and interaction with protective cells of the immune system is normally observed [5, 6]. Currently, there are certain treatment methods followed for cancer, namely, surgical method, radiation therapy and chemotherapy. Conventional chemotherapy, although a very effective method, have the drawbacks of non-selectivity, thereby affecting healthy cells and low accessibility at the site of tumour. However, the emergence of nanotechnology in the past few years have hinted at a prospect of a safer and highly efficient way of cancer therapy. [7] Ovarian cancer is a type of malignancy which is a major cause of death for women. It is not only limited to ovary but also includes fallopian tube and primary peritoneal cancers. [8] It is a threat because there's no method of early detection leading to late diagnosis. Oral contraceptives have been widely used for treatment only. So, it is imperative to work on more pharmaceutical compounds which would successfully treat the condition.[9, 10, 11] HEK293T cells are immortalized human embryonic kidney cells, expressing SV40T antigen gene, which are widely used for toxicological testing of drugs and in cancer research. [12] So, this study focuses on the development and

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

evaluation of a liposomal formulation (QTLF) of a drug of natural origin named Quercetin and thereby analyzing its activity on human ovarian cancer cell line SKOV-3 and human embryonic kidney cell line HEK293T.

MATERIALS AND METHODS

Chemicals used

Quercetin (Loba Chemie, India), Ethanol absolute (Balaji Drugs Pvt. Limited, India), Chloroform (Balaji Drugs Pvt. Limited, India), Soya Lecithin (Himedia, India), Hydrogen Chloride (Rankem , Avantor, India), Potassium chloride, Potassium di-hydrogen phosphate, Sodium hydroxide (Merck Specialties Pvt. Ltd., Mumbai), DMEM media (Gibco), Foetal calf serum (FCS), Penicillin-Streptomycin, Gentamycin, MTT [3-(4,5-dimethylthiozol-2-il) 2,5-2,5-dipheniltetrazoliumbromide], Dimethyl sulphoxide (DMSO).

Cell Culture

Human Ovarian Cancer cell line (SKOV-3) and Human Embryonic Kidney cell line (HEK293T) cell lines were obtained from National Centre for Cell Science, Pune for *in-vitro* studies. These cells were sub-cultured as per the requirement of the experiment at an initial concentration of 1 x 10^6 cells/ml. SKOV-3 and HEK293T cells were maintained in sterile DMEM medium supplemented with 10% heat activated FCS. Cultures were maintained at 37° C in a humified atmosphere containing 5% CO_2 in air.

Preparation of Standard Curve of Quercetin in Ethanol at 370nm

50 mg Quercetin was measured and taken into a 50 ml volumetric flask. 50ml ethanol was added into the flask to solubilise the Quercetin (QTF) properly and concentration becomes 1000µg/ml. 5ml of solution was taken and dissolved in 50ml ethanol to make 100µg/ml. From this stock solution different concentrations of QTF solution i.e., 2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml were prepared using ethanol. The Quercetin solutions were scanned in UV Visible Spectrophotometer (UV 1800, Shimadzu, Japan) at 200-400nm. Then absorbance was measured by UV Spectrophotometry at 370nm wavelength using ethanol as blank.

Preparation of Standard Curve of Quercetin in Phosphate buffer pH 7.4 at 370nm

25 mg quercetin was measured and taken in a 100ml volumetric flask. Around 1ml of ethanol was added into the flask to solubilize the Quercetin properly and the volume was made up to 100ml with phosphate buffer solution pH 7.4 to make a concentration of 250µg/ml. From this stock solution different concentrations of QTF solution 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml were prepared using phosphate buffer pH 7.4. The quercetin solution was scanned in UV Visible spectrophotometer (UV 1800, Shimadzu, Japan) at 200-400nm. Then absorbance was measured by UV Spectrophotometry at 370nm wavelength using phosphate buffer as blank [13].

Preparation of Liposomal Formulations of Quercetin

Blank liposomes were prepared using lipid and the organic solvents only and the consistency of the formulation was checked. After that lipid mixture of phospholipid and Quercetin was dissolved in two organic solvents which were present in a fixed ratio and shaken continuously for some time under a temperature of 50-55ºC. The film formed was then hydrated by an aqueous buffer solution and the dispersion was then sonicated. The liposome dispersion was transferred into a tube and then placed in a bath Sonicator. Controlling the temperature of the lipid dispersion is usually easier in this method. The dispersion was then kept in a sterile container and stored for evaluation [14].

Table 1: Formulations of Liposome Preparation

INGREDIENT	QTLF1	QTLF2	QTLF3	QTLF4	QTLF5	QTLF6	QTLF7	QTLF8
Quercetin/mg	80	75	150	270	200	234	270	200
Soya Lecithin/mg	20	25	20	20	20	20	40	30
Ethanol/ ml	10	10	10	10	10	10	10	10
Chloroform/ ml	10	10	10	10	10	10	10	10

Drug Identification Study by FTIR

Fourier Transform Infrared Spectroscopy (FTIR) of pure drug using FTIR spectrophotometer. The sample is prepared with potassium bromide and data are collected at a spectral range of 450-4000 cm^{-1} [13].

Entrapment Efficiency (%EE)

The mixture was centrifuged for 70 minutes at 14000 rpm, the supernatant containing free Quercetin was obtained, and the absorbance was measured using HPLC. The entrapment efficiency of liposomes was determined by the following formula: EE (%) = $\{(C_i - C_f)/C_i\} \times 100$ where EE is the concentration of entrapped sample (mg/mL), C_i is the initial concentration of sample used in formulating the liposomes (mg/mL), C_f is the concentration of sample in the supernatant (mg/mL), and EE (%) is the percentage of the sample's entrapment [15].

Cytotoxicity Study on Ovarian Cancer and Human Embryonic Kidney Cells

SKOV-3 and HEK293T (1x10^5) cells (100µl of cell suspension per well) were seeded in 96-well plates and incubated inside a CO_2 incubator for 24 hours before

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

treatment. They were treated separately with freshly prepared 1mg/ml stock solution of Liposomal formulations of Quercetin (QTLF1-QTLF8), with doses of 50µg, 100µg and 200µg for 24 hours at 37° C in a humified atmosphere containing 5% CO_2 in air. Untreated cells served as control. At the end of treatment, 20µl of MTT [3-(4,5-dimethylthiozol-2-il)-2,5-2,5-diphenyltetrazoliumbromide] was added to each well and incubated for another 4 hours at 37-degree C in a CO_2 incubator. The MTT assay is a colorimetric assay for measuring the activity of enzymes that reduce MTT to formazan dyes, giving a purple color. A solubilizing solution DMSO (Dimethyl sulphoxide) 100µl is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance was taken at 570 nm by micro plate manager (Reader Type: Model 680 XR Bio-Rad laboratories Inc.) [16].

Statistical Analysis

This was done by Student's t-test $P < 0.05$ was considered as significant. The percentage cell inhibition was calculated by the following formula:
% Cell Inhibition: 100 X (O. D of Control – O. D of Treated)/ O. D of Control Where O. D= Optical Density

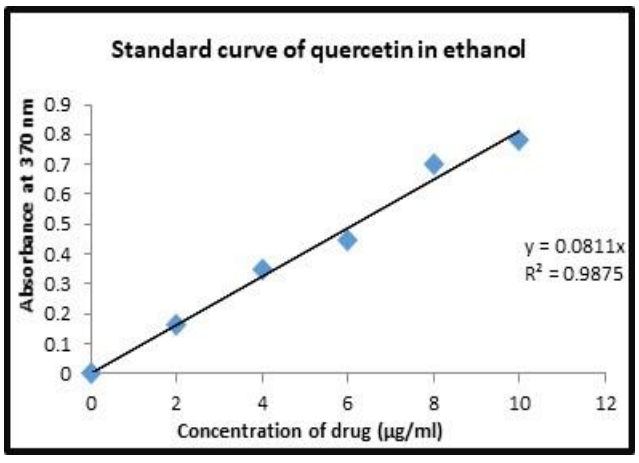
RESULTS

Preparation of Standard Curve of Quercetin in Ethanol at 370nm

The Quercetin solutions (2µg/ml, 4µg/ml, 6µg/ml, 8µg/ml and 10µg/ml) dissolved in ethanol were scanned in UV Visible Spectrophotometer (UV 1800, Shimadzu, Japan) at 200-400nm. Then absorbance was measured by UV Spectrophotometry at 370nm wavelength using ethanol as blank. A graph was plotted keeping the concentrations in the X-axis and the absorbance found in the Y-axis.

Preparation of Standard Curve of Quercetin in Phosphate buffer pH 7.4 at 370nm

The quercetin solution (5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml dissolved in phosphate buffer pH 7.4) was scanned in UV Visible spectrophotometer (UV 1800, Shimadzu, Japan) at 200-400nm. Then absorbance was measured by UV Spectrophotometry at 370nm wavelength using phosphate buffer as blank.



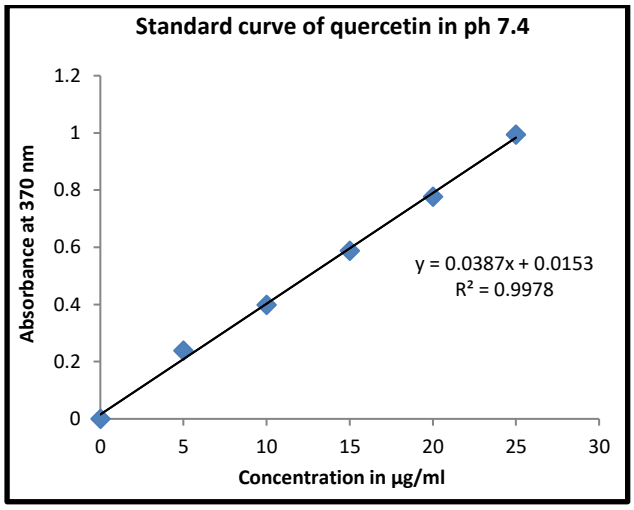


Figure 1: Standard Curves of Quercetin in Ethanol and pH 7.4. The concentration is plotted in the X-axis and the Absorbance in the Y-axis. The graphs give us R^2 values of 0.9875 and 0.9978 respectively.

Table 2: Standard Curve of QTF in Ethanol

Concentration (µg/ml)	Absorbance			Average ± S.D.
	1st	2nd	3rd	
2	0.156	0.162	0.169	0.162± 0.005
4	0.331	0.353	0.360	0.348± 0.012
6	0.439	0.452	0.445	0.445±0.005
8	0.697	0.699	0.707	0.701± 0.004
10	0.775	0.781	0.797	0.784± 0.009

Table 3: Standard Curve of QTF in Phosphate Buffer pH 7.4

Concentration (µg/ml)	Absorbance			Average ± S.D.
	1st	2nd	3rd	
5	0.237	0.238	0.240	0.238±0.0012
10	0.403	0.405	0.389	0.399±0.007
15	0.583	0.589	0.592	0.588±.0037
20	0.771	0.778	0.782	0.777±.0045
25	0.996	0.994	0.993	0.994±0.0012

Identification of Quercetin by FTIR

Fourier Transform Infrared Spectroscopy (FTIR) of pure drug was taken using FTIR spectrophotometer. The sample was prepared with potassium bromide and data were collected at a spectral range of 500-4000 cm^{-1}.

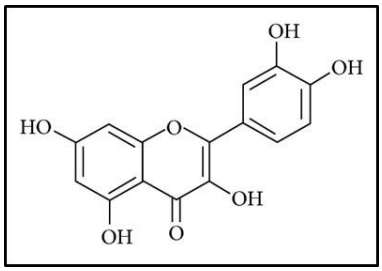


Figure 2: Structure of Quercetin

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

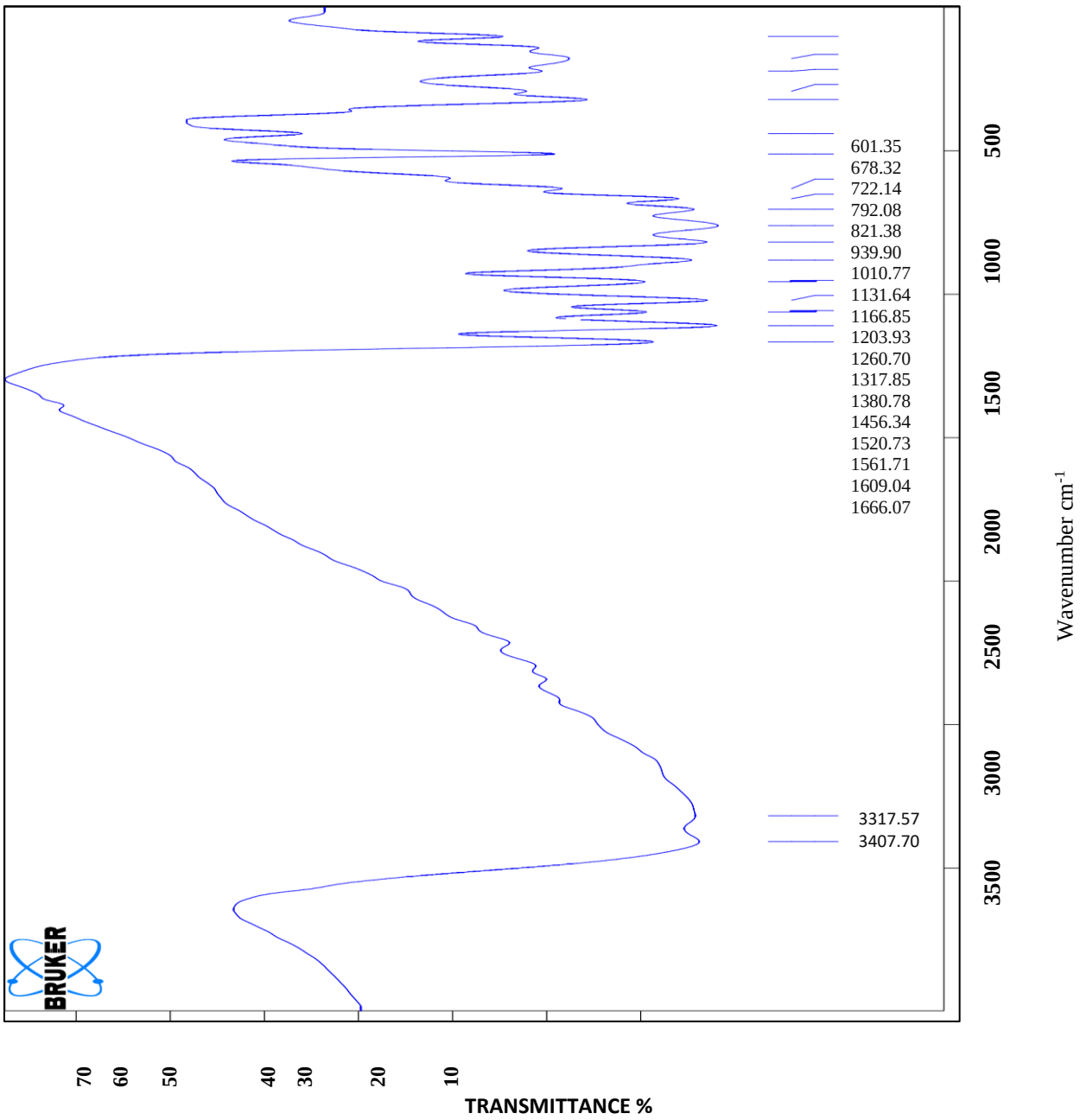


Figure 3: FTIR Spectrum of Quercetin

The peaks obtained were at 3407.70 cm^{-1}, 3317.57 cm^{-1}, 1666.07 cm^{-1}, 1609.04 cm^{-1}, 1561.71 cm^{-1}, 1520.73 cm^{-1}, 1456.34 cm^{-1}, 1380.78 cm^{-1}, 1317.85 cm^{-1}, 1260.70 cm^{-1}, 1203.93 cm^{-1}, 1166.85 cm^{-1}, 1131.64 cm^{-1}, 1010.77 cm^{-1}, 939.90 cm^{-1}, 821.38 cm^{-1}, 792.08 cm^{-1}, 722.14 cm^{-1}, 678.32 cm^{-1} and 601.35 cm^{-1}. The peak at 1380.78 cm^{-1} shows O-H bending and that at 3407.70 cm^{-1} show O-H stretching of phenolic groups. The peak at 1520.73 cm^{-1} points out the presence of aromatic C=C stretching and that at 939.90 cm^{-1} is responsible for aromatic C=C bending. The peaks at 1456.34 cm^{-1} is responsible for in-plane aromatic C-H bending and at 722.14 cm^{-1} leads to presence of out of plane bending of aromatic C-H bond. C=O stretching is detected by the presence of sharp peak at 1666.07 cm^{-1}.

Entrapment Efficiency

The entrapment efficiency of liposomes was determined by the following formula:

$$EE\ (\%) = \{(C_i - C_f)/C_i\} \times 100,$$

where EE is the concentration of entrapped sample (mg/mL), C_i is the initial concentration of sample used in formulating the liposomes (mg/mL), C_f is the concentration of sample in the supernatant (mg/mL), and EE (%) is the percentage of the sample's entrapment. The entrapment efficiencies were found to decrease with the increase in the amounts of lipid.

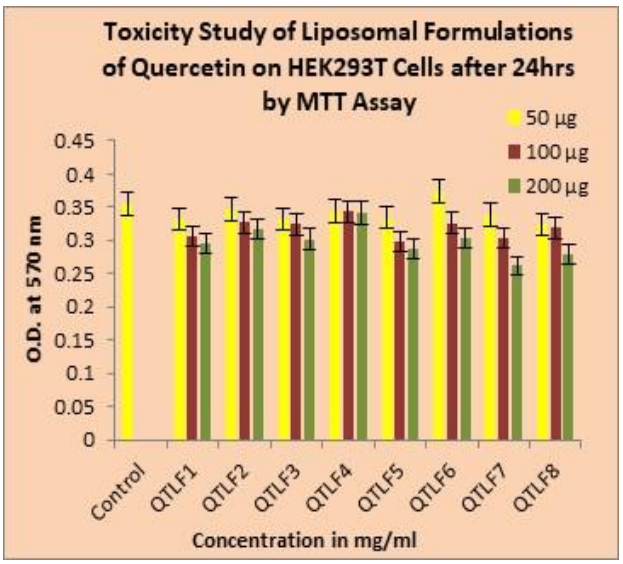
©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

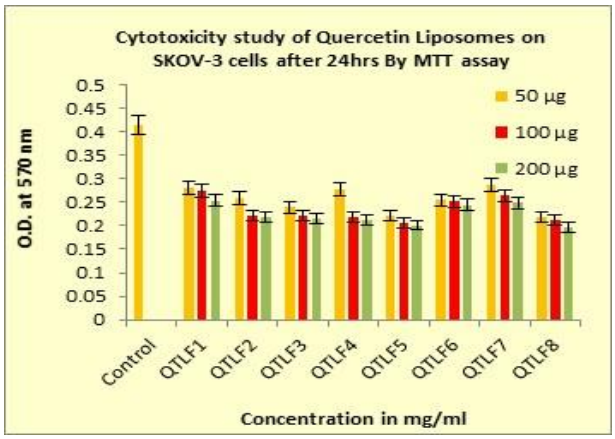
Table 4: Entrapment Efficiency of Quercetin Liposomal Formulation

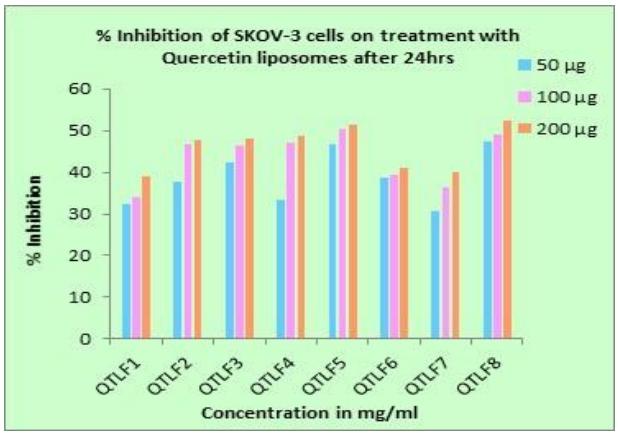
Formulation			Initial Conc. in mg/ml (C_i)	Final Conc. in mg/ml (C_f)	EE (%)
Code	Drug (mg)	Lipid (mg)			
QTLF1	20	80	1.00	0.1214	87.86
QTLF3	20	150	1.00	0.1653	83.47
QTLF5	20	200	1.00	0.1705	82.95
QTLF6	20	234	1.00	0.1912	80.88
QTLF4	20	270	1.00	0.2997	70.03
QTLF2	25	75	1.25	0.3049	75.60
QTLF7	40	270	1.00	0.2093	79.07
QTLF8	30	200	1.00	0.1681	83.19

Cytotoxicity Study on Ovarian Cancer and Human Embryonic Kidney Cells

The cytotoxic effects of the formulations (QTLF1-QTLF8) were checked on human ovarian cancer cells SKOV-3 by MTT Assay. All the formulations were found to have inhibitory effect on ovarian cancer cells after 24 hours of treatment. QTLF5 and QTLF8 showed slightly higher cytotoxicity as compared to the rest. So, it is reasonable to assume that if treated for more hours, a stronger inhibitory action will be observed. Whereas cytotoxic effects of the formulations (QTLF1-QTLF8) were checked on human embryonic kidney cells HEK293T by MTT Assay to analyze the effects of drug on normal cells. The formulations showed insignificant toxic effect on the cells. The O.D. values at 570 nm were plotted against the concentrations of 50 µg, 100 µg and 200 µg. All eight formulations were found to show an inhibition of below 30% which is considered safe for administration. Hence, one can conclude that it does not cause toxicity to healthy human cells.







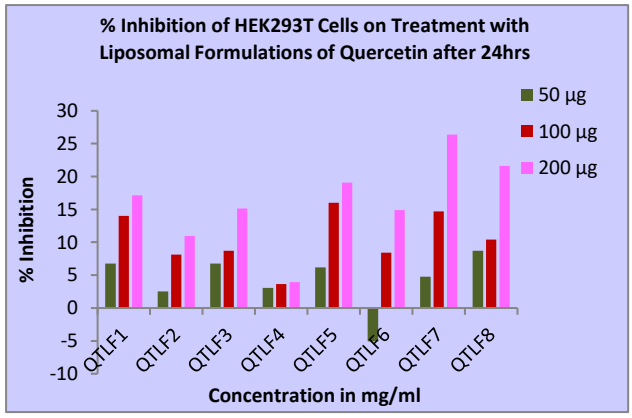


Figure 4: Histograms show the effect of QTLF1-QTLF8 on SKOV-3 cells and on HeK293T cell line by MTT Assay after 24 hours. Reduction in the O.D. values and increase in %

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

inhibition is observed in a concentration dependent manner. Data are mean ± S.E.M. The highest inhibition on SKOV-3 cells was shown by QTLF5 and QTLF8 whereas the rest showed moderate cytotoxicity. All the formulations show effects below toxicity level on HEK293T cells, QTLF4 being the least toxic.

DISCUSSION

Quercetin is a chemo preventive agent of natural origin and is known to cause apoptosis or cell cycle arrest. It has shown efficiency of various types of cancer such as that of prostrate, cervical, lung, breast and colon. [16] Quercetin is a potential candidate for treatment of ovarian cancer but the disadvantages associated with it are low water solubility and hence poor systemic bioavailability. So, it is necessary to incorporate it into suitable delivery system and yield a suitable pharmaceutical dosage form. [17] Liposomes are carriers which can provide effective cancer treatment on incorporation of flavonoids, eliminating adverse effects of chemotherapy. Liposome bound drugs show an increase in bioavailability and hence leads to proper cancer treatment. [18, 19] Hence, in this study, a liposomal formulation of Quercetin is prepared to check the activity on human ovarian cancer cell line, SKOV-3. The toxicological screening of the formulations are done on human embryonic kidney cell line, HEK293T, which is often used in research fields for determination of adverse effects of drugs on normal healthy cells. [12]

On determining the entrapment efficiency, QTLF1 showed the maximum percentage whereas QTLF4 showed the least value. The cytotoxic activity was observed on human ovarian cancer cells SKOV-3.The formulations (QTLF1-QTLF8) showed a satisfactory cytotoxic activity on SKOV-3 cell line after 24 hours which indicates stronger effect after 48 or 72 hours of exposure. QTLF5 and QTLF8 were found to show slightly higher inhibitory effect on the ovarian cancer cells. Further, a toxicity study was performed on Human Embryonic Kidney cells (HEK293T). The formulations (QTLF1-QTLF8) have not shown a toxic effect on healthy cells and hence were considered safe for administration into body. QTLF4 shows the least toxicity among all the formulations.

Summary & Conclusion

Quercetin is a poly-phenolic compound which bears high antioxidant, anti-tumor and cytotoxic activity which is crucial to fight different types of cancer. It is useful in treatment of quite a number of cancers like breast, colon, cervical, etc. However, it belongs to BCS Class IV which is why it has low solubility in aqueous media and limited bioavailability in systemic circulation. Being a natural flavonoid, Quercetin is devoid of extreme harmful effects and can be considered a much safer candidate for treatment of cancer. So, overcoming its limitations is the most essential approach in giving rise to a major breakthrough in cancer therapy.

Novel Drug Delivery is a unique approach which leads to formulations of small particle size with better solubility, higher bioavailability and efficient targeting at the site of action. Novel drug delivery formulations can be prepared by modifying the release kinetics of drug in the body by incorporation into lipid matrices or in suitable polymers such as liposomes, nanoparticles, phytosomes etc. The drug under consideration, Quercetin can be considered a good candidate to be converted into a novel formulation by incorporation into a lipid carrier. The choice of novel carrier was Liposome, a lipid matrix composed of phospholipids with a hydrophilic head and a lipophilic tail. Liposomes structurally represent the biological membranes and hence are biocompatible and non-immunogenic. It is capable of incorporating both lipophilic and hydrophilic drugs into it and gets accumulated in the vicinity of tumors once administered. This results in a strong and prolonged pharmacological action thereby making liposomes, the novel carrier of choice for the study.

Cancer is a result of gene mutations characterized by abnormal cell growth and proliferation leading to some serious health hazards. This occurs by various factors like gene mutations, smoking, environmental factors etc. This study focuses on ovarian cancer. It is a type of cancer frequently seen in women affecting the gynecological processes, although this type of cancer whose early detections are yet to be discovered and thereby is a prime cause of death in women. Oral contraceptives and certain synthetic anti-cancer drugs have been used for the treatment but have given rise to side effects. Hence, efforts are frequently given by researchers to increase natural drug formulations in market which can be equally effective but with reduced side effects.

The free drug Quercetin had to undergo initial identification tests wherein standard curves were prepared and FTIR spectra of the drug was analyzed. Eight liposomal formulations of Quercetin within different drug and lipid ratios were prepared by hand shaking method and stored for characterization. The entrapment efficiencies of the formulations were determined. The prepared liposomes (QTLF1-QTLF8) were then used for *in-vitro* studies to check the cytotoxic activity on the human ovarian cancer cell (SKOV-3). Toxicity studies of all eight formulations were conducted on normal human cells, namely human embryonic kidney cells (HEK293T). Kidney is a vital organ involved in filtration of blood, formation of urine, maintaining homeostasis etc. in our body. Kidney cells are delicate and prone to drug adverse effects thereby disrupting the normal functioning of the human body. Thus, the human embryonic kidney cells were selected for examining the toxicity causing capabilities of the liposomal formulations of Quercetin.

Hence, it can be concluded that in the ovarian cancer cells, the liposomal preparations of Quercetin show cytotoxic activity after a treatment for 24 hours, so it is found to be effective for treating ovarian cancer. The toxicity studies on normal human cells i.e., human embryonic kidney cells, proved the formulations to be safe for administration into human body. So, a further conclusion can be drawn that by

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.

incorporating the drug into the lipid carrier, the activity of the drug was found to improve and hence the solubility enhancement effectively intensifies the cytotoxic activities of Quercetin by correcting the solubility issues.

Acknowledgement: The authors of this paper are very much thankful to Council of Scientific and Industrial Research, Indian Institute of Chemical Biology (CSIR-IICB), Kolkata for providing the funding to perform the research work and also NSHM Knowledge Campus, Kolkata-Group of Institutions, where a portion of the study was conducted.

REFERENCES

1. Das S., Hussain A. , Verma P.R.P, Imam S. S., Altamimi Mohammad A, Alshehri S., Singh S. K., Recent Advances in Liposomal Drug Delivery System of Quercetin for Cancer Targeting: A Mechanistic Approach. *Current Drug Delivery,* 17(1), 2020, 6.
2. Faheem A. M. and Abdelkader D. H., Novel drug delivery systems. *Engineering Drug Delivery Systems,* 2020, pp: 1-16.
3. Pucci C., Martinelli C., and Ciofani G., Innovative approaches for cancer treatment: current perspectives and new challenges. *Ecancermedical science*, 13, 2019, 961.
4. He K., Tang M., Safety of Novel Liposomal Drugs for Cancer Treatment: Advances and Prospects. *Chemico-Biological Interactions*, 2017, pp: 1-23.
5. Kakde D., Jain D., Shrivastava V., Kakde R. and Patil A. T., Cancer Therapeutics- Opportunities, Challenges and Advances in Drug Delivery. *Journal of Applied Pharmaceutical Science,* 01 (09), 2011, 01-10.
6. Hassanpour S. H., Dehghani M., Review of cancer from perspective of molecular. *Journal of Cancer Research and Practice*, 4 (4), 2017, 127-129.
7. Senapati S., Mahanta A. K., Kumar S. and Maiti P., Controlled drug delivery vehicles for cancer treatment and their performance. *Signal Transduction and Targeted Therapy*, 3(7), 2018, 1-19.
8. Lee Jung-Min, Minasian Lori, Kohn Elise C., New strategies in ovarian cancer treatment. *Cancer*, 125 (S24), 2019, 4623-4629.
9. Song Y. S., Kim H. S., Aoki D., Dhanasekaran D. N. and Tsang B. K., Ovarian Cancer. *BioMed Research International*, 764323, 2014, 1-2.
10. Chien J. and Poole E. M., Ovarian cancer prevention, screening and early detection: Report from the 11th Biennial Ovarian Cancer Research Symposium. *Int J Gynecol Cancer*, 27(9), 2017, S20–S22.
11. Modugno F. and Edwards R. P., Ovarian Cancer: Prevention, Detection and Treatment of the Disease and Its Recurrence. Molecular Mechanisms and Personalized Medicine Meeting Report, *Int J Gynecol Cancer*, 22(8), 2012, S45–S57.
12. Hu, J., Han, J., Li, H., Zhang, X., Liu, L. lan, Chen, F., & Zeng, B., Human Embryonic Kidney 293 Cells: A Vehicle for Biopharmaceutical Manufacturing, Structural Biology, and Electrophysiology. *Cells Tissues Organs*, 205, 2018, 1-8.
13. N. Kendre Prakash, V. Pande Vishal and M. Chavan Kishori, Novel Formulation Strategy to Enhance Solubility of Quercetin. *Pharmacophore,* 5 (3), 2014, 358-370.
14. Rao Monica R. P. and Laxmi S. B., Liposomal Drug Delivery for Solubility and Bioavailability Enhancement of Efavirenz. *Indian J Pharm Sci*. 80(6), 2018, 1115-1124.
15. Bahareh S., Ibrahim N. M., Shaharuddin, Development and Characterization of Liposomal Doxorubicin Hydrochloride with Palm Oil. *BioMed Research International,* 2, 2014, 765426.
16. Besra A. R., Pal K., Basu S., Besra S. E., Anti-tumor activities of secretion extract of *Bellamya bengalensis* in human hepatocellular carcinoma cell lines is mediated by caspase-dependent apoptosis and cell cycle arrest. *World Journal of Pharmacy and Pharmaceutical Sciences*, 4 (10), 2015, 2326-2344.
17. Jeong J. H., An J. Y., Kwon Y. T., Rhee J. G., and Lee Y. J., Effects of low dose quercetin: Cancer cell-specific inhibition of cell cycle progression. *J Cell Biochem*, 106(1), 2009, 73–82.
18. Xu, G., Li, B., Wang, T., Wan, J., Zhang, Y., Huang, J., & Shen, Y., Enhancing the anti-ovarian cancer activity of quercetin using a self-assemblin. *RSC Adv*., 8, 2018, 21229.
19. Das, A., Konyak, P. M., Das, A., Dey, S. K., & Saha, C., Physicochemical characterization of dual action liposomal formulations: anticancer and antimicrobial. *Heliyon*, 5, 2019, e02372.

Source of Support: None declared.

Conflict of Interest: None declared.

For any question relates to this article, please reach us at: editor@globalresearchonline.net
New manuscripts for publication can be submitted at: submit@globalresearchonline.net and submit_ijpsrr@rediffmail.com

©Copyright protected. Unauthorised republication, reproduction, distribution, dissemination and copying of this document in whole or in part is strictly prohibited.