Research Article

Evaluation of Liposomal Formulations of Quercetin *on* Promonocytic Human Myeloid Leukemia Cell Line

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ABSTRACT

Novel Drug Delivery System is a recent and advanced field of study which can be used to modify the pharmacokinetic and pharmacodynamics properties of a drug. Quercetin is a flavonoid of natural origin known to have anti-cancer and antioxidant properties. It belongs to BCS Class IV and hence challenges are faced to combat the solubility issues of the drug. Therefore, this study aims to preparing a liposomal formulation of Quercetin to improve its solubility profile and thereby intensify its pharmacological activity. Initial characterizations of the prepared Quercetin liposomal formulations (QTLF) were evaluated on promonocytic human myeloid leukemia (U937), cell by MTT assay. QTLF produced notable cytotoxic effect on U937. The Quercetin liposomal formulations showed greater efficacy than the free drug in U937. Thus, it can be considered safe for administration into the human body which proved that the solubility enhancement of drug produced a more potent form of the drug.

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

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INTRODUCTION

Conventional drug delivery systems have limited efficiency in this medical world of large number of diseases and complications that affect the human body on a regular basis. Hence, the needs for faster and sustainable therapies arise. Novel drug delivery system is an advanced delivery system which appears suitable for fulfilling such requirements. The novel drug delivery system (NDDS) consists of carriers which maintain the drug concentration in therapeutic range for longer period of time and improve the bioavailability [1]. Novel drug delivery follows the method of drug release modification by using modified polymers and also different carriers which release the drug at the site of action. Carriers include nanoparticles, liposomes, phytosomes etc. [2]

Liposomes are a group of novel vesicular systems which can incorporate a variety of drugs of both hydrophobic and hydrophilic natures [3]. They are biocompatible as they resemble the structure of a biological membrane [4]. It primarily consists of phospholipids, which are molecules that have a head group and a tail group. The head is attracted to water, and the tail, which is made of a long hydrocarbon chain, is repelled by water [5]. Researchers all over the world have been working on natural products, keeping in mind the harmful effects of synthetic drugs and have succeeded in establishing their affectivity on various diseases. Flavonoids are a group of natural products which are present in quite a number of fruits and vegetables and can be useful in treating various pathological conditions. Quercetin is one such flavonoid possessing cytotoxic activity [6]. Its pharmacological actions are limited by hydrophobicity, instability in physiological media, poor gastrointestinal dissolution rate and absorption and extensive xenobiotic metabolism at intestines and liver. Hence, its formulation in a suitable delivery system may improve its oral bioavailability, ensuring protection from degradation and preventing premature release [7, 8].

Cancer is the biggest cause of mortality in the world and happens when the tumor suppressing genes are obstructed by various environmental or lifestyle factors [9]. The types of cancer are sarcoma which includes growth of malignant tumours in connective tissues, lymphoma which causes proliferations in lymphocytes, leukaemia which leads to uncontrolled and exaggerated growth of blood cells and sarcoma which results in uncontrolled division of cells in the skin or tissue which cover the internal organs [10, 11, 12, 13]. Leukemia being one of the most dangerous types of cancers is associated with serious impact on human body[14].

So, this study focuses on the development and evaluation of a liposomal formulation (QTLF) of a drug of natural origin named Quercetin and thereby analyzing its activity on human myeloid leukemia cell line U937.

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MATERIALS AND METHODS

Chemicals

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), Distilled water, RPMI1640 medium with L-glutamine (GIBCO), Foetal calf serum (FCS), Penicillin-Streptomycin, Gentamycin, MTT [3-(4,5-dimethylthiozol-2-il) 2,5-2,5-diphenilteltrazoliumbromide], Dimethyl sulphoxide (DMSO).

Cell Culture

Human leukemic cell line U937 cell line was 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. U937 cells were maintained in sterile RPMI 1640 medium supplemented with 10% heat activated FCS and cultures was 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 solubilise 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 [15].

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 [16].

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}. [15]

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. [17]

Cytotoxicity Study by MTT Assay

For cytotoxicity analysis, 1 x 10^5 concentrations of human leukemic cell U937 in log phase were used as stock suspension. 100 µl of these cell suspensions were seeded in each well of 96 well tissue culture plates. They were treated separately with freshly prepared 1mg/ml stock solution of free Quercetin as well as the Liposomal formulations of Quercetin (QTLF1-QTLF8), with doses of 25µg, 50µg, 100µg and 200µg for 24, 48, 72 hours at 37° C in a humified atmosphere containing 5% CO_2 in air. Untreated cells served as control. At the end of treatment,

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20µl of MTT [3-(4,5-dimethylthiozol-2-il)-2,5-2,5-diphenilteterazoliumbromide] 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 colour. A solubilisation solution DMSO (Dimethyl sulphoxide) 100µl is added to dissolve the insoluble purple formazan product into a coloured solution. The absorbance was taken at 490 nm by micro plate manager (Reader Type: Model 680 XR Bio-Rad laboratories Inc.). From the absorbance values obtained at 490 nm the % inhibition was determined using the relation:

% Cell Inhibition: 100 X (O. D of Control − O. D of Treated)/ O. D of Control [18].

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.

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

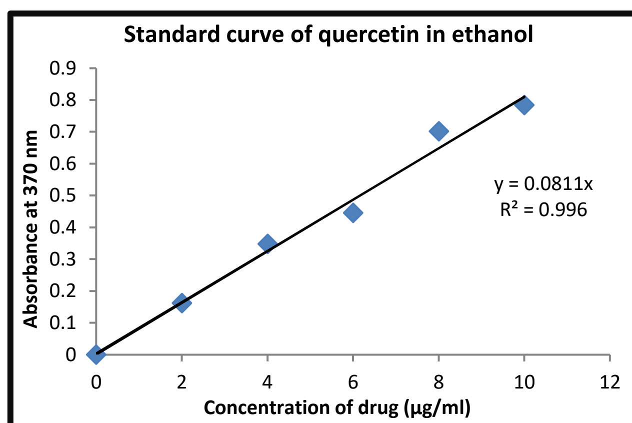

Figure 1: Standard Curve of Quercetin in Ethanol. The concentration is plotted in the X-axis and the Absorbance is plotted in the Y-axis. The graph gives us an R^2 value of 0.9875.

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.

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

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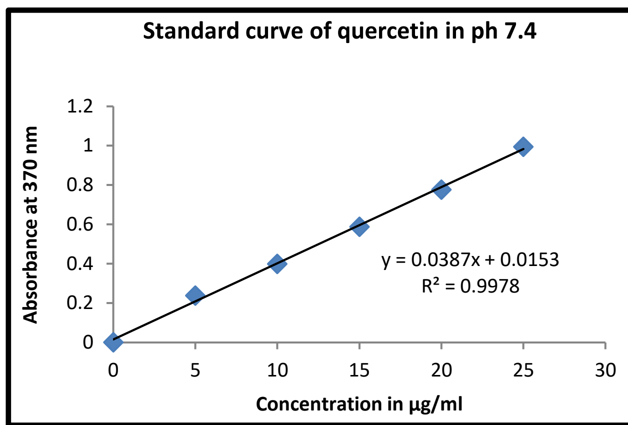


Figure 2: Standard Curve of Quercetin at pH 7.4. The concentration is plotted in the X-axis and the Absorbance is plotted in the Y-axis. The graph gives us an R2 value of 0.9978.

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}.

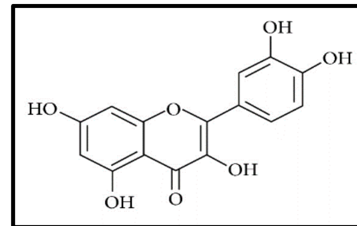
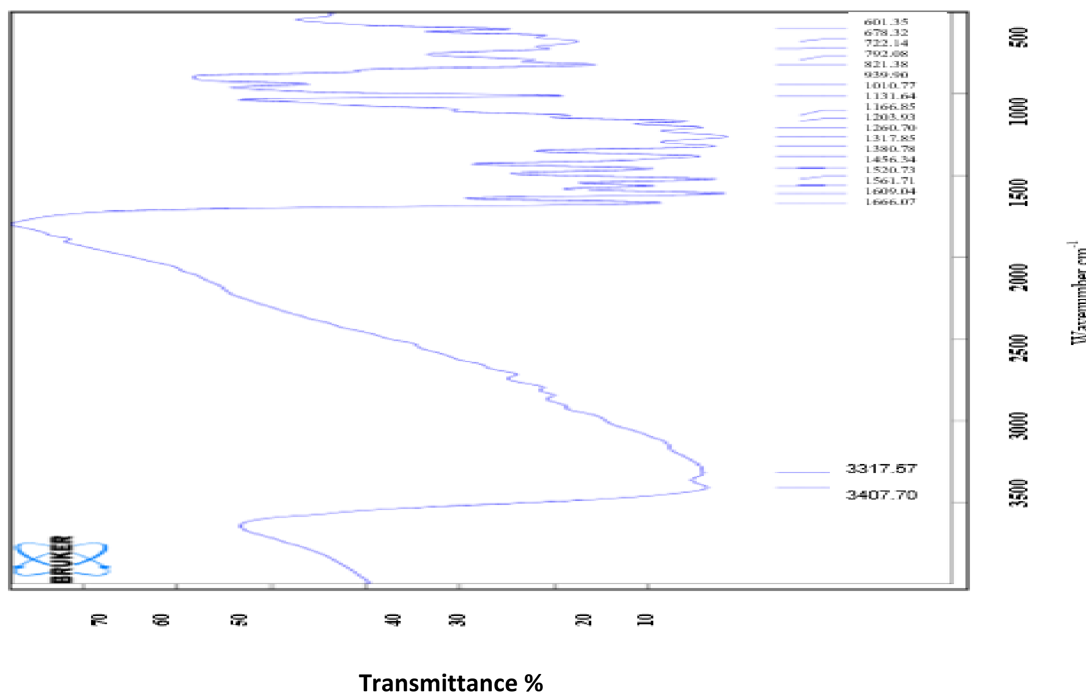


Figure 3: Structure of Quercetin



Transmittance %

Figure 4: 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.

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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

Determination of IC_{50} values of Quercetin Liposomal Formulations

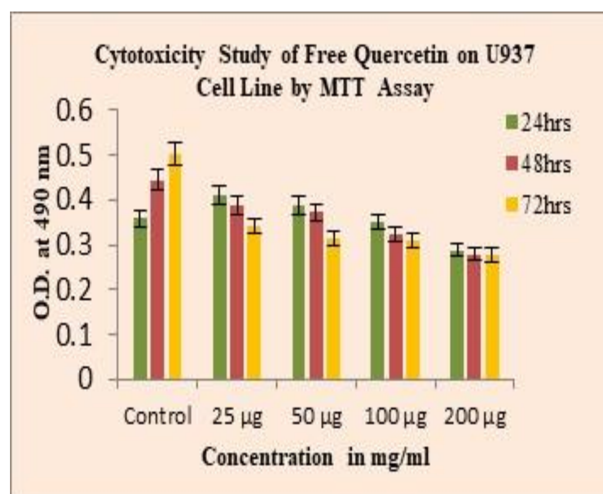
IC_{50} value is the concentration of a drug which inhibits 50% of the cancer cell growth. This value was determined for the drug QTF as well as the formulations QTLF1-QTLF8. The results for the same are represented in a tabular form as follows:

Table 5: IC_{50} Values of the Liposomal Formulations

Sl. No.	Formulation	24hrs IC_{50} value (µg/ml)	48hrs IC_{50} value (µg/ml)	72hrs IC_{50} value (µg/ml)
1	QTF	240	-	-
2	QTLF1	106.70	-	-
3	QTLF2	169.32	-	-
4	QTLF3	-	35.36	-
5	QTLF4	-	10.41	-
6	QTLF5	-	175.95	-
7	QTLF6	-	-	63.45
8	QTLF7	-	-	51.33
9	QTLF8	-	-	37.25

Cytotoxicity Study by MTT Assay

The raw drug (QTF) and the liposomal formulations (QTLF1-QTLF8) inhibited the growth and produced significant cytotoxicity of leukemic cell lines. Reduction in the mean OD of cells treated with increasing dose of drug was observed as compared to control for 24hrs, 48hrs and 72hrs respectively. The % inhibitions were also plotted against the increasing concentrations of the formulations. On determining the entrapment efficiency, QTLF1 showed the maximum percentage whereas QTLF4 showed the least value. The formulations (QTLF1-QTLF4) showed a satisfactory activity on U937 cell line after 24 hours which indicates proper effect after 48 or 72 hours of exposure. The liposomal formulations have shown a decrease in IC_{50} values as compared to the free drug in U937. The IC_{50} value for free Quercetin was 240µg/ml whereas the respective IC_{50} values of formulations QTLF1-QTLF8 were **106.81µg/ml, 169.32µg/ml** at 24 hours, **35.36µg/ml, 10.41µg/ml, 175.95µg/ml** at 48 hours, **63.45µg/ml, 51.33µg/ml and 37.25µg/ml** at 72 hours respectively. In U937 cells, formulations QTLF5-QTLF8 were found to show gradual release of Quercetin with negligible action after 24 hours but notable effect after 72 hours of treatment whereas QTLF1-QTLF4 showed cytotoxic action right from the very beginning. This proved that in QTLF5-QTLF8 the lipid matrix had kept Quercetin entrapped for a longer span of time. QTLF1 was found to have the best IC_{50} value among all the eight prepared formulations.



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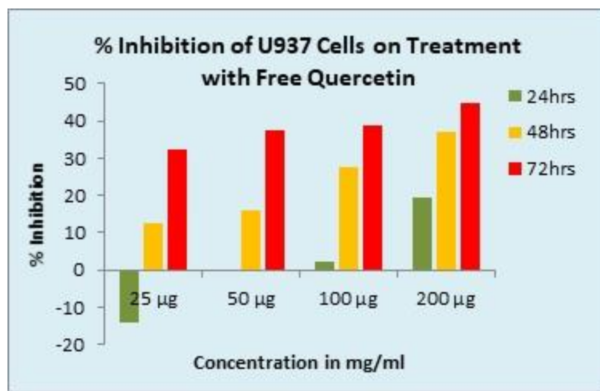
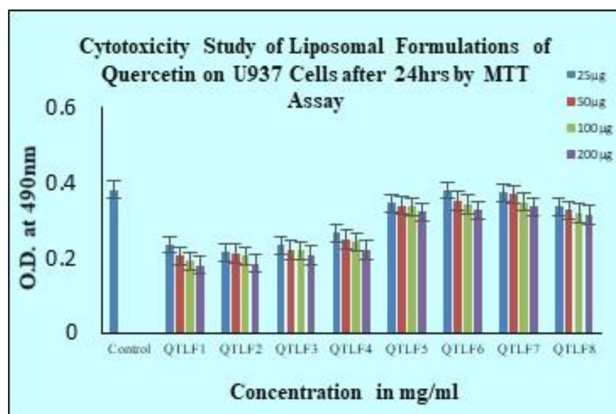
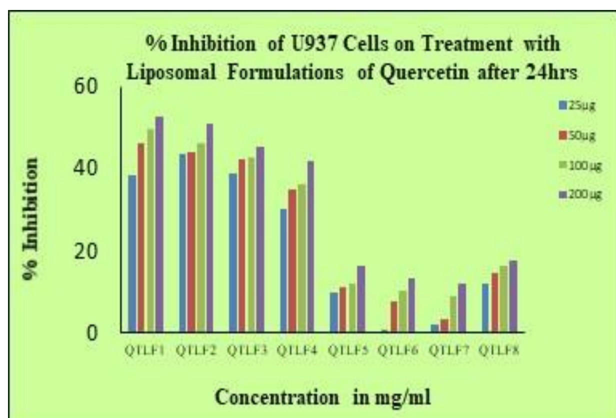
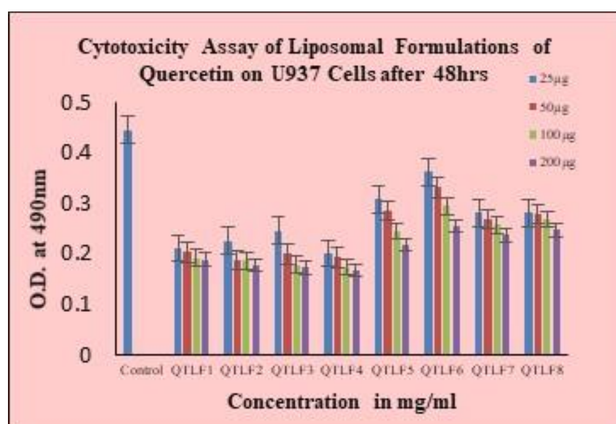
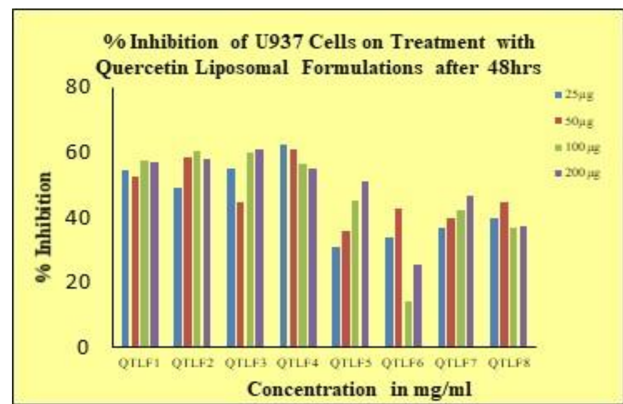


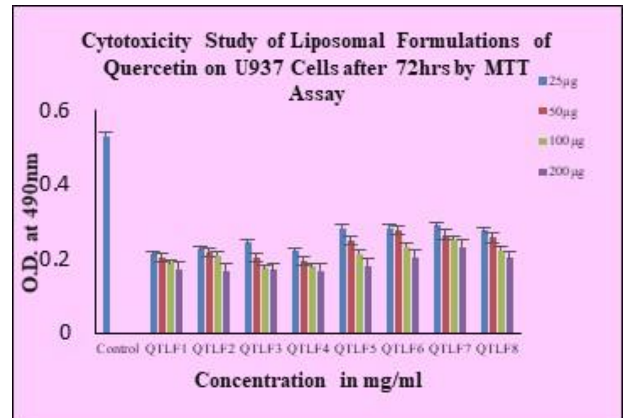
Figure 5: Both the histogram shows the effect of QTF on U937 cell line. Reduction in O.D. value and increase of % inhibition was observed in a time and concentration dependant manner. Data are mean ± S.E.M.











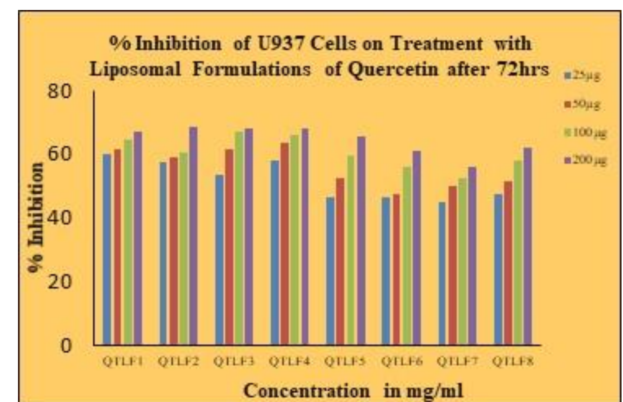


Figure 6: Histograms show the effects of QTLF1-QTLF8 on U937 by MTT Assay after 24, 48 and 72 hours respectively. Reduction in the O.D. values and increase in the % inhibition of cells is observed in a time and concentration dependant manner. Data are mean ± S.E.M. QTLF1-QTLF4 shows marked reduction in O.D. value and notable increase in the % inhibition at 24hrs whereas QTLF5-QTLF8 show delayed action on both O.D. values and % inhibition with maximum effect after 72 hours.

DISCUSSION

Quercetin manifests low solubility which posed a problem in its effectiveness after administration into the body in spite of having strong anti-cancer and antioxidant properties [4]. Liposomes are novel carriers which are known to increase bioavailability of hydrophilic as well as lipophilic components, thereby making Quercetin a proper candidate for incorporation into it [2]. Cancer being one of the biggest causes of mortality is a huge threat to mankind and as a result continuous efforts are being given on

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development of anti-cancer drugs and formulations. About 60% of drugs currently used for cancer treatment have been isolated from natural products, majorly from plant sources due to efficacy and reduced side effects [19]. Quercetin is a naturally occurring flavonoid which has been used in cancer prevention and to prevent the spread of various cancers, such as lung, prostate, liver, breast, colon, and cervical cancers [20]. This study thereby deals with the tackling of the solubility issues of Quercetin by incorporating it into liposomes and determination of its anti-cancer activities.

On determining the entrapment efficiency, QTLF1 showed the maximum percentage whereas QTLF4 showed the least value. The cytotoxic activity was observed in U937 cell line isolated from the histolytic lymphoma of a 37-year-old male patient. The liposomal formulations have shown a decrease in IC_{50} values as compared to the free drug in U937. The IC_{50} value for free Quercetin was 240µg/ml whereas the respective IC_{50} values of formulations QTLF1-QTLF8 were 106.81µg/ml, 169.32µg/ml at 24 hours, 35.36µg/ml, 10.41µg/ml, 175.95µg/ml at 48 hours, 63.45µg/ml, 51.33µg/ml and 37.25µg/ml at 72 hours respectively. In U937 cells, formulations QTLF5-QTLF8 were found to show gradual release of Quercetin with negligible action after 24 hours but notable effect after 72 hours of treatment whereas QTLF1-QTLF4 showed cytotoxic action right from the very beginning. This proved that in QTLF5-QTLF8 the lipid matrix had kept Quercetin entrapped for a longer span of time. QTLF1 was found to have the best IC_{50} value among all the eight prepared formulations.

SUMMARY & CONCLUSION

Natural products are of prime importance in the pharmaceutical industries in the current scenario where synthetic drugs have considerable amounts of adverse effects which affect mankind. A wide number of natural drug products are currently under study for their respective pharmacological uses. One such group of active natural products are the flavonoids. Flavonoids are poly-phenolic plant extracts present in the daily diet and contribute to the flavour and colour of fruits and vegetables. Quercetin is a flavonoid which bears high antioxidant and cytotoxic activity which can be of much use to fight the battle against the most feared disease of the current century, cancer. However, it belongs to BCS Class IV with solubility and permeability limitations which hamper the effectiveness of the drug. This establishes a need to modify the drug's solubility by using carriers.

Novel Drug Delivery is a unique approach for preparing drug formulations with better bioavailability and release at the preferred 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. There are quite a number of novel carriers which are in use for creating new formulations of drugs like liposomes, nanoparticles, phytosomes etc. Novel formulations of some drugs like doxorubicin, amphotericin B are already in market, showing good amount of patient compliance. Similarly, the drug Quercetin was 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 represent the biological membranes as far as the structure is considered, and as a result is highly compatible for administration into the body. It has another advantage of being able to incorporate both lipophilic and hydrophilic drugs into it, thereby becoming the carrier of choice for the study.

Cancer is a disease 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 two of the various types of cancer namely leukaemia and ovarian cancer. Leukaemia is a type of cancer characterized by increased number of white blood cells. Synthetic anti-cancer drugs have been proved to be effective for the treatment but alongside the drugs were found to show serious side effects. Hence, approaches were made 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 analysed. 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 were then used for *in-vitro* studies to check the cytotoxic activities on two types of cancer cells namely the monocytic myeloid leukaemia cell (U937).

Hence, it can be concluded that the liposomal preparations of Quercetin showed better cytotoxic activity on U937 cells as compared to free Quercetin showing lower IC_{50} values. It thereby possesses anti-leukemic activity. A further conclusion can be drawn that by 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.

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REFERENCES

1. Khan Mohd. Gayoor. , The novel drug delivery system. *World Journal of Pharmacy and Pharmaceutical Sciences*, 6(7), 2019, 477-487.
2. Kotturi Nagashree., Novel Drug Delivery System. *Journal of Pharmaceutics and Nanotechnology*, 3(2), 2015, 33-36.
3. Guo Yu, Shen Li-xian, Lu Yan-feng, Li Hai-yan, Min Ke, Li1Lan-fang, Yu Cui-yun, Zheng Xing., Preparation of Rutin-liposome Drug Delivery Systems and Evaluation on Their in vitro Antioxidant Activity; *Chinese Herbal Medicines*, 8(4), 2016, 371-375.
4. BaiJ.,Yanga E., Changa Pahn-Shick, Ryu Sangryeol., Preparation and characterization of endolysin-containing liposomes and evaluation of their antimicrobial activities against gram-negative bacteria. *Enzyme and Microbial Technology,* 128, 2019, 40–48.
5. Daraee Hadis,, Etemadi Ali, Kouhi Mohammad, Alimirzalu Samira & Akbarzadeh Abolfazl., Application of liposomes in medicine and drug delivery. *Artificial Cells, Nanomedicine, and Biotechnology*, 44, 2014, 381-391.
6. Toniazzo Taise, Peres Marina S., Ramos Ana Paula, PinhoSamantha C., Encapsulation of quercetin in liposomes by ethanol injection and physicochemical characterization of dispersions and lyophilized vesicles. *Food Bioscience*, 19, 2017, 17-25.
7. Caddeoa Carla, Gabrieleb Morena, Fernàndez-Busquetscd Xavier, Valentia Donatella, Faddaa Anna Maria, Puccib Laura, Manconia Maria., Antioxidant activity of quercetin in Eudragit-coated liposomes for intestinal delivery. *International Journal of Pharmaceutics*, 565, 2019, 64-69.
8. Khor Chia Miang, Ng Wai Kiong, ChanKok Ping, DongYuancai., Preparation and characterization of quercetin/dietary fiber nano formulations. *Elsevier*, 161, 2017, 109–117.
9. Dutta S., Mahalanobish S., Saha S, Ghosh S., Sil P.C. Natural Products: An upcoming approach to Cancer. *Food and Chemical Toxicology*, 128, 2019, 240-255.
10. Gao Peng, Seebacher Nicole A., Hornicek Francis, Guo Zheng, Duan Zhenfeng, Advances in Sarcoma Gene Mutations and Therapeutic Targets. *Cancer Treatment Reviews*, 2018,62: 98-109.
11. Kojo S.J. Elenitoba-Johnson, Megan S. Lim, New Insights into Lymphoma Pathogenesis.*Annual review of Pathology*: *Mechanism of Disease*, 9, 2017, 1-25.
12. Nelson Hamerschlak, Leukemia: genetics and prognostic factors. *Journal de Pediatria*, 84, 2008, 52-57.
13. Dey Biplab, Kumar Arun, A Review Article on Breast Cancer. *International Journal of Pharmacy and Pharmaceutical Research*, 11(2), 2018, 284-298.
14. Hwang Dongwon, Kim Minsun, Park Hyejin, Jeong Myung In, Jung Woojinand Kim Bonglee., Natural Products and Acute Myeloid Leukemia: A Review Highlighting Mechanisms of Action. *Nutrients*, 11, 2019, 1010.
15. N. Kendre Prakash, V. Pande Vishal and M. Chavan Kishori, Novel Formulation Strategy to Enhance Solubility of Quercetin. *Pharmacophore,* 5 (3), 2014, 358-370.
16. R. P. Rao Monica and S. Babrekar Laxmi, Liposomal Drug Delivery for Solubility and Bioavailability Enhancement of Efavirenz. *Indian J Pharm Sci*. 80(6), 2018, 1115-1124.
17. Sabeti Bahareh, Noordin Mohamed Ibrahim, Shaharuddin, Development and Characterization Sof Liposomal Doxorubicin Hydrochloride with Palm Oil. *Bio Med Research International,* 2, 2014, 765426.
18. Subhadeep Roy, Nilanjana Deb, Santanu Basu, Shila Elizabeth Besra, "Apoptotic activity of ethanolic extract of *Moringa oleifera* root bark on human myeloid leukemia cells via activation of caspase cascade" *World Journal of Pharmacy and Pharmaceutical Sciences*, 2014, 3 (10), 1138-1156.
19. Dave Shreya, Modi HA and Jain NK., Assessment of anticancer properties of few medicinal plants. *Journal of Pharmacognosy and Phytochemistry*, 2016, 5(4), 46-52.
20. Xu Dong, Hu Meng-Jiao, Wang Yan-Qiu and Cui Yuan-Lu., Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application. Molecules, 24, 2019, 1123.

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