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STUDY OF ANTIOXIDANT ACTIVITY OF MONOOLEIN BASED LIQUID CRYSTALLINE NANOPARTICLE OF QUERCETIN

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ABSTRACT

The objective of the study was to entrap Quercetin into the monoolein and to conduct antioxidant studies.Liquid-crystalline state is an intermediate between an ordered crystal and a disordered isotropic liquid. It has properties related to both liquids and crystals.Monoolein, is a polar unsaturated monoglyceride, they form thermodynamically stable self- assembled structures like liquid crystalline nanoparticles by ultrasonication in presence of aqeous phase. 2,2 Diphenyl-1-picryl hydrazyl (DPPH) and nitrogen oxide free radical scavenging activity was studied. IC_{50}value of nanoparticles was found to be 0.1188µg/ml and that of standard was 0.7001 µg/ml in DPPH assay. Inhibitory concentration 50 (IC_{50}) value of nanoparticle was found to be 7.86 µg/ml and that of standard was 13.68 µg/ml. Quercetin entrapped nanoparticle has a promising effect as an antioxidant.

Keywords: Quercetin, Monoolein, Liquid crystalline nanoparticles (LCN), Antioxidant.

INTRODUCTION

The liquid-crystalline state is an intermediate between an ordered crystal and a disordered isotropic liquid. It has properties related to both liquids and crystals. i.e. these molecules in a crystal are highly ordered, while molecules in a liquid are free to diffuse in a random way [1,2]. In general liquid crystals systems can be classified in two categories, i.e. thermotropic and lyotropic mesophases. Thermotropic liquid crystal phases are formed by a change of temperature, whereas lyotropic phasesare formed when it is mixed with a solvent. The lyotropic liquid crystalline nanoparticles are of two types, Hexosomes and Cubosomes. The Hexosomes and cubosomes are named after their highly ordered crystalline structure or shape. Quercetin, a topical antioxidant, is known to have the ability to defend ultraviolet radiation-mediated oxidant injury and cell death by scavenging oxygen radicals, by terminating the chain-radical reaction[3,4]. Monoolein, is a polar unsaturated monoglyceride. Monoolein is a nontoxic, biodegradable, and biocompatible material and is listed in the Food and Drug Administrationn (FDA)'s Inactive Ingredients Guide. When

exposed to aqueous environment,amphiphiliclipids spontaneously form thermodynamically stable self- assembled structures[5].

Liquid crystalline nanoparticles (LCNs) have attracted significant attention due to their potential improvements in physicochemical stability, improved skin retention and hydrophobic drug loading. Due to its improvement in the physicochemical stability drugs and plant extracts can be entrapped and may have a better action potential than its crude form. The aim of the study is to formulate liquid crystalline nanoparticles of Quercetin using ultrasonication method and to evaluate its antioxidant activity[6].

MATERIALS AND METHODS

Chemicals Required

Quercetin was purchased from otto chemie pvt ltd,Mumbai and Monoolein was procured as gift sample from Abitech chemical supplier,Mumbai. All other chemicals used were of analytical grade.

Instruments Used

Ultra bath sonicator 1.5L(H-Pci analyte), Vortex mixer REMI CM-101, UV-Visible spectrophotometer V-630 Jasco.

Formulation of liquid crystalline nanoparticle of Quercetin by ultrasonication method

The accurately weighed quantity of monoolein,propylene glycol, and oleic acid was taken and melted in a water bath at 60 °C followed by Quercetin addition with continuous stirring using the vortex mixer to complete dissolution followed by ultrasonication[7].

STUDY OF ANTIOXIDANT ACTIVITY OF LIQUID CRYSTALLINE DISPERSION

DPPH FREE RADICAL SCAVENGING ASSAY[8]

1 ml of different concentration of Quercetin was prepared in ethanol (10,20,30,40 µg/ml) were added to 1 ml of 0.1m Methanolic solution of DPPH. After 30 minutes incubation in darkness at room temperature,the absorbance was measured at 517nm. 1ml Ethanol and 1 ml of 0.1mM ethanolic solution of DPPH was kept as control. Ethanol served as blank. Similarly the formulation of LCN was also tested. Radical scavenging activity was expressed as percentage inhibition and was calculated using the following formula[8] :

Percentage of scavenging activity (%) = [$(A_{Control} - A_{Sample})/ A_{Control})$]* 100

Where $A_{Control}$ = Absorbance of control

A_{Sample} = Absorbance of sample (both test and standard)

All the tests were performed in triplicates and the results were reported as $IC_{50.}$

NITRIC OXIDE FREE RADICAL SCAVENGING ASSAY[9]

To 2 ml of sodium nitroprusside in phosphate buffer 0.5 ml of different concentration of Quercetin standard and LCN were added and incubated at 25^0c for 150 minutes. To this add 0.5ml of Griess reagent was added and absorbance was measured at 546 nm.Phosphate buffer pH 7.4 was served as blank.The experiment was performed in triplicate.The nitric oxide scavenging assay was also conducted for blank liquid crystalline nanoparticles.

Nitric oxide scavenging activity was expressed in %Percentage inhibition and was calculated using the following formula[10]

Percentageof scavenging activity % = [($A_{Control}$ - A_{Sample})/ $A_{Control}$)]* 100

Where $A_{Control}$ = Absorbance of control

A_{Sample} = Absorbance of sample (both test and standard)

All the tests were performed in triplicates and the results were reported as $IC_{50.}$

RESULTS

DPPH FREE RADICAL SCAVENGING ASSAY

Percentage DPPH scavenging activity of different concentrations of LCN) was determined. DPPH free radical scavenging activity was found to be concentration dependent.IC_{50} value of LCN was found to be 0.1188µg/ml and that of standard Quercetin was 0.7001 µg/ml.

NITRIC OXIDE FREE RADICAL SCAVENGING ASSAY

Percentage Nitric oxide scavenging activity of different concentrationsof LCN was determined. Nitric oxide scavenging activity was found to be concentration dependent.IC_{50} value of LCN was found to be 7.86 µg/ml and that of standard Quercetin was 13.68 µg/ml. The Quercetin has a predefined topical antioxidant potential which is limited due lack of solubility[11] ,which overcomes by entrapping it in liquid crystalline nanoparticle using monoolein.

Table 1: Formula of liquid crystalline nanoparticle

Monoolein (g)	Oleic acid (ml)	Propylene glycol (ml)	Water added (ml)
1.5	0.51	0.27	7.5

Table 2: Observations and calculations of DPPH free radical scavenging assay

Drug	Concentration in µg/ml	Absorbance Mean ± SD	Percentage scavenging activity % (Mean ± SD)
Control	-	1.0578±0.013	-
Standard	10	0.4612±0.002****	56.43±0.009
	20	0.4091±0.002****	61.32±0.008
	30	0.3259±0.023****	69.19 ±0.010
LCN	10	0.4569±0.0032****	56.80±0.006
	20	0.4014±0.0044****	62.09±0.009
	30	0.3198±0.0051****	69.76±0.007

Percentage scavenging activity expressedas Mean ± SD(n=3).**** P<0.0001,Absorbance of standard and liquid crystalline nanoparticle (CA) were compared with the control (One way ANOVA followed by Dunnet test)

Table 3: Observations and calculations of nitric oxide free radical scavenging assay

Drug	concentration in µg/ml	Absorbance (mean ± SD)	Percentage scavenging activity %(mean ± SD)
Control	-	0.6589±0.102	-
Standard	10	0.3454±0.002***	47.57±0.175
	20	0.3009±0.005****	54.33±0.020
	30	0.2798±0.09***	57.53 ±0.203
LCN	10	0.3239±0.004***	50.84±0.015
	20	0.2998±0.004****	54.49±0.041
	30	0.2745±0.003***	58.33±0.122

Percentage scavenging activity expressed as Mean ± SD(n=3). **** P<0.0001, *** P<0.001 Absorbance of standard and liquid crystalline nanoparticle (CA) were compared with the control (One way ANOVA followed by Dunnet test).

Fig.1: DPPH scavenging activity of LCN

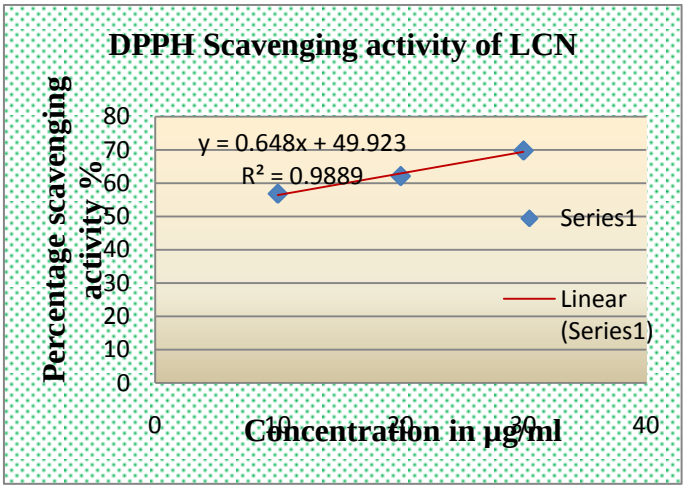


Fig.2: DPPH scavenging activity of standard

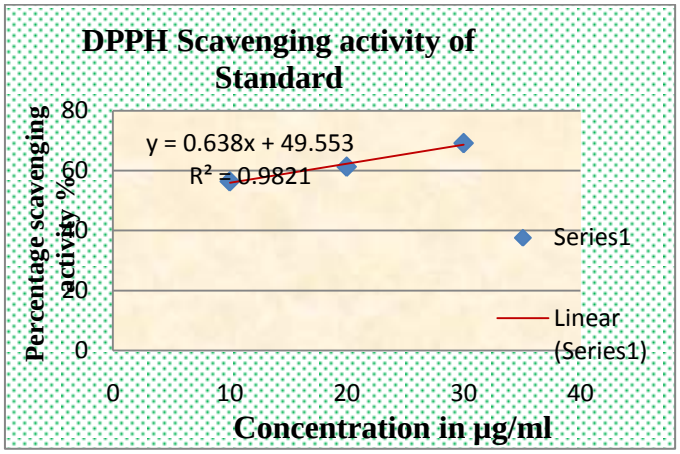


Fig.3: Bar graph representing comparison of DPPH free radical scavenging activity of Standard Vs LCN

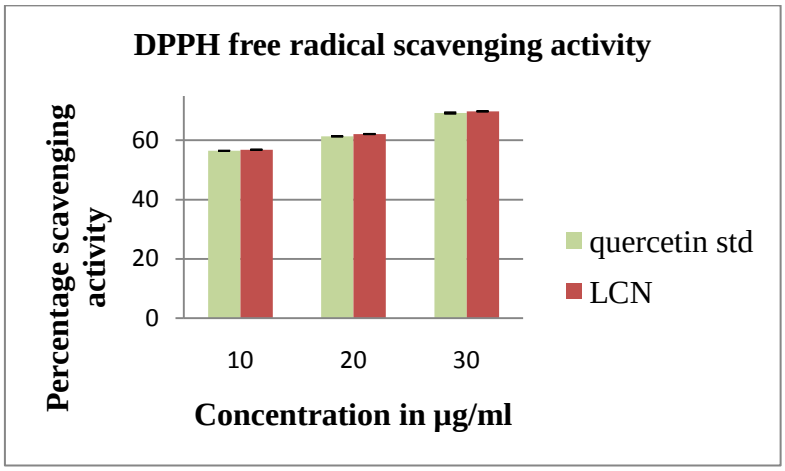


Fig.4: Nitric oxide free radical scavenging activity of LCN

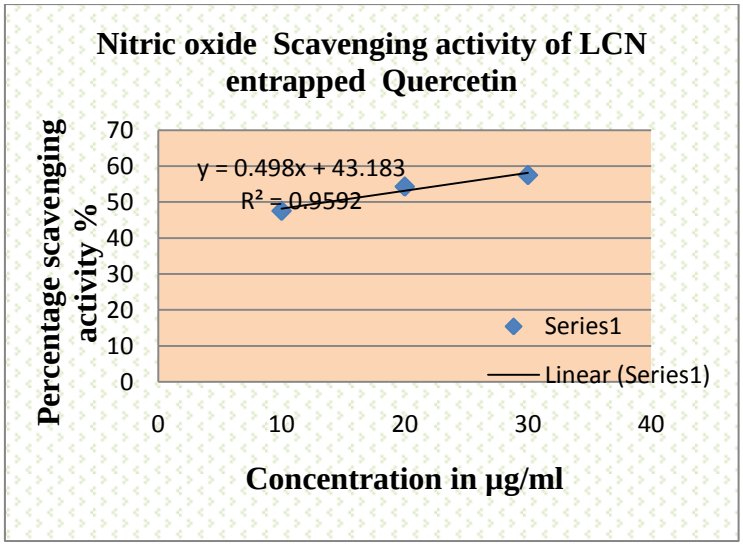


Fig.5: Nitric oxide free radical scavenging activity of standard

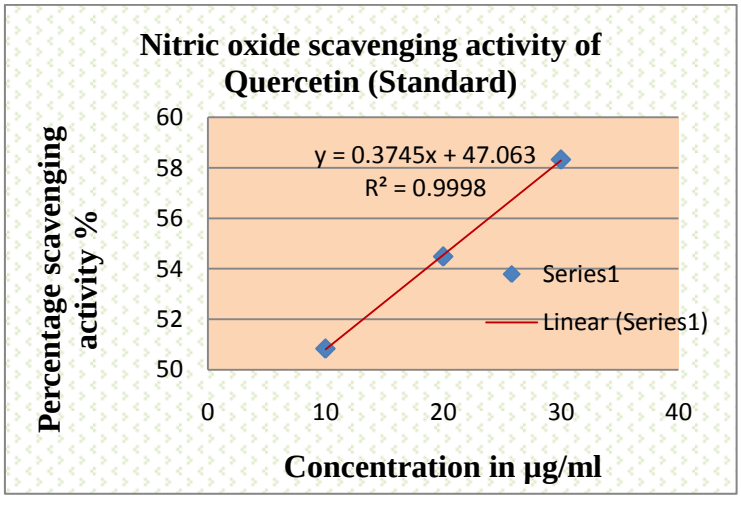
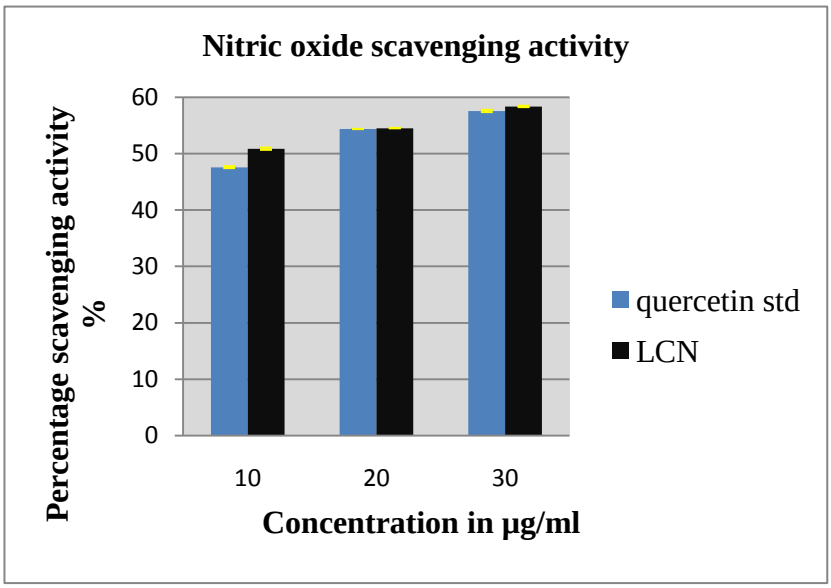


Fig.6: Bar graph representing Comparisonof Nitric oxide free radical scavenging assay of Standard Vs LCN



DISCUSSION

Quercetin is natural drug having tremendous potential as a topical antioxidant activity, because of its hydrophobic nature topical deliveryand availability through the skin is a troublesome process.Liquid crystalline nanoparticle possess excellent hydrophobic drug loading capacity[12] in which the quercetin can be loaded. Monoolein is a monoglyceride which can self-assemble into nanoparticles, apart from that its having a good skin retention property[13,which acts as a sunscreen and makes the availability of quercetin for its antioxidant action. The antioxidant activity of quercetin was found to be improved Nanoparticles of quercetin formulated using monoolein showed improved antioxidant action,when compared with the crude drug, due to the availability of drug at site of action. The better functional ability of Quercetin is due its entrapment into monoolein due its increased skin retention property and stability[14].

CONCLUSION

The Quercetin entrapped nanoparticle has a promising effect as topical antioxidant. The encouraging results obtained from this study states that liquid crystalline system could be proposed as a UVprotectant or may be incorporated in to gel or cream base[15]. Incorporation of such nanoparticle may offer greater stability. Increased antioxidant activity was observed for the nanoparticles when compared to the standard.Further thorough study is required for comparing,the Quercetin liquid crystalline nanoparticles with available topical formulations.

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