

Original Research Article

Formulation and evaluation of Microemulsion of Curcumin in Thymol-Menthol carrier system.

ABSTRACT

Aim: To formulate and evaluate the Micro emulsion of curcumin in thymol-menthol carrier system.

Study design: Curcumin dissolves in mixture of thymol & menthol to reasonable extent. 100 mg of curcumin in 1 g of thymol and 0.6 g of menthol mixture was finalized as product mixture. The solution of curcumin in eutectic mixture being oily liquid, it gave the idea for development for the micro emulsion

Place and duration of study: Department of Pharmaceutics and Department of Pharmacology, STES's Smt. Kashibai Navale College of Pharmacy, Kondhwa (BK), National Chemical Laboratory, Pune between June 2016 and May 2017

Methodology: Micro emulsion system with eutectic mixture of thymol and menthol was chosen as oil phase and carrier for curcumin, tween 80 as surfactant and ethanol as co-surfactant. Ternary phase diagrams were constructed to obtain the optimum concentration range of oil phase, surfactant and co-surfactant. The micro emulsion of 100 mg curcumin containing 4.95 % oil phase, 33.39 % surfactant, 11.13% co-surfactant, 50:50% of water was optimum. Micro emulsion of curcumin was prepared by water titration method and evaluated for globule size, drug content, pH, viscosity, conductivity and *In vitro* drug release study. The *ex-vivo* permeation study for micro emulsion of curcumin was carried out on excised mice skin using Franz Diffusion apparatus. The cytotoxicity study for thymol, menthol was performed on healthy L929 murine fibroblast cell line. Anticancer activity of thymol-menthol eutectic mixture alone and with curcumin was performed on HeLa cell lines.

Result: The preformulation studies did clearly indicate good compatibility of drug with thymol menthol carrier system. The cytotoxicity study was done on thymol – menthol(TM) as a preformulation aspect to ascertain the toxicity of these two excipients to be tried. It was found that TM was not cytotoxic in 120ug/mL concentration and thus was safe to be used in concentration below 120ug/mL. Batch B1 was optimised. The average globule size of micro emulsion was found to be 131.54 nm, zeta potential was found to be -0.57 mV. The drug content in micro emulsion batch B1 was determined and found to be 74.6%. Permeability study across mice skin *ex-vivo* model showed 52.38% permeability compared with curcumin alone which was 22.41%. So permeability of curcumin is enhanced substantially with micro emulsion made in thymol and menthol, owing to fact that thymol and menthol act as good permeation enhancers. The *In vitro* drug release study of micro emulsion was compared with curcumin alone. About 48.75% of curcumin was released from micro emulsion showed increase in release rate.

Conclusion: Eutectic mixture of thymol and menthol showed promising results as penetration and solubility enhancer for curcumin. Solubility of curcumin was improved so as its rate of permeation which was used to develop formulation of micro emulsion with proven anticancer activity. The solubility of curcumin formulated with combination of eutectic mixture was increased up to 50 %.

Keywords: Curcumin, thymol, menthol eutectic mixture, ternary phase system, micro emulsion of curcumin, cytotoxicity, anticancer cell line study.

1. INTRODUCTION

The micro emulsion term is applied to a system prepared by emulsifying oil in an aqueous surfactant and then adding a fourth component known as co-surfactant which is generally intermediate chain length alcohol like butanol, isopropyl alcohol, pentanol. [1] Curcumin is BCS class IV drug with severe limitations of solubility in water and permeability across biological membranes. This restricts the use of curcumin as a drug in clinical practice. Curcumin is reported to have around 10-15 pharmacological activities but clinically this becomes a challenge to make curcumin bioavailable. Currently only oral dosage forms of curcumin are available with very high dose (4-5gm in a day). This shows that there is wide scope to develop effective formulation of curcumin with increased bioavailability and decreased dose. Literature survey done under this study shows there is lots of research done and is going on in the area of development of successful drug delivery system for curcumin. The concept of hydrotropy was one of the not much tried efforts with curcumin. The concept of eutectic mixture of thymol and menthol as excipients seemed untouched area yet. There had been various reports that various phenolic compounds can act as good hydrotropic agents and also may be beneficial as permeation enhancers. [2] 1gm thymol formed eutectic mixture with 0.6 gm of menthol and resulted into clear oily liquid at room temperature. The increasing amount of curcumin was dissolved into this eutectic mixture to get maximum of 100 mg of curcumin in 1.6 gm of eutectic mixture. This was basic clear liquid mixture further used in design and development of micro emulsions

2. MATERIALS AND METHODS

2.1 Materials:

Curcumin (purity 90%) was purchased from Amsar Goa Pvt.Ltd.India. Thymol crystals (AR) and Menthol crystals (AR) was purchased from Research Lab Fine Chem.India. Tween 80 (AR) was obtained from Research Lab Fine Chem.India, Ethanol (AR) was obtained from Research Lab Fine Chem.India, Citric acid (AR) was obtained from Research Lab Fine, India, Sunflower oil and Linseed oil- from local vendors of edible oils Pune, Maharashtra, India; Propylene glycol from Research Lab Fine Chem, India.

2.2 Methods:

2.2.1 Drug excipient compatibility studies:

The drug excipient compatibility was done by studying the FTIR (IR Affinity -1 SHIMADZU)[2,20], DSC(Perkin Elmer DSC 40000), NMR(Bruker) and TLC[3](Chloroform: Ethanol: Glacial acetic acid=95:5:1) of the curcumin, thymol-menthol eutectic mixture and mixture of thymol-menthol and curcumin to determine the compatibility and interaction with each other.

2.2.2 Anticancer activity of thymol-menthol eutectic mixture:

Thymol: Menthol: Curcumin (TMC) and Thymol: Menthol (TM) were sterilized by 30 minutes UV exposure inside bio-safety cabinet. A stock solution was made in DMSO, later it was diluted using complete DMEM media. The concentrations were 100, 120, 140, 160, 180, 200 µg/mL. Healthy L929 murine fibroblast cell lines (passage number 61/62) were maintained using complete DMEM. 10,000 cells were plated in each well of a 96 well plate. It was incubated in 5% CO₂ at 37°C for 1 day. At 1d samples of above concentrations were added in triplicates. After 1d incubation in 5% CO₂ at 37°C, 100 µl of 200 µM resazurin solution (in complete DMEM media) was added. It was incubated for 6 hours in 5% CO₂ at 37°C. From each well 100 µl media was taken out and read in a plate reader (excitation 530 to 560 nm and emission at 590 nm). The standard MTT assay was modified only for sample preparation by exposing to UV light. [3]

2.2.3 Cyto-toxicity study of thymol-menthol:

TMC and TM were sterilized by 30 minutes UV exposure inside biosafety cabinet. A stock solution was made in DMSO, later it was diluted using complete DMEM media. The concentrations were 100, 120, 140, 160, 180, 200 µg/mL. Healthy L929 murine fibroblast cell lines (passage number 61/62) were maintained using complete DMEM. 10,000 cells were plated in each well of a 96 well plate. It was incubated in 5% CO₂ at 37°C for 1 day. At 1d samples of above concentrations were added in triplicates. After 1d incubation in 5% CO₂ at 37°C, 100 µl of 200 µM resazurin solution (in complete DMEM media) was added. It was incubated for 6 hours in 5% CO₂ at 37°C. From each well 100 µl media was taken out and read in a plate reader (excitation 530 to 560 nm and emission at 590 nm). The standard MTS cell viability assay was modified only for sample preparation by exposing to UV light. 9% [4]

2.2.4 Solubility of curcumin in various oils:

2 ml of sunflower oil was taken in test tube (25°C) to which curcumin was added and sonicated until no more curcumin is dissolved. Furthermore quantity of curcumin was added to make it 100 mg mixture was sonicated to 15 min covered with aluminium foil and kept overnight. Later this mixture was centrifuge to get clear supernatant liquid which was removed without disturbing settled curcumin at bottom. The settled curcumin was filtered and washed with ample of water and dried, the weight of dry curcumin obtained was subtracted from 100mg that determined solubility of curcumin in Sunflower oil, similar experiment was carried out using linseed oil and Thymol- menthol eutectic mixture [5].

2.2.5 Screening of surfactants:

The method adopted by Adnan Azeem, Mohammad Rizwan, Farhan J. Ahmad, Zeenat Iqbal et al was used for screening of surfactants. Two types of surfactants were screened for micro emulsion formulation which included Tween 60 and Tween 80. In water, 2.5 ml of 15% v/v surfactant solution was prepared in water, and 4µL of oil was added with micropipette with vigorous vortexing. If one-

phase clear solution was obtained, the addition of the oil was repeated until the solution became cloudy [5, 6].

2.2.6 Screening of co-surfactants:

The method adopted by Romica Cretu, Cristian Dima, Gabriela Bahrim was used for screening of co-surfactants. Few modifications were done for screening of co-surfactants. The pseudoternary diagrams were constructed to find the mixing ratio of the corresponding micro emulsion state components. Tween 80 was combined with two solubilizers as co-surfactants namely, ethanol, and propylene glycol. At a fixed surfactant mixture (S mix) ratio of 1:1, the pseudo ternary phase diagrams were constructed. Nine different combinations(B1 to B9) in different weight ratios of oil an S mix, 1:9, 2:8, 3:7, 4:6, 5:5,6:4, 7:3, 8:2, 9:1 were utilized so that maximum ratios were covered to delineate the boundaries of phases precisely formed in the phase diagrams[7].

2.2.7 Construction of pseudo-ternary phase Diagram:

From solubility studies of curcumin in various oils and thymol-menthol eutectic mixture, tween 80 and ethanol were selected as oil, surfactant and co-surfactant respectively for preparation of micro emulsion. Micro emulsion region was identified by constructing pseudo ternary phase diagram containing different proportion of surfactant: co-surfactant (S/CoS) (1:1, 2:1 and 3:1), and oil, water [7]. S/CoS mix and oil were mixed in ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 [7]. To the resultant mixtures, water was added drop wise till the first sign of turbidity in order to identify the end point and after equilibrium; if the system became clear then the water addition was continued.

2.2.8 Physicochemical characterization of curcumin loaded micro emulsion:

2.2.8.1 Optical transparency:

Optical transparency of formulation was determined by inspecting the sample in clear and transparent container under the presence of good light against reflection into the eyes, and viewed against black and white illuminated background. [8]

2.2.8.2 Phase separation:

Micro emulsion system were subjected to centrifugation at 15000 rpm for period of 15 min. and examined for any change in phase separation. [9, 10]

2.2.8.3 Drug Content:

1 ml of micro emulsion was dissolved in 10ml of methanol. Solution was sonicated for 10-15 min and estimated by UV at 420nm. [9, 10]

2.2.8.4 Measurement of globule size:

The average globule size was measured using NANOPHOX (NX008), cross correlation. The measurement was performed at 25°C. [11, 12]

2.2.8.5 Polydispersity index:

Polydispersibility which determines size range of particles in the system, it is expressed in term of Polydispersibility index (PDI). An ideal micro emulsion should be widely distributed with particles less than 100 nm and so PDI should be less than 0.3 or in other words particles having size more than 100 nm should be maximum up to 23%. [11, 13]

$(D_{0.9}-D_{0.1})/D_{0.5}$

i.e. $(X_{90} - X_{10})/X_{50}$

2.2.8.6 Viscosity measurement:

The viscosity of micro emulsion was measured using a Brookfield viscometer equipped with the spindle no.63 at speed of 5 rpm. The measurement was performed at ambient temperature for all formulation batches. [14, 15]

2.2.8.7 pH measurement:

The pH value of micro emulsion was determined in triplicate using digital pH meter. [9, 11, 16]

2.2.8.8 Conductivity measurement:

The electric Conductivity of micro emulsion was measured with a conductivity meter 306. This was done by using conductivity cell consisting of two platinum plates separated by desired distance and having liquid between the platinum plates acting as a conductor. Conductivity determinations are carried out in triplicates. [9, 17]

2.2.8.9 Scanning electron microscopy:

Scanning electron microscopy (SEM) was used to characterize microstructure of emulsions. SEM of samples was measured using (Jeol JSM-6510, USA) [18].

2.2.8.10 In-vitro drug release study:

The release of curcumin from curcumin +water and from micro emulsion formulation was compared. Release of drug from micro emulsion employed a dialysis bag to study drug release..It was first activated using release medium. Phosphate buffer of pH 7.4 was used as release medium. The dialysis bag was suspended in a beaker containing 200 ml of phosphate buffer solution which was kept on magnetic stirrer. For overall experiment temperature of 37°C was maintained, 5 ml formulation (ME)and curcumin +water mixture containing same quantity of curcumin was transferred to dialysis bag.1ml sample was removed from the beaker containing phosphate buffer at time interval of 1 hr,2

hr ,3 hr ,4hr ,5hr ,6hr ,7hr ,8hr,22 hr,23 hr,24hr and was diluted to 10ml with methanol and absorbance was noted at 420nm[19].

2.2.8.11 Ex-Vivo Study:

2.2.11.1 Preparation of skin for Ex-vivo permeation study:

Mice Skin: Swiss albino mice weighing 80-100 gm were selected for preliminary permeation study and the study was conducted with the approval of institutional animal ethical committee. The mice were sacrificed using anaesthetic ether. Then the hair from their abdominal region was removed using animal hair clipper, and, subsequently, full thickness of skin was harvested. The fatty layer, adhering to the dermis side, was removed by surgical scalpel. [20]

Procedure for permeation study: *Ex vivo* skin permeation studies were carried out using Franz diffusion cell. The cell consists of two chambers, the donor and the receptor compartment with a diffusion area of 1.43 cm². The donor compartment was open at the top and was exposed to atmosphere. The excised mice skin was mounted between the compartments of the diffusion cells with stratum corneum facing the donor compartment and clamped into position. Magnetic stirrer bars were added to the receptor chambers and filled with the receptor phase. Phosphate buffer saline, pH 7.4, was used as receptor medium. The entire setup was placed over magnetic stirrer, and the temperature was maintained at 37± 0.5°C. The skin sections were initially left in the Franz diffusion cells for 2 hours in order to facilitate hydration of the excised skin. After this period, 1 ml of ME(B1) formulation was applied onto the surface of the skin. 1 ml of medium was collected from receptor compartment at 1 hr, 2 hr, 3 hr, 4hr, 5hr, 6hr, 7hr, 8hr, 22 hr, 23 hr, 24 hr intervals. In 24 hrs study period and replaced with the same amount of fresh buffer. The amount of permeated drug was estimated using UV spectrophotometer by measuring absorbance at 420 nm [20].

2.2.8.12 Anti-cancer activity of micro emulsion:

Samples (ME and Curcumin) were sterilized by 30 minutes UV exposure inside the bio-safety cabinet. Samples were initially dissolved in sterile DMSO (Dimethylsulfoxide) and further dilutions (5, 10, 15, 20 µg/100 mL) were carried out using complete DMEM (Dulbecco's Modified Eagle Medium). HeLa cervical cancer cell lines (passage number 89/90) were maintained using complete DMEM. 10,000 cells were plated in each well of a 96 well plate. It was incubated in 5% CO₂ at 37°C for 1 day. At 1d, samples of above concentrations were added in triplicates. After 1d incubation in 5% CO₂ at 37°C, 100 µl of 200 µM resazurin solution (in complete DMEM media) was added. It was incubated for 6 hours in 5% CO₂ at 37°C. Entire plate was read in a plate reader (excitation 530 to 560 nm and emission at 590 nm). [3]

3. Result and discussion

3.1 FTIR:

In fig.1 and 2 all reported frequencies are found identical in TM sample and TMC sample. The thymol+ menthol forms eutectic mixture that showed decrease in intensity of peak but not vanished, that showed physical interaction occur between thymol and menthol, But in TMC there was no chemical interaction was found between TM eutectic mixture and curcumin.

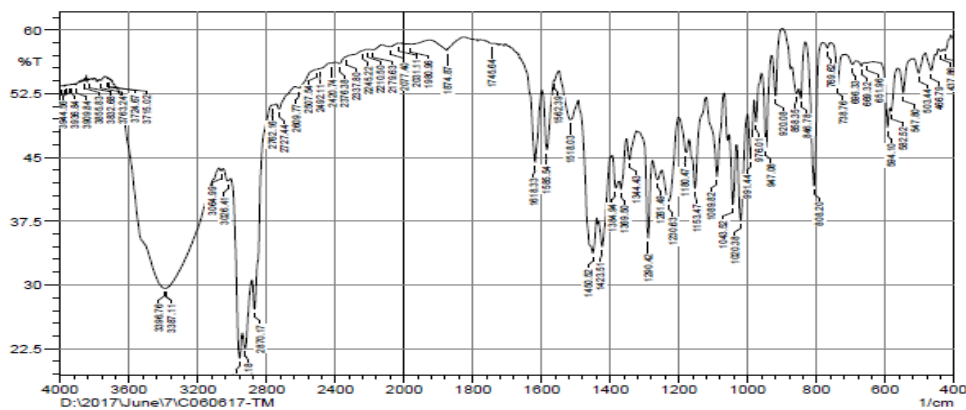


Fig.1- IR spectra of Thymol+ Menthol

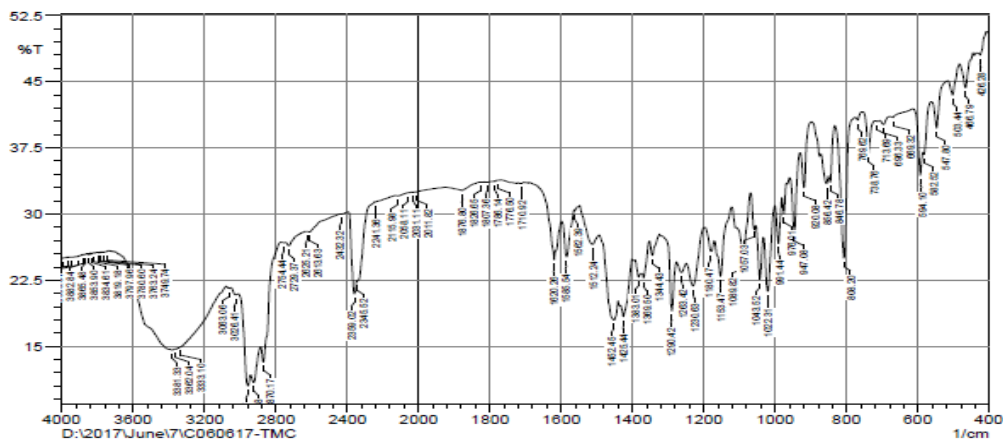


Fig.2- IR spectra of Thymol+ Menthol+ Curcumin

3.2 DSC Study:

All melting points of Thymol, menthol and curcumin vanished in eutectic mixture because Thymol + menthol melt at room temperature when mixed being eutectic and curcumin is dissolved in it. In the Eutectic mixture+ curcumin, the endotherm of melting points of Thymol, menthol and curcumin are not seen, because thymol and menthol melted into which curcumin is dissolved [Fig.3].

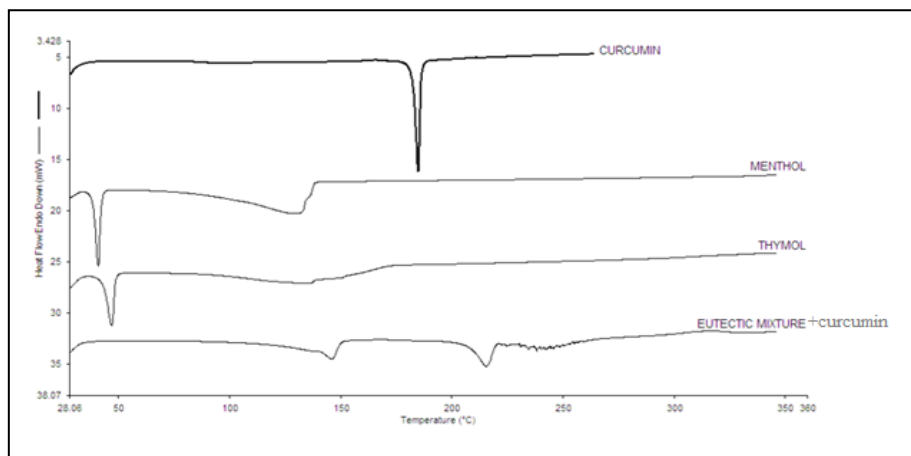


Fig.3-DSC Overlay

3.3 Thin layer Chromatography:

As shown in fig.5 the R_f value of TM was found to be 0.8 which is different from R_f values of thymol and menthol that clearly showed that the thymol and menthol forms the physical mixture (eutectic mixture) at the ratio of 1:0.6. In the TLC of TMC the spot of TM and curcumin was observed at different R_f value showed that there was no chemical interaction found between curcumin and eutectic mixture TM.

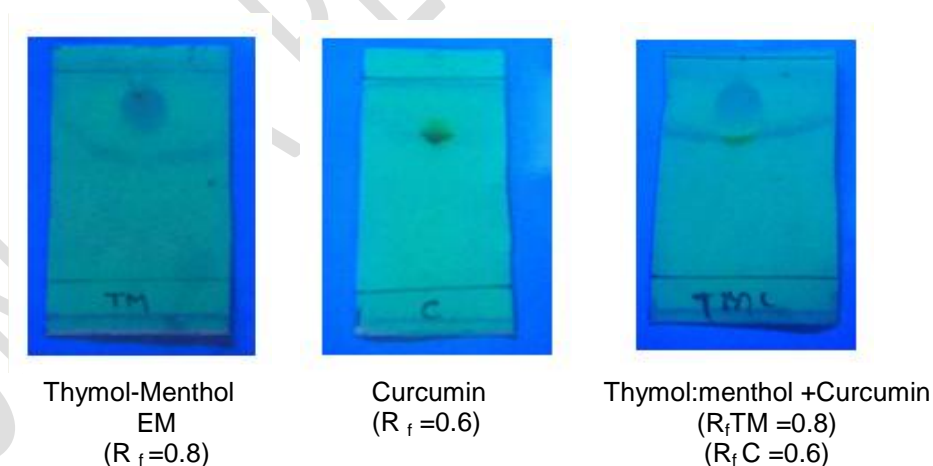


Fig.4- TLC plate Images of different component

3.4 NMR Study:

As seen from fig 5, 6, 7 NMR interpretation there was no chemical interaction seen between thymol, menthol and curcumin. Individual functional peaks were observed in the mixture too. This indicates

The anti cancer activity of Thymol : Menthol eutectic mixture can be clearly seen which does not increase beyond 120µg/ml concentration and anti cancer activity of curcumin along with thymol and menthol increases from 100µg/ml to 120µg/ml in which concentration of curcumin is 5.8µg/ml to 11.8µg/ml. The scope of using thymol and menthol as an excipient has its threshold limit to 120µg/ml in development of **micro emulsion** this was taken into consideration.

It implies that curcumin is potential anti- cancer agent and its response is quantitative as anti-cancer agent. Whereas thymol and menthol can be used in maximum 120 µg/ml concentration and thus it can potentiate the anti- cancer response of curcumin. It was also indicated in the cyto-toxicity pre-formulation study that thymol and menthol can be used till 120 µg/ml concentration and beyond this it proves cyto-toxic.

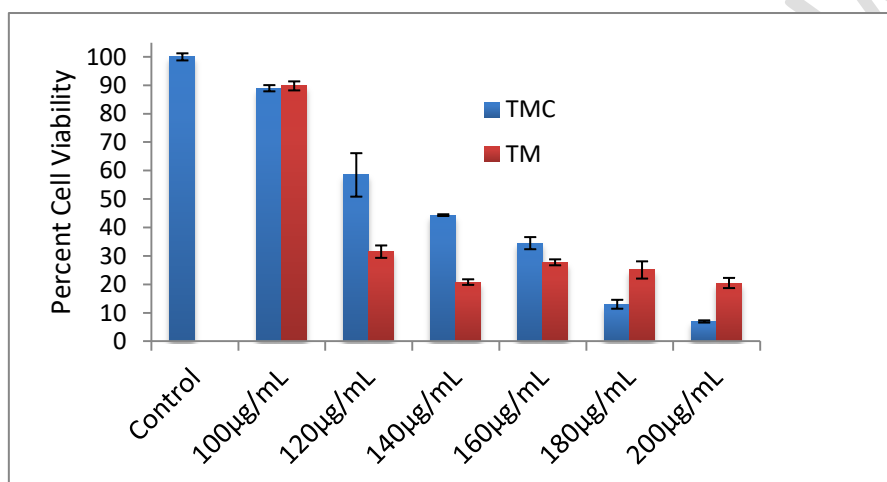


Fig.8-Anticancer activity of TM and TMC

3.6 Cyto-toxicity study of Thymol: Menthol (TM) eutectic mixture and Thymol: Menthol: Curcumin (TMC):

It was necessary to carry out cyto-toxicity study especially for thymol-menthol eutectic mixture being phenolic compounds. The maximum effective concentration of thymol and menthol in anticancer study is 120 µg/ml, so cyto-toxicity study was carried out for this concentration. In this study it has been seen that thymol and menthol in concentration 120 µg/ml is safe to be used but proves cyto-toxic in concentration 140 µg/ml [Fig.9].

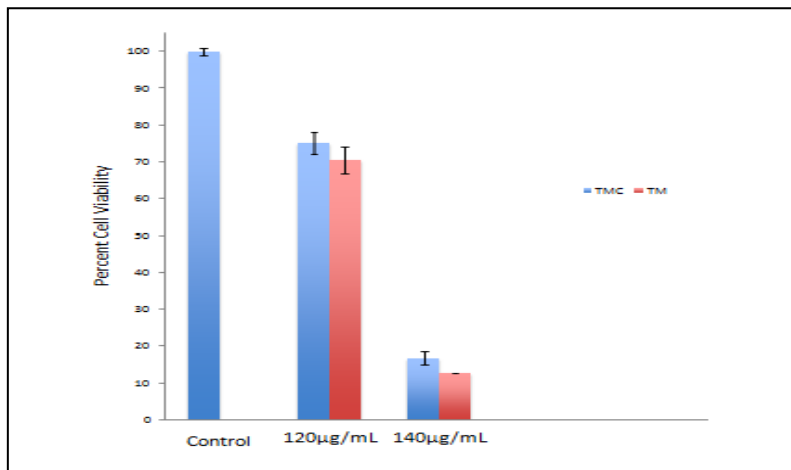


Fig.9-Cyto-toxicity study of TM and TMC

3.7 Screening of oils:

Table 1 - Solubility of Curcumin in Different oils

Sr. No.	Oils	Saturation Solubility of curcumin(mg/ml)
1	Sunflower Oil	32.5
2	Linseed Oil	26
3	Thymol-Menthol Eutectic Mixture(1:0.6)	62.5

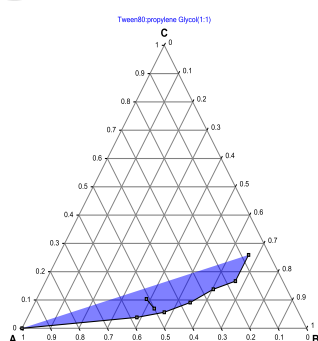
3.8 Screening of Surfactant:

Table 2 –Miscibility of oily mixture of thymol and menthol with Tween 80 and Tween 60

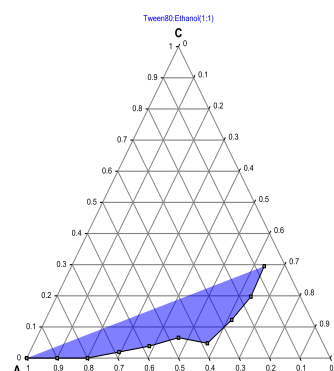
Sr. No	Surfactant	Oil (T:M)%
1	Tween 80	31.25
2	Tween 60	18.75

3.9 Screening of co-Surfactant:

Figure 10 presents the pseudo-ternary Phase diagrams constructed for thymol-menthol eutectic mixture (oil phase), water, tween 80 (surfactant), and different co-surfactant at a fixed ratio of S:CoS 1:1. based on area of micro emulsion formation in ternary phase diagram, ethanol was selected and used for further studies as co-surfactant.



a)Phase diagram of Tween 80: Propylene Glycol



b)Phase diagram of tween 80: Ethanol

Fig.10-Pseudo-ternary phase diagram of micro emulsion composed of T:M mix , tween 80 , water and co-surfactant a)propylene glycol ; b) Ethanol

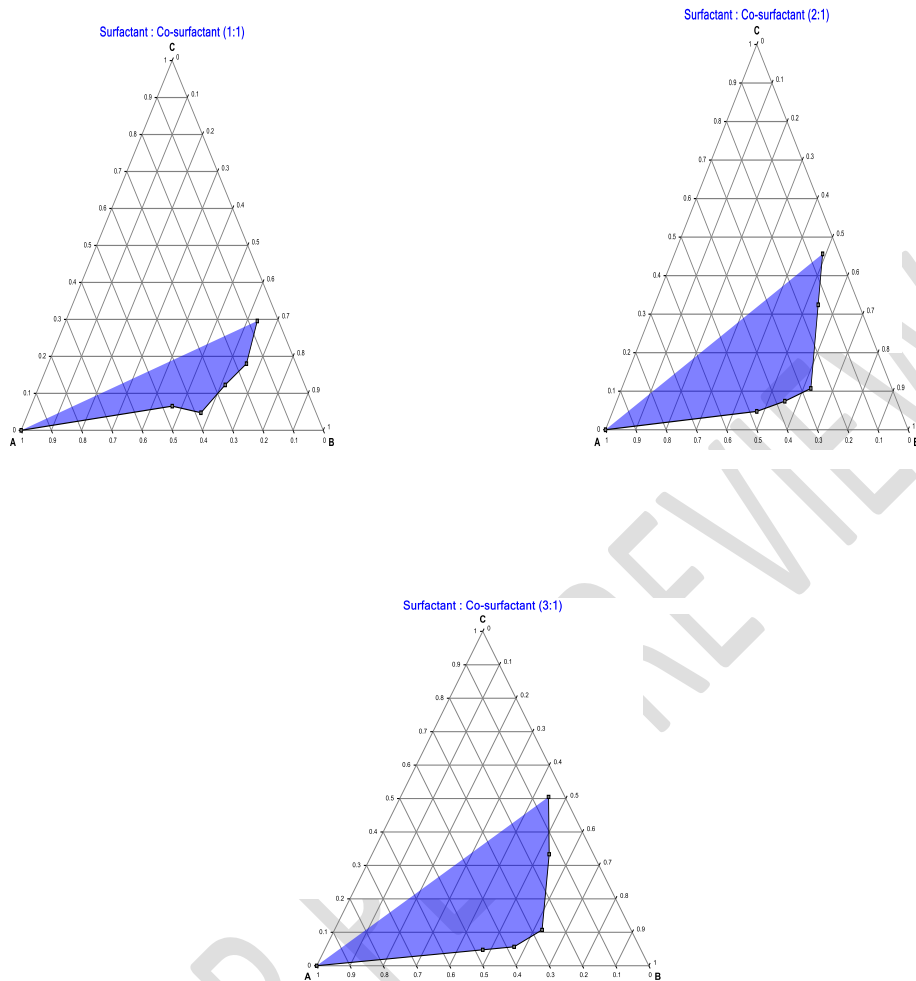


Fig.11- Pseudo ternary Phase diagram using T:M eutectic mixture as oil, Tween 80 as surfactant, Ethanol as co-surfactant and water (Tween80:Ethanol=1:1,2:1,3:1)

Table 3 -Formulation design of micro emulsion containing curcumin with 3:1 ratio(S: Co-S mix)

Batch code	Curcumin (mg)	(Oil phase)T:M Eutectic mix (1:0.6) %	Tween 80 %	Ethanol %	Water %
B1	100	4.95	33.39	11.13	50.50
B2	100	13.5	40.14	13.36	33
B3	100	27	46.89	15.63	10.3
B4	100	38	42	14	6
B5	100	47	35.25	11.75	6
B6	100	57	28.5	9.5	5
B7	100	68	21.75	7.25	3

B8	100	78.4	14.7	4.9	2
B9	100	90	7.5	2.5	0

3.10 Physicochemical parameters of ME:

Batch B1 was found to be optimised batch because it consumes more amount of water and more percentage of drug content as compared it with rest 8 batches. So, batch B1 used for further study.

Table 4 –Composition of optimised micro emulsion B1

Component	B1
Curcumin(mg)	100
TM eutectic mixture%	4.95
Tween 80%	33.39
Ethanol%	11.13
Water%	50.50

*TM=Thymol-menthol eutectic mixture

Table 5 –Drug Content

Batch code	Drug content in %(n=3, ±S.D)
B1	74.6±0.0060
B2	41.84±0.0095
B3	30.75±0.0084
B4	21.70±0.0118
B5	19.59±0.0108
B6	9.94±0.0077
B7	12.17±0.0084
B8	9.34±0.0051
B9	6.19±0.008

The micro emulsion should have good physical stability which was examined by phase separation/flocculation and optical transparency. This can be achieved when zeta potential values are negative. The pH, viscosity, conductivity, globule size and zeta potential of prepared formulations are shown in table 6. Result of globule size indicated that smallest globule size with the PDI 0.2802, which is close to zero indicating that the ME(B1) had uniform globule size. The pH of ME is within the normal range of 6-6.8. The conductivity of the results confirmed the formation of solution type ME with water in continuous phase. Viscosity of ME was found to be 76.66cps. Zeta potential was negative which indicated stability of formulation as there was less chances of globules aggregation. After

centrifugation cycle it was found that micro emulsion of T:M:C was stable and no separation was seen which indicate centrifugation stability. The ME remained clear and transparent even after a month of storage. The *In-vitro* drug release and *Ex-vivo* permeation study was performed with optimised micro emulsion which showed promising results.

Table 6 –Physicochemical parameters ME (B1)

Parameter	B1
% Drug content	74.6±0.0060
pH	6.8±0.0816
Globule size(nm)	131.54
Conductivity(ms/cm)	8.7±0.25
Viscosity	76.66±3.39
Zeta potential(mV)	-0.57
Optical transparency	Transparent
Phase separation	No Phase separation

3.10.1 Globule size measurement:

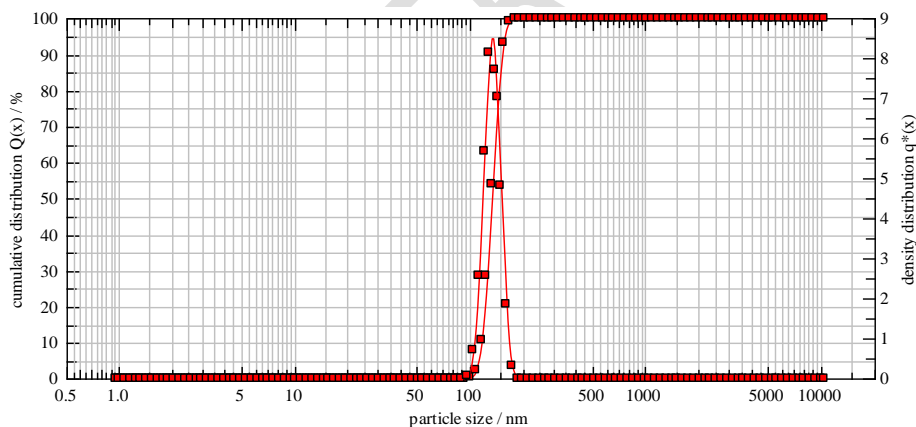


Fig.12-Droplet size distribution of micro emulsion for batch B1

3.10.2 Zeta potential:

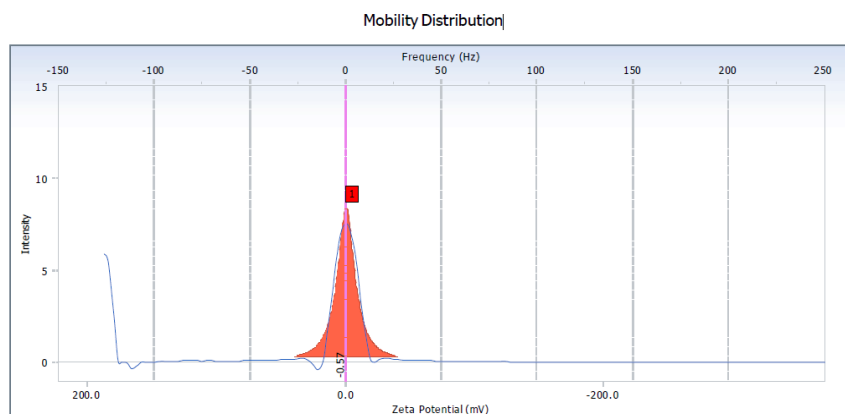


Fig.13-Zeta potential of micro emulsion of batch B1

3.10.3 SEM study:

Batch B1 contains more amount of water compared to other batches. At water concentration 50.50 % w/w, globular structures were observed as given in figure [Fig.15]. Image A showed the surface structure of the micro emulsion where Image B,C,D showed the globular structure present in micro emulsion[18].

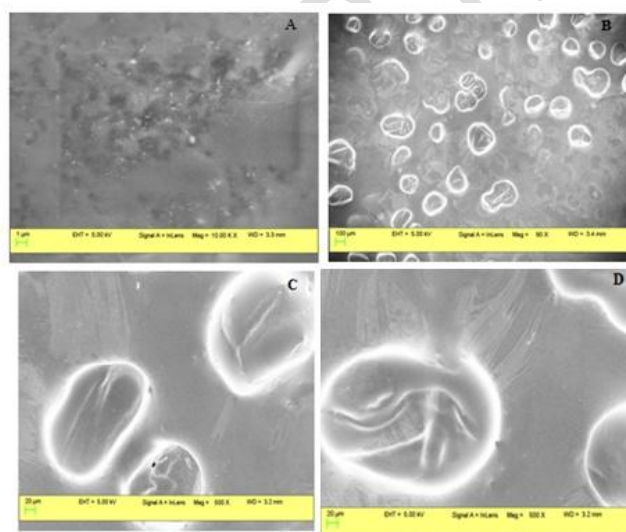


Fig.14-SEM images of batch B1 micro emulsion

3.10.4 In-vitro Drug release studies:

The result of drug release study is shown in figure no.16. The in vitro study of microemulsion formulation and curcumin + water was compared with each other. It can be seen that curcumin released from micro emulsion formulation and curcumin +water are different. As seen in figure nearly 48.75 % of drug was released from micro emulsion formulation and 19.97 % of drug was released from curcumin + water after 24 hours. The *in-vitro* drug release study showed that drug released at a faster rate from the micro emulsion (ME) system than from the curcumin + water (C)[14].

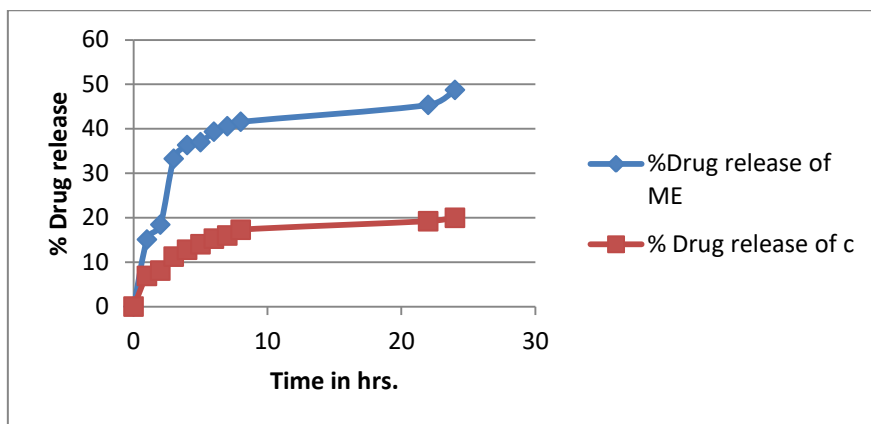


Fig.15-Comparative *in-vitro* drug release profile of curcumin (—◆—) for micro emulsion (ME) And (—■—) for curcumin + water.

3.10.5 Ex-Vivo permeation study:

The *Ex-Vivo* permeation of curcumin was carried out for micro emulsion and for the curcumin dissolved in water as reference. After 24 hr, the amount of curcumin permeated from micro emulsion formulation and from water + curcumin was compared that given in figure17 [20].

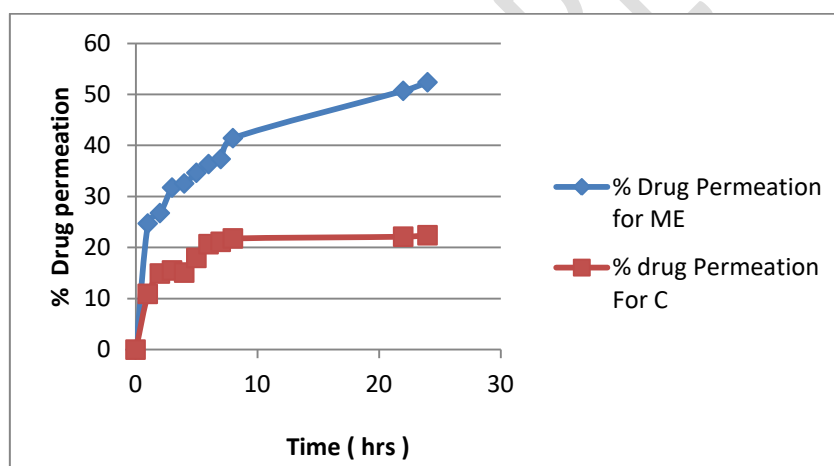


Fig.16-Comparative *ex-vivo* permeation profile of curcumin through mice skin (—◆—) for micro emulsion (ME) and (—■—) for curcumin + water.

3.10.6 Anticancer activity of micro emulsion formulations:

The micro emulsion was diluted to get concentration of curcumin in the range of 5 μ g/ml, 10 μ g/ml, 15 μ g/ml, and 20 μ g/ml and compared with standard curcumin in same concentration range. The result indicates that micro emulsion is successful in achieving anticancer activity as compared with curcumin as standard. The anticancer activity of micro emulsion of curcumin in 20 μ g/ml concentrations was found to be 55%, In vivo better results will be obtained with ME of curcumin than curcumin alone as it has poor bioavailability.

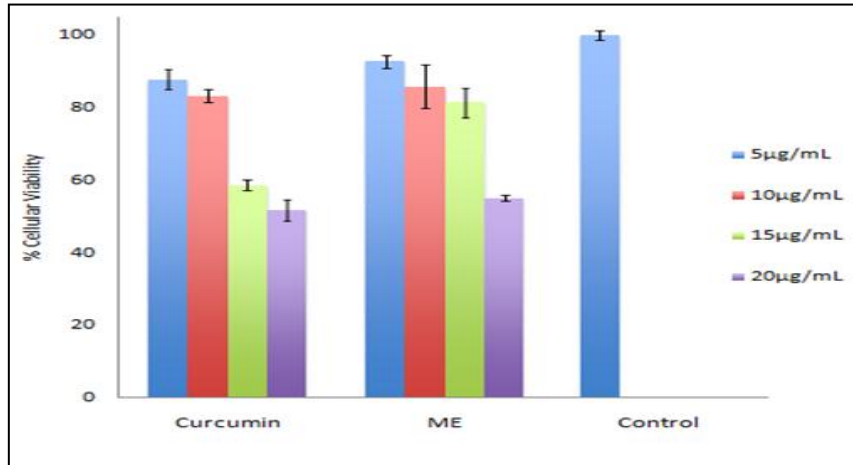


Fig.17-Anticancer activity of micro emulsion on HeLa cell lines

4. Conclusion:

Curcumin micro emulsion was prepared in thymol-menthol eutectic mixture carrier as novel technique to improve solubility and permeability of curcumin. The preformulation studies clearly indicate good compatibility of drug with the thymol menthol carrier system. The cytotoxicity study was done on thymol: menthol(TM) as a preformulation aspect to ascertain the toxicity of these two excipients to be tried. It was found that TM was not cytotoxic in 120ug/mL concentration and thus was safe to be used in concentration below 120ug/mL Batch B1 was optimised. The average globule size of micro emulsion was found to be 131.54 nm, zeta potential was found to be -0.57 mV. The drug content in micro emulsion batch B1 was determined and found to be 74.6%. Permeability study across mice skin ex-vivo model showed 52.38% permeability compared with curcumin alone which was 22.41%. So permeability of curcumin is enhanced substantially with micro emulsion made in thymol and menthol, owing to fact that thymol and menthol act as good permeation enhancers. The *In vitro* drug release study of micro emulsion was compared with curcumin alone. About 48.75% of curcumin was released from micro emulsion showed increase in release rate. Curcumin in micro emulsion of thymol menthol carrier system showed promising anticancer activity, the extension of this research can be *in-vivo* bioavailability study of the same.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any

litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

References

- 1) Ram Chand Dhakar et al. Microemulsion as carrier for nose to brain targeting: a review and update. International journal of pharmaceutical sciences-20112(2).
- 2) M.K.Modasiya and V.M.Patel.Studies on solubility of curcumin.International journal of Pharmacy and life sciences.Mar.2012 Vol.3(3):1400-1497.
- 3) S. L. Deore, S.S. Khadabadi.Antiproliferative activity of saponin fractions of Chlorophytum borivilianum . Pharmacognosy Journal,2010;2(16):33-37
- 4) Vanicha Vichai and Kanyawim Kirtikara. Sulforhodamine B colorimetric assay for cytotoxicity screening Nature Protocols 1, - 1112 - 1116 (2006)
- 5) Mukherjee, Pulok. (2002). Quality Control of Herbal Drugs: An Approach to Evaluation of Botanicals. Business Horizons.
- 6) Nguyen HuyKhuong, Tran Van Thanh. Formulation of microemulsion-based gel for skin delivery of curcumin. ResearchGate, Conference paper-December 2013:105-111.
- 7) Adnan Azeem,MohammadRizwan,FarhanJ.Ahmad et al.Nanoemulsion components screening and selection:a technical note.AAPS PharmSciTech,2008 vol.10(1).
- 8) Masthan Rao CH.N.V.S, Ram Bramha Reddy.Formulation development and evaluation of Diclofenac sodium microemulsion. Indo American journal of Pharmaceutical sciences,2015,Vol. 2(12):1673-1688.
- 9) Rashmin B.Patel , Mrunali Patel.Formulation and evaluation of microemulsion drug delivery system for intranasal administration of olanzapine. International journal of biomedical and pharmaceutical science.(2012).
- 10) Asit R Sahu, Sunil B Bothara et al.Formulation and evaluation of self – microemulsifying system of curcumin for enhanced solubility and dissolution.Asian journal of pharmaceutical education and research. Jan-March 2015.Vol. 4(1):48-59.
- 11) Patel V. A., Makwana S. B. et al. Formulation and Characterization of Microemulsion based gel of Curcumin for the Management of Plaque Psoriasis.
- 12) Adwoa O. Nornoo et al. Oral microemulsions of Paclitaxel: In situ and pharmacokinetic studies. European journal of pharmaceuticals and biopharmaceutics.2009 7(1) 310-317.
- 13) Chirag Raval et al.Enhanced oral bioavailability of Olmesartan by using novel solid self emulsifying drug delivery system. International journal of advanced pharmaceuticals.2005:2(2):82-92.

- 14) Sajal kumar jha, roopa karki, geetha lakshmi et al. Formulation development and characterization of microemulsion drug delivery systems containing antiulcer drugs(2011)
- 15) G.R. Dixit and A.B.Shende. Formulation and evaluation of anthralin microemulsion gel using karanj oil. International journal of pharmaceutical sciences and research:2320-5148.
- 16) Romica cretu, Cristian dima et al. Improved solubilization of curcumin with a microemulsification formulation. The Annals of the University Dunarea de Jos of Galati Fascicle VI – Food Technology 35(2):46-55
- 17) Asit R Sahu, Sunil B Bothara et al. Formulation and evaluation of self – microemulsifying system of curcumin for enhanced solubility and dissolution. Asian journal of pharmaceutical education and research. Jan-March 2015. Vol. 4(1):48-59.
- 18) Praporn Boonme, Karen Krauel et al. Characterization of microemulsion structures in the pseudoternary phase diagram of isopropyl palmitate/water/Brij 97:1-butanol. AAPS PharmSciTech. 2006 Jun; 7(2): E99–E104.
- 19) Sajalkumarjha, roopakarki, geethalakshmi et al. Formulation development and characterization of microemulsion drug delivery systems containing antiulcer drugs(2011)
- 20) Jadupati malakar suma oomen sen et al. Development and evaluation of microemulsions for transdermal delivery of insulin. International scholarly research network ISRN pharmaceuticals (2011). Article ID 780150:7 Pages.