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OPEN PEER REVIEW

Targeted Solid Lipid Nanoparticles Formulation for Colon Cancer Treatment and Cytotoxicity Assessment Using HT29 Cell Line

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1 Original submission

3 comments · 28 Feb 2023 – 5 Mar 2023

Anayatollah Salimi Reviewer

ACCEPT 28 Feb 2023

Mp is very strong in English writing and technical results.

Referee 1 Reviewer

MINOR REVISION 4 Mar 2023

Needs English revision.

Equation 1 should be written correctly.

In DSC, it might be better to include the rate of increase in temperature.

Figure 4: The 24 h time point might not be significant for assuming that thymol-SLNs have a sustained release profile because no intermediate time points have been measured.

Figure 6: error bars?

Since thymol is a natural ingredient, it would be better to include other studies of similar natural ingredients on cancer, especially in the studied cell line.

The presented data are very valuable, but more explanation and discussion would make this study noteworthy.

Editor-in-Chief

MAJOR REVISION 5 Mar 2023

Dear Authors,
Based on the reviewers' comments, significant changes are required before making a final decision. We invite you to revise the manuscript according to the provided comments and upload point-by-point answers or any rebuttals to the comments with the revised manuscript. Remember to highlight changes.

Kind regards,
JJNPP EIC

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor

Sincerest thanks for your reviewing our manuscript. We have revised the manuscript according to the comments and suggestions. Please find attached a point-by-point response to reviewer's concerns. We would be glad to respond to any further questions and comments that you may have.

Yours Sincerely

Reviewers' Comments:

Reviewer 1:

The manuscript entitled " Targeted SLN Formulation for Colon Cancer Treatment and Cytotoxicity Assessment using HT29 Cell Line" suffers from some major defects that should be addressed as follow:

• The purpose of various tests is not fully described.

The purpose of various tests is included in the MS revised (page 5 & 6) as:

- Differential scanning calorimetry (DSC) was performed to investigate the interaction between nanocarriers and molecules of drug.
- Cytotoxicity assays measure the ability of cytotoxic compounds to cause cell damage or cell death.
- The hemolysis test is used to study the degree of RBCs destruction induced by the nanocarriers
- The *in vitro* release study is a critical test to assess the rate of drug release at different times

In the formulation, the components and percentages of each component are not mentioned or specified.

Thanks for your comment. The amount of each component for preparation of SLN was selected according to the preliminary studies. SLNs were prepared using a microemulsion method. Briefly, stearic acid (300 mg) and GMS (100 mg) were heated at 70 ˚C. Then, thymol (10 mg) was incorporated in the molten lipid. For preparation of aqueous phase, Tween 80 (300 mg/10 mL) was heated at 70 ˚C.

• How is the percentage of formulation components optimized?

The percentage of formulation components was optimized based on the highest encapsulation efficiency (EE%) and the lowest particle size after preliminary studies.

• There would have been a benefit to summarizing the various characteristics of SLN in one table

The table is included in the in the MS revised (page 8) as:

Particle size (nm)	EE %	Formulation
145	63	Th-loaded SLNs

• A more detailed explanation of the rationale for choosing SLN as a carrier is needed in the introduction

Thanks for your comment. The reason for choosing SLN for this study was explained in the introduction as follow (page 3):

" According to the previous reports, SLNs do not display the restrictions associated with polymeric nanoparticles and liposomes including, sterilization, toxicity and long-term stability. Moreover, these carriers show advantages over other drug delivery systems, such as high encapsulation

efficiency, improved physical stability, sustaining the drug release, easy large-scale production and increased bioavailability."

• There is a lack of clarity in the interpretation of DSC test results.

It is added.

• An explanation of how to calculate the IC50 of the compounds is not provided, and it would have been better to compare the IC50 in a table

Thanks for your comment. IC_{50} value was calculated by linear approximation regression of the percentage survival versus the drug concentration. The table containing of IC_{50} was included in the MS revised (page 13).

Table 3. The IC_{50} values for thymol, SLNs and Th-SLNs

IC_{50} (µM)	Formulation
39.22 ± 0.9	Thymol
94.87 ± 1.1	SLNs
7.88 ± 0.7	Th-SLNs

• For the cytotoxicity test, it was preferable to use the normal cell line as a control.

The non-toxicity of SLN components on normal cells has been proven in previous studies. Therefore, based on the published results, in the current study, the toxicity activity was only investigated on cancer cells.

Reviewer 2:

Needs English revision.

It is revised.

Equation 1 should be written correctly.

It is corrected in the MS revised as (page 4):

$$EE\,\% = \frac{W_a - W_s}{W_s} x\,100$$

In DSC it might be better to include the rate of increase in temperature.

It is mentioned in the MS revised (page 5):

The samples (5 mg) were sealed in aluminum pans and analyzed at a heating rate of 10 $^{\circ}$C min^{-1} from 0 to 250 $^{\circ}$C for 30 min.

Figure 4: 24 h time might not be significant in assuming thymol-SLNs have a sustained release profile because no time in between has been measured.

While confirming your opinion, according to the obtained results and previous studies, it seems that a sustained release behavior for thymol from SLNs can be predicted because of the gradual release of thymol from particles into the diffusion medium.

Figure 6: error bars?

Error bars are included in Fig 6 (page 14)

Since thymol is a natural ingredient, it would be better to include other studies of similar natural ingredients on cancer, specially the studied cell line.

It is added (page 12):

Additionally, several studies reported that encapsulation of essential oil in SLNs leads to increasing anti-cancer activity. Valizadeh et al. prepared SLNs containing *Zataria multiflora* essential oil to improve anti-cancer activity against breast and skin cancer. They found that essential oil loaded in SLNs had a remarkable anti-cancer effect than free essential oil on cancer cells [17]. Dousti et al. evaluated anti-cancer activity of *Pistacia atlantica* essential oil loaded in SLNs against breast cancer. It was observed that essential oil loaded in nanocarriers could trigger more apoptosis in breast cancer cell than free essential oil [39].

The presented data is very valuable, but more explanation and discussion would make this study noteworthy.

It is added (page 7 & 12):

According to the previous study, stearic acid is used for preparation of SLNs due to increasing the highest entrapment efficiency in nanocarriers. Therefore, in this study, it was chosen as a lipid core for SLNs preparation [26].

Additionally, several studies reported that encapsulation of essential oil in SLNs leads to increasing anti-cancer activity. Valizadeh et al. prepared SLNs containing *Zataria multiflora* essential oil to improve anti-cancer activity against breast and skin cancer. They found that essential oil loaded in SLNs had a remarkable anti-cancer effect than free essential oil on cancer cells [17]. Dousti et al. evaluated anti-cancer activity of *Pistacia atlantica* essential oil loaded in SLNs against breast

cancer. It was observed that essential oil loaded in nanocarriers could trigger more apoptosis in breast cancer cell than free essential oil [39].

Reviewer 3:

Mp is very strong in english writing and technical results.

2 Revision 1

0 comments

No public comments in this round.