April 10, 2021

Dear Drs. Patel and Safe,

Attached please find our revised manuscript entitled " **Therapeutic potential of thymoquinone in combination therapy against cancer and cancer stem cells**" (Manuscript NO.: 65070, Review)**.** We have provided a point by point response to the comments of the reviewer and the editorial office. We uploaded the manuscript with tracked changes as a supplementary material. We also uploaded the original figures as a PowerPoint file along with the tables as a Word file.

We believe that our paper is now in a form that is acceptable, and we look forward to your positive response.

Sincerely,

Hala Gali-Muhtasib

Answers to Reviewer Comments

Comment 1: In the abstract, the conclusion should be added.

Answer 1: We thank the reviewer for this comment. We have added a conclusion to the abstract stating that: "In conclusion, the ability of TQ to potentiate the anticancer activity of many chemotherapeutic agents and sensitize cancer cells to radiotherapy makes it a promising molecule that could be used in combination therapy to overcome resistance to standard chemotherapeutic agents and reduce their associated toxicities".

Comment 2: Introduction: It is necessary to narrow down the specific mode of action of thymoquinone. The theoretical background that thymoquinone alone induces various anticancer mechanisms seems to be lacking in expertise. And anticancer drugs must be able to provide the target clearly.

Answer 2: Thymoquinone has been shown to target nine of the ten hallmarks of cancer and to have multiple modes of action with multiple targets which explains why we have opted to review comprehensively its mode of action. The exact molecular target of TQ is not known yet and a statement that "its exact molecular target is not known yet" has been added to the introduction section, page 6 lines 11-12, for clarification.

Comment 3: The MOA for the chemotherapeutic agents introduced in Table 1, but some information was omitted in the related paragraph. For example, MOA was provided that cyclophosphamide increased the percentage of cells in G1 and sub-G1 phases in Table 1, but it did not describe in the main text. All must be supplemented.

Answer 3: We included the detailed cellular and molecular mechanisms of TQ action in the tables and highlighted some but not all mechanisms in the main text to make it clear and simple. Including details of TQ molecular mechanisms for all the studies (around 62 of them) in the main text would make the paper very long.

Comment 4: Are there no studies for the anticancer effect using the xenograft mouse model?

Answer 4: There are studies that used human esophageal (40), lung (42, 147), gastric (44, 48), pancreatic (57), leukemic (83, 117), multiple myeloma (99), breast (85, 107, 127) cancer xenografts in mice. These types of xenografts were mentioned in the tables.

Comment 5: The specific cell line name like MDA-MB-231 breast cancer for the indication did not specify in many paragraphs.

Answer 5: Throughout the review, we included the types of cell lines in the tables except for the cancer stem cells studies and the studies that used apoptosis defective cell lines.

As such, we removed "MDA-MB-231" from the "Cabazitaxel" section page 12 line 13 in the revised paper.

Comment 6: Combination antitumor effects with second and third-generation drugs have not been reviewed (eg. PD-1 and PD-L1 inhibitors, trastuzumab, gefitinib, lapatinib, or erlotinib, etc.). Is there any reason?

Answer 6: There is only one study investigating the anticancer effects of TQ in combination with Imatinib which is a tyrosine kinase inhibitor. This study was published in March 2021 after submitting our review.

Thus, we added the following paragraph to page 14 lines 25-29 and page 15 lines 1-7:

"***Tyrosine kinase inhibitor***
Imatinib
Imatinib is a potent tyrosine kinase inhibitor that was approved for treating chronic myeloid leukemia and gastrointestinal stromal tumors[100]. Resistance to Imatinib was reported to develop in cancer patients through several mechanisms including the modulation of the expression of drug efflux and influx transporters[101,102]. In a study conducted by Thabet et al[103], TQ was found to improve the anti-proliferative and apoptotic effects of Imatinib in colorectal cancer cells *in vitro*. Interestingly, this was accompanied by a significant decrease in the expression of the drug transporters ATP-binding cassette (ABC) subfamily B member 1 (ABCB1), ABC subfamily G member 2 (ABCG2) and human organic cation transporter 1 (hOCT1) leading to a significant increase in Imatinib uptake/efflux ratio compared to Imatinib alone".

Comment 7: It would be desirable to provide information on cases in which clinical studies were conducted.

Answer 7: There is only one clinical study that investigated the anticancer effects of TQ in combination with Tamoxifen in breast cancer patients which is now included in the revised version.

We added the following paragraph to page 15 lines 19-27:

"Treating breast cancer patients with a combination of TQ and Tamoxifen resulted in greater increase in 5-year survival rate and decrease in relapse rate of patients compared to single treatments.
At the molecular level, the dual treatment induced a higher increase in tumor tissue antioxidant enzymes (catalase and superoxide dismutase) and increased caspase 3 expression compared to individual treatments. Moreover, the combination of TQ and Tamoxifen enhanced the decrease in tumor tissue Bcl-2, TGF-β1, lipid peroxidation product malondialdehyde, and pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) compared to each treatment alone[108]".

After adding this study, we modified one sentence in the conclusion and added the following sentence on page 22 line 14 "studies reporting TQ anticancer therapeutic potential in clinical settings are still limited".

Answer to the Editorial Office's comment

Comment: References: A total of 152 references are cited, including 38 references published in the last 3 years; (5) Self-cited references: There are 7 self-cited references. The self-referencing rates should be less than 10%. Please keep the reasonable self-citations that are closely related to the topic of the manuscript, and remove other improper self-citations. If the authors fail to address the critical issue of self-citation, the editing process of this manuscript will be terminated.

Answer: The total number of references is now 157 after including new studies as per the reviewer's comment. The self-referencing rate is 4.5% (7 references out of 157) which is less than 10%. All the self-cited papers are closely related to the topic and thus need to be cited in the review.