# Format for ANSWERING REVIEWERS

May 30, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript NO:7666).

**Title:**

**Author:**
**Name of Journal:** *World Journal of Gastrointestinal Oncology*

**ESPS Manuscript NO:** 7666

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers

1. **00503405**: The abstract has been expanded with the main results and the conclusion of the study. The text has been reformatted according to the guidelines of WJGO for the Brief Article. The manuscript has been polishing for the English language by the AmEditor-English language editing company.

2. **00070821**: The manuscript has been enriched with images of the *in vitro* cell culture experiments on the study of the effects of quercetin and genistein on the cell growth of the colon cancer cellular models used for this research. The structure of the article has been modified in the part of the DISCUSSION and of the INTRODUCTION, which has been reduced. The manuscript has been polishing for the English language by the AmEditor-English language editing company. The bibliography has been updated as much as possible and the references were reviewed and formatted as the guidelines of WJGO for the Brief Article.

3. **02543800**: The Abstract was reviewed and figure legends were separated by the text, in a separate page as request by the guidelines of WJGO for the Brief Article, making them simple to read.

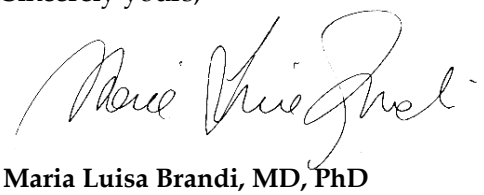
The manuscript presents a preliminary study on two cellular models of colon carcinoma with the aim to evaluate *in vitro* the effects of two phytoestrogens, genistein and quercetin, which, as documented by epidemiological studies were found to be possible preventive agents in the development of colorectal cancer. βStudies made up to now on the two isoforms of the estrogen receptor have shown that the estrogen receptor type β ( ERβ ) , expressed at high levels of the colonic mucosa healthy , is not present, if not perhaps in very slight traces, in the colonic mucosa subject to neoplastic transformation. This receptor has as substrate the human estrogen , 17beta - estradiol . Quercetin and genistein are two natural molecules with molecular structure similar to estrogen that can act as a substrate for this ERβ. Therefore, we investigated, using the cell line HCT8-β8 (a colorectal cancer cell line engineered in our laboratories for overexpressing ERβ), the possible effects of phytoestrogens on growth and cell viability compared with 17β-estradiol, which is been documented to be an agent inhibitor of tumor growth in CRC. Based on the results obtained from experiments, we carried out to evaluate if indeed the activation of ERβ was involved in the mechanism of action of phytoestrogens, and in relation to the results obtained about this we also went to assess if the two compounds could have any effect on gene expression level. The experiments have actually shown a growth inhibitory effect and a decrease in cell viability , which for now, on the basis of literature data and the results obtained from our experiments, might actually be mediated by molecular mechanisms involving the ERβ . In this work we used as a control cell model similar to HCT8-β8, being engineered in turn , but also analogous to the cellular model of colon cancer expressing very low levels , of ERbeta . Of this we have included data related to cell proliferation and viability , not shown in the previous version . On the basis of the comparison of the data of the various tests between them , we assume that ERβ may have a role in inhibiting the growth of colon cancer cells . To confirm our hypothesis , as suggested by the Reviewer, in the future take the same experiments presented here on the same cell line engineered to over- express ERbeta in the presence of an agent blocking the estrogen receptor , which ICI 182.170 . In conclusion, our study does not prove with certainty that genistein and quercetin inhibit the growth of colon cancer cells by binding ERbeta certainly , but it is a preliminary figure on the molecular mechanism that could be activated really , and that will be the subject of our future study .

In light of the data obtained , however , in view of the studies in the literature , our research applies the positive effects in vitro and inhibitors observed by clinical and epidemiological data in preventing the development and progression of CRC in the presence of a diet rich in phytoestrogens.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastrointestinal Oncology*

Sincerely yours,



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