**Author’s response to reviews**

**Title:** Castanea Sativa Mill. bark extract exhibits chemopreventive properties triggering extrinsic apoptotic pathway in Jurkat cells

**Authors:**

Monia Lenzi (m.lenzi@unibo.it)

Marco Malaguti (marco.malaguti@unibo.it)

Veronica Cocchi (veronica.cocchi4@unibo.it)

Silvana Hrelia (silvana.hrelia@unibo.it)

Patrizia Hrelia (patrizia.hrelia@unibo.it)

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"The authors' response letter has been included also as a supplementary file" file name: "Cover letter R1"

Answer to Reviewers’ comments:

1- Reviewer 1: “Provide the HPLC chromatogram of Castanea Sativa Mill. (CSM) extract confirming the presence of phenolic compounds”.

Answer: In a previous publication (Oxid Med Cell Longev. 2013;2013:471790. doi: 10.1155/2013/471790) we demonstrated, by HPLC-DAD-MS analyses, the presence of phenolic compounds in the same batch of CSM bark extract used in the present manuscript. This point has been better clarified in the “Methods” section as follow: “We previously characterized, by HPLC-DAD-MS analysis, the same batch of CSM bark extract used in this manuscript demonstrating that it is rich in phenolic compounds such as: castalin, vescalin, castalgin, vescalgin, ellagic acid and gallic acid” (page 6 lines 24-30).

Since CSM bark extract HPLC analyses had been the object of a previous publication and to avoid any possible plagiarism accusation we think that the proper bibliographic reference could be exhaustive.

2- Reviewer 1: “Include the morphological analysis (micrographs) of Jurkat cells”.

Answer: In the present manuscript all endpoints have been evaluated by flow cytometry analysis that represents the golden standard to evaluate apoptosis especially in suspension cell culture. However, according to the Reviewer request, to further confirm that CSM bark extract induces
apoptosis in Jurkat cells we introduced the fluorescence microscopy images of morphological changes after Jurkat treatment with 50 microg/ml CSM bark extract for 72h as additional file (additional figure 1). “Results” section has been updated with a sentence to highlight the introduction of a new figure (Additional file 1) (page 11 lines 24-28).

3- Reviewer 1: “Provide the schematic mechanism of anticancer activity of the extract”.

Answer: A schematic image (Figure 7) describing that CSM bark extract induces apoptosis in Jurkat cells by activating caspase-8 has been added to the Discussion section (page 14 lines 56-59).

4- Reviewer 1: “Provide the JC-1 ratio along with red and green fluorescence images for mitochondrial membrane potential”.

Answer: As suggested by the Reviewer, Figure 5 has been improved showing JC-1 ratio along with orange and green fluorescence.

5- Reviewer 1: “Though CSM bark extract was purchased commercially for this study, specify the method for synthesis of CSM bark extract”.

Answer: The preparation method of CSM bark extract was previously published by Budriesi R. et al., in 2010 (J Med Food 2010: 13, 1104-1110). According to the Reviewer, the extraction procedure has been briefly described in the manuscript with a new reference (page 6 lines 19-24).

6- Reviewer 1: “Kindly avoid sentences like "thank to their phytochemical content", "thanks to its ability" (in the abstract) and usage of thanks in the manuscript (Page-12)”.

Answer: As requested, the usage of the word “thanks” has been avoided, and it has been substituted everywhere it appeared in the manuscript.

1- Reviewer 2: “I felt it is too much of exagenation of the activity and I am first time seeing someone is thanking the extract for it chemopreventive properties. It was mentioned in the conclusion para of the abstract (Conclusions: Our data suggest that CSM bark extract might be considered an interesting and partially selective cytotoxic agent thanks to its ability to induce apoptosis in transformed cells at concentrations significantly lower than in nontransformed ones.) Authors should explain this if they are correct. Similarly, prevention and counteraction are two different contradictory words. How these works can be correlated to chemopreventive action? Chemoprevention, generally means preventing the cancer growth. The Aspirin, is a chemopreventive agent in colorectal cancer, Tamoxifen is in breast cancer, etc. In this manuscript how and in what contest the term 'chemoprevention' is used? This factor they can explain in introduction so as to increase the flavour of the article”.

Answer: The term “thanks to...” was previously used with the meaning of “due to...” and has been now modified.
Moreover, to better explain the concept of chemoprevention, the “background” section has been improved including a brief description of carcinogenesis and a description of the main chemopreventive mechanisms such as the prevention of cancer initiation or counteraction of its promotion and progression (page 3 lines 2-13; 19-48).

2- Reviewer 2: “Under the subheading, Background, the sentence 'Numerous studies, in fact, demonstrated that alterations in cell death induction pathways are important for cancer development and are influencing the response to chemotherapy.' have to be modified (remove 'in fact'. For the facts you are citing the references.)”.

Answer: As requested, “in fact” has been deleted.

3- Reviewer 2: “Under the same subheading, there are few sentences overlooked. What is the meaning of 'CSM wood extract containing 73% tannic agents'? What are tannic agents? Is it tannic acid? or tannins”?

Answer: Since the sentence: “Frankic et al. [20] demonstrated that a commercially available CSM wood extract containing 73% tannic agents…” was introduced to describe a previous research, in the first version of the manuscript we maintained the same definition “tannic agents” used in the original paper. However, as requested, it has been removed and substituted with “tannins”.

4- Reviewer 2: “Kindly explain how p53 and Bax protein levels are correlating the chemopreventive activity of the extract or for any given substance? This can be explained in introduction or in the discussion of the manuscript”.

Answer: p53 and Bax are two proteins involved in development and progression of cancer. The dysregulation of tumour suppressor gene p53 and the dysfunction of pro-apoptotic protein Bax can lead to uncontrolled growth and carcinogenesis. Conversely, the up-regulation of their levels can be exploited as chemopreventive mechanism. We introduced these considerations in the “background” section together with two new references (page 5 lines 13-20).

5- Reviewer 2: “There are many spelling and grammatical corrections, should be done by consulting the language specialists”.

Answer: A naturally English speaking colleague checked spelling and grammar, all detected mistakes have been corrected.

6- Reviewer 2: “Authors should cross check the references and the eustis used in the manuscript”.

Answer: We cross checked the references.