Author's response to reviews

Title: Effects of Ginkgo biloba on chemically-induced mammary tumors in rats receiving tamoxifen

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Author's response to reviews: see over
Dear Editor:

We would like to thank the reviewers for the careful reading of the manuscript and suggestions to improve it. All modifications performed are outlined in yellow in the present revised manuscript.

Regarding reviewer's suggestions on the above manuscript, our comments to each one of the raised points are as follows:

Reviewer: Jiaren Liu

Point 1: “Authors divided female SD rats bearing palpable mammary tumors (> 1 cm in diameter) into 4 groups and given different treatment Control, TAM, TAM with GbE. Before the beginning of treatments, all animals bearing palpable tumors were submitted to excision biopsies to evaluate the histopathological pattern of PCNA and cleaved-caspase-3, estrogen receptor (ER-alpha) and p63. These data were mammary tumors which were before treatments. My concerns are about how about the data of after treatments. Had these rats new mammary tumors during 4-week treatment? If yes, authors should supply new data after treatment.”

At necropsy, some non-palpable tumors were detected as follows: 02 tumors in the TAM alone group and 02 tumors in TAM+GbE50 group (This information was included in the Results section, page 9).
Point 2: “As table 1, there had 7 tumors in control, 11 in TAM, etc. It seems new tumors in the rats. Authors should give the information about how many rats in each group and how many tumors each rat.”

The difference between initial and final number of rats/group was due to the dead or sacrifice of moribund animals (05 animals in untreated group), or development of mammary tumor with less than 5% of positivity for ER (01 and 03 animals in TAM alone and TAM+GbE50, respectively) or complete regression of tumors (03 animals in TAM+GbE100). Two animals from each group presented two mammary tumors before the beginning of treatments. (This information was included in Table 1 footnote).

Point 3: “About expression of cell proliferation, apoptosis and ER- and p63 in mammary tumor, authors should have the data of statistical analysis.”

Only the statistical difference (p < 0.05) for PCNA biomarker was included in the Figure 3.

Point 4: “Figure 4 should be cleaved-caspase-3.”

The Figure 4 was revised, as suggested.

Reviewer: Jin-Rong Zhou

Point 1: “Animal and treatment: It is unclear why GbE was administered via ip injection, but not oral administration which is more relevant to human situation; Not including the GbE alone in the experimental groups was also a flaw of design.”

GbE contains 24% flavone glycosides (i.e., quercetin, kaempferol and isorhamnetin). The biological fate of the dietary flavonoid glycosides into gastrointestinal system has been an elusive and controversial issue over the years. It was long believed that these fairly large and
highly polar molecules could not be absorbed after oral ingestion, but are hydrolyzed to their aglycones forms by bacterial enzymes in the lower part of the intestine (Walle, 2004). The aglycones might then be partially absorbed or may undergo further biotransformation by gut bacteria. In general, flavonoids are absorbed as their aglycones forms after prior hydrolysis of the glycosides along the gastrointestinal system. As the bacterial microflora in the colon also plays a critical role in the metabolism of polyphenols (Blaut et al., 2003; Walle, 2004) and the microbial metabolism is significantly different between human and rodent (Blaut et al., 2003; Walle, 2004), we investigated only the i.p. administration of GbE.

The main objective the present study was to assess only the association between Tamoxifen and GbE treatments rather than to investigate a potential therapeutic action of GbE alone. Moreover, we tested the administration of GbE alone on the development of DMBA-induced tumors in a 25-weeks mammary carcinogenesis assay (manuscript in preparation).

Additional Reference:

Point 2: “Measurement of mammary tumor volume and area: The authors did not provide sufficient details of protocols for determination of tumor areas (live, degenerative and necrotic). Specifically, it is unclear, and this reviewer doubt if the measured areas in the HE slide represent the true proportions of these areas in the tumor. The authors should cite any reference(s) for the equation used to calculate areas, if there is any.”

The determination of tumor areas (live, degenerative and necrotic) in each tumor mammary was made by a veteran pathologist (M.A.M Rodrigues, co-author). More detailed information and one reference was included (see material and Methods section, page 7).
Point 3: “The authors stated that 10 rats/group were used in the experiment, but the data in Table 1 showed different animal numbers, especially only 5 rats in the control group. The authors should provide explanation why only part of the experimental animals was included in the final results.”

The difference between initial and final number of rats/group was due to the dead or sacrifice of moribund animals (05 animals in untreated group), or development of mammary tumor with less than 5% of positivity for ER (01 and 03 animals in TAM alone and TAM+GbE50, respectively) or complete regression of tumors (03 animals in TAM+GbE100). Two animals from each group presented two mammary tumors before the beginning of treatments. (This information was included in Table 1 footnote).

Point 4: “Statistical results should be clearly and consistently labeled. The labeling should represent the statistical results of the control vs. the treatment groups, and the TAM alone vs TAM+GbE groups in the figures and table.”

The labeling statistical (untreated vs. TAM-treated groups and TAM alone vs. TAM+GbE50 and 100) were introduced in the Table 1 and Figures, as suggested.

Point 5: “The authors defined the live tumors as the TAM-resistant or ER-alpha-resistant breast tumors, but did not provide any experimental evidence to support it.”

In fact, more than 60% of human breast tumors continued to express ER even while progressing in the face of tamoxifen treatment. Thus, the TAM-resistant or ER-alpha-resistant terms were replaced by live areas (page 10-13).

Point 6: Minor compulsory revisions

All revisions suggested were introduced in the manuscript.
Thanking you beforehand for your attention I remain,

Kind regards,

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