Dear Editor,

Thank you very much for your critical comments on our manuscript. The reviewers had positive responses to our study. There were some revisions needed to be made. Now, we have revised as suggested by the Reviewers. Revisions (marked in red color) have been made according to the Reviewers' comments, which were very helpful in improving presentation. Please refer to the list of **Responses to Reviewers**.

We hope that the revised manuscript will be satisfactory for publication in the ***World Journal of Gastroenterology*** and look forward to hearing from you about the acceptability of the review paper.

With best personal regards.

Sincerely yours,

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Format for ANSWERING REVIEWERS

Dec. 23, 2021

Dear Editor,

Title: Anti-inflammatory effects of quercetin via inhibition of TNF-α-induced matrix metallopeptidase 9 expression in normal human gastric epithelial cells

Author: Hsi-Lung Hsieh[#], Ming-Chin Yu[#], Li- Ching Cheng[#], Mei-Yi Chu, Tzu-Hao Huang, Ta-Sen Yeh and Ming-Ming Tsai

Name of Journal: *World Journal of Gastroenterology (WJG)*

Manuscript ID: 71180

Reviewers' comments:

Reviewer #1: Thank you for the insightful review and excellent suggestions from the anonymous reviewers of the manuscript ID: 71180, entitled " Anti-inflammatory effects of quercetin via inhibition of TNF-α-induced matrix metallopeptidase 9 expression in normal human gastric epithelial cells ". The revisions requested by the reviewers are now complete. The reviewer's criticisms, suggestions and comments have been addressed and the manuscript revised accordingly. The changes are indicated by the lines/page numbers and colored font. We hope that you and the reviewer will be satisfied with the revised manuscript. Thanks again for your valuable suggestions. I look forward to your favorable reply.

Reviewer #2: In this manuscript, the authors used TNF-α-induced GES-1 cells to investigate the in vitro antimetastatic and anti-inflammatory activities of quercetin. MMP9 expression damages the extracellular matrix including components of basement membrane, inducing gastric disease. The manuscript indicated that TNF-α induced MMP9 expression and through c-SRC/ERK1/2/NFκB pathway, and quercetin suppressed the TNF-α-induced MMP9 expression and could be a potential food supplement for preventing gastric inflammation. However, there are many issues with the current form of the manuscript as indicated below: Major revision for

consideration.

1. In the paragraph under "Omics approach to identifying inflammation-related proteins involved in GC", the website "https://www.abcam.com/human-inflammation-antibody-array (40 Targets)" is empty, please check it again.

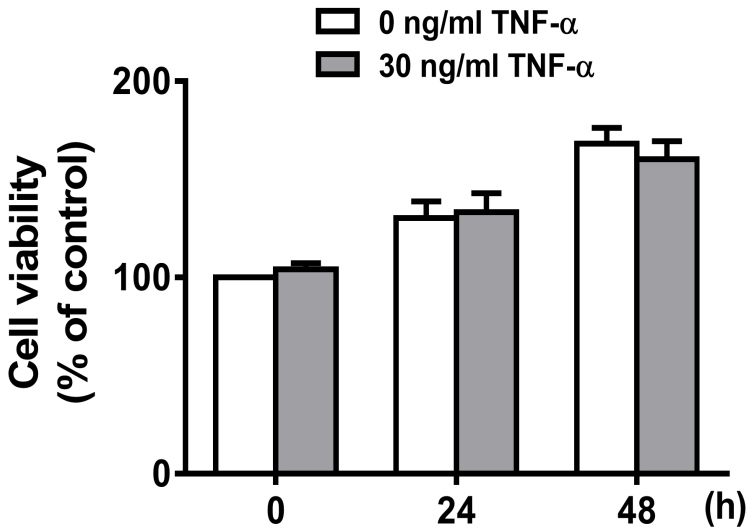
   Author Response 1-1:

   Thank you for your suggestion. I have corrected the website (https://www.abcam.com/human-cytokine-antibody-array-membrane-174-targets-ab193657.html) and Figure 1A. (line 2-4, page 15)

2. The author used TNF-α to induced MMP9 expression, but there are no data showing that MMP9 did cause cell damage under 30 ng/ml TNF-α. And the data from Figure1 also only show the relevance between MMP9 and gastric cancer.

   Author Response 1-2:

   Thank you for your suggestion. I have evaluated the influence of TNF-α-induced MMP-9 expression on the physiological activity of GES-1 cells was evaluated based on a cell viability assay, and GES-1 cells were observed at 0, 24 and 48 hours after TNF-α treatment. We found that TNF-α-induced MMP-9 expression did not affect GES-1 cell survival and proliferation (data as shown below, Supplemental Figure 1). Our data showed that TNF-α-induced GES-1 cell migration significantly increased by upregulation of MMP-9 (Figure 8).



Supplemental Figure 1. Effects of TNF-α-induced GES-1 cell viability**.** GES-1 cells were

treated with TNF-α for various times in the cell viability assay, and the number of cells were evaluated by CCK-8 assay. Cell viability was expressed as the percentage of living cells relative to the control. A T test was used to compare the two groups. Data are expressed as mean ± SEM of three independent experiments. GES-1: normal human gastric mucosa epithelial cell line; TNF-α: tumor necrosis factor-α

3. What is MMP inhibitor? The method part did not mention the catalog and supplier, please add the information.

Author Response 1-3:

Thank you for your suggestion. We have added the MMP-9 inhibitor information (the catalog and supplier) in the text. Moreover, we also provided all reagents' information in the "Materials and Methods" section. (line 2-27, page 10)

4. In figure2, MMP9 inhibitor did decrease the expression of MMP9 induced by TNF-α, but actually in order to prove the effect of TNF-α, TNF-α inhibitor instead of MMP9 inhibitor should be used.

Author Response 1-4:

Thank you for your suggestion. As the reviewer's suggestion, we have used TNF-α inhibitor instead of MMP9 inhibitor in the study. The results showed that pretreatment of TNF-α antagonist (SC-356159) significantly attenuated TNF-α-induced MMP-9 expression in GES-1 cells (Figure 2C), demonstrating that TNF-α-induced MMP-9 expression is indeed through TNF-α action in GES-1 cells. Moreover, we also added the influence of TNF-α on these signaling molecules by pretreatment of TNF-α antagonist. The results showed that TNF-α is indeed can cause these signaling molecules activation, including TNFR, c-Src, ERK1/2, c-Fos, and NF-κB, and then induce the MMP-9 expression and cell migration in GES-1 cells (Figure 2C, 4C, 5C, 6C, 7C, 7F and 8). Therefore, we have corrected the summary figure (Figure 9).

5. Please check the grammar of sentence "…if so, the mechanisms responsible" under the paragraph "Effects of quercetin on the expression of MMP-9 induced by TNF-α in GES-1 cells".

Author Response 1-5:

We have checked and modified the sentence as the Reviewer's suggestion. (line 13, page 16)

6. In figure6D, the abbreviation “Q” did not explain in the legend.

   Author Response 1-6:

   We have explained it in the legend of Figure 6D. (line 19, page 36)

7. In figure7D, the response of TNF-α-induced NF-κB translocation reduced in 60min and did not follow the time dependent manner, why?

   Author Response 1-7:

   Thank you for your suggestion. The data showed that TNF-α induced NF-κB translocation in a time-dependent manner in these cells. A maximal response was achieved within 30 min and sustained over 60 min during the period of observation (Figure 7D). (line 3-5, page 19)

8. The figure9 overclaimed the effect of quercetin, the experiments above did not prove that quercetin directly inhibit the c-Src or MAPKs pathway, the changes in this pathway may cause by upstream pathway.

   Author Response 1-8:

   As the Reviewer’s speculated, quercetin may indeed directly inhibit upstream molecules (such as TNFR or c-Src) and then cause the inhibition of downstream molecules, such as ERK1/2, c-Fos, and NF-κB (Figure 2C, 4C, 5C, 6C, 7C and 8) in GES-1 cells. Therefore, we have corrected the summary figure (Figure 9), indicating that quercetin suppresses TNF-α-induced MMP-9 expression and migration activity in GES-1 cells. (line 17-19, page 21)

9. The author said “…quercetin has been reported to inhibit the lipopolysaccharide-induced MMP-9 expression observed in lung inflammation and the production of pro-inflammatory cytokines”, in this paper, author change the cell line and inducer, so what is the innovation of this paper?

   Author Response 1-9:

   Thank you for your suggestion. Previous studies have shown that quercetin is a flavonoid polyphenol compound widely found in fruits, vegetables, and grains, and has anti-inflammatory, anti-cancer, and immune-regulating effects. Although the anti-inflammatory and anti-cancer functions of quercetin have been studied in different fields, and several signaling molecules have established widespread attention (such as eye epidermal cells, cardiomyocytes, vascular endothelial cells, bronchial epithelial cells, fat cells and mouse

small intestine epithelial cells) [1-5], showing that quercetin is really important. However, our manuscript is different from these studies. So far, the pharmacological effects and the molecular mechanism of quercetin in TNF-α-induced inflammation of human normal gastric mucosal epithelial cells, which still has not been studied. Therefore, the purpose of this manuscript is to evaluate whether quercetin can protect normal human gastric mucosal epithelial cells from inflammation by TNF-α-induced, related gastritis symptoms and provide related mechanisms, which has high clinical importance in preventive medicine. Therefore, this research has its novelty and importance in this field.

Reviewer #2: These results indicated that TNF-induced MMP-9 expression mediated the anti-inflammatory effects of quercetin in gastric mucosal epithelial cells, both in a dose- and time-dependent manner. These new findings suggest that quercetin significantly downregulates TNF-induced MMP-9 expression in GES-1 cells via the c-Src–ERK1/2 and c-Jun or NF-B pathways. The works are informative and interesting. With a dose- and time-dependent manner, how about the safety of the use of Quercetin and quercetin-rich diets as food supplements? Quercetin can suppress the expression of MMP-9 and prevent the early pathological changes associated with gastric inflammation. Does an overdose of quercetin aggravate gastric injures else, such as ulcer, GC and so on?

Author Response 1:

Thank you for your suggestion. In Figure 3A and 3B, we evaluated the effects of different concentrations of quercetin on the normal GES-1 cell line using the cell viability assay. We found that cells treated with quercetin at a concentration ≤ 10 μM for 24 to 48 h had almost no effect on GES-1 cell viability. Additionally, many studies have confirmed that the safety and efficacy of quercetin remain inconclusive [6]. In 2010, the American Food and Drug Administration (FDA) considered that the utilization of high-purity quercetin as a food ingredient in different food products is GRAS ("Generally Recognized As Safe") (https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=GRASNotices&sort=GRN_No&order=DESC&startrow=1&type=basic&search=341)(GRN No. 341). Based on several published human intervention studies, daily doses of oral quercetin are usually in the range of up to 1000 mg/day (they are the safest at 500 mg/day). Therefore, the daily supplement doses of oral quercetin were noticeably higher than the background daily doses of oral quercetin. Side effects subsequent to supplemental quercetin intake have rarely been described naturally. Moreover, supplemental isolated quercetin at doses clearly exceeding dietary quercetin intake

will not aggravate gastric injuries, ulcers or GC [7-9]. However, based on several published *in vivo* data, quercetin promotes insulin secretion, and insulin can increase tumor growth. Oral quercetin products have a few potential crucial safety behaviors, and the potential of quercetin to have opposite effects in diabetes, those who have developed nephropathy or estrogen-dependent cancer, and the use of quercetin should be tracked and researched in the future [10-15]. Additionally, I have also provided above description in the "Discussion" section. (line 5-24, page 22)

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