Dear Editors and Reviewers,

The authors of the manuscript appreciate the opportunity to have entered the revision process. Below is a point-by-point reply to the reviewers' comments. The insightful comments and suggestions, and the corresponding amendments we have made to the manuscript, greatly improve the quality of our work. We hope that our amendments meet with your approval, and look forward to any further suggestions which could enhance the quality of the manuscript.

Sincerely,

Sheng-Teng Huang

Reviewer #1:

Specific Comments to Authors:

The manuscript describe herbal medicine may decrease HCC risk in DM patients with insulin use. But Taiwan is not a nation and it is not a nationwide study.

Reply:

We appreciate your comment. Accordingly, we have deleted the term "nationwide" from our manuscript.

Page 8, lines 17-18: This large-scale retrospective cohort study reveals that herbal medicine may decrease HCC risk by 12% in DM patients with regular insulin use.

Page 9, lines 5-7: We aimed to investigate whether regular herbal medicine use can decrease HCC risk in DM patients with regular insulin control in this propensity score-matched, population-based cohort study.

Page 20, lines 4-5: …this is the first large-scale cohort study indicating the efficacy of herbs at decreasing HCC risk by 12% in these patients…

Page 27, lines 4-6: …while the prescription patterns identified in this large-scale cohort study provide clinical guidance and warrant further clinical studies and pharmacological investigation.

Page 27, line 11: This is the first large-scale cohort study revealing that…

Page 31, line 9: This large-scale retrospective cohort study reveals that…

1 Hepatocellular carcinoma (HCC) is the fifth [a1] most common cancer where is the fifth from? There is no citation.

Reply:

We have added a reference and the necessary citation as follows:

Page 31, lines 17-19: 1 Chidambaranathan-Reghupaty S, Fisher PB, Sarkar D. Hepatocellular carcinoma (HCC): Epidemiology, etiology and molecular classification.

Adv Cancer Res 2021; **149**: 1-61 [PMID: 33579421 PMCID: PMC8796122 DOI: 10.1016/bs.acr.2020.10.001]

2 Treatments for DM fail to significantly reduce the risk of HCC occurrence, and insulin increasing HCC risk. But the literature also indicated that in the last decade, increasing evidence has suggested that metformin may reduce the risk of HCC. The evidence was insufficient in the manuscript.

Reply:

Thank you for your comment. We have added text regarding metformin to the Discussion section.

Page 23, lines 1-8: The biguanide metformin is an oral anti-hyperglycemic agent which has been identified as a potential hepatoprotective drug against HCC development [21]. Metformin activates AMPK and inhibits the downstream mTOR pathway, which plays a key role in preventing hepatocarcinogenesis [22]. Indeed, previous studies have reported a metformin treatment group with a 43-76% reduced risk of HCC and reduced overall mortality risk [8, 9]; however, our subgroup analysis showed no difference between biguanides users and non-users (adjusted HR= 1.16; 95% CI=0.84, 1.61; p-value=0.368).

3 The index date for herb-use group and non-exposure was not inconsistent which may induce a significant bias and influence the results of HCC occurrence and survival: The prior was set at the first day of herb use after insulin prescription; The latter was assigned a random date [a2] after insulin prescription for non-exposure patients

Reply:

Thanks for your insight. We have amended the study group as below.

Page 14, lines 7-9: The index date for the exposure and non-exposure groups were matched, with both post-indexed more than 3 months to account for insulin exposure.

4 GLP [a3] -1 is an insulin secretagogue which reduced HCC risk, while insulin increases the risk of HCC. The conclusion of GLP-1 should be cautious.

Reply:

Your note is appreciated. Accordingly, we have modified the GLP-1 text in the Discussion.

Page 23, lines 16-19: However, it should be noted that GLP-1 receptor agonists elevate the insulin level in patients, thereby potentially elevating the risk of HCC. Thus, more high-quality clinical evidence is required to clarify the association between GLP-1 receptor agonist and HCC occurrence.

Reviewer #2:

Specific Comments to Authors: The authors conducted an interesting report. My main concerns are as follows.

1. Since the insulin treatment was considered as a risk factor for HCC incidence. It would be better if the dosages of insulin treatment in DM patients were provided and analyzed.

Reply:

Thank you for the suggestion. We added the insulin use analysis to Table 1.

Page 40-43, Table 1:

	DM patients with regular insulin use				
	Herb non-users		Herb users		
	N=140,547		N=46,849		
Variables	n	%	n	%	SMD
Insulin dose (mg),					
Mean, (SD)	9449.92 (135.59)		8248.88 (115.34)		0.071

2. In the Introduction section, the authors declared that "While these medications generally provide satisfactory control of blood sugar levels, they fail to significantly reduce the risk of HCC occurrence". This conclusion is inadequate. Evidences have been emerged that metformin could decrease hepatocellular carcinoma risk (pmid: 22773548, 26013675).

Reply:

Thank you for your comment. We have amended our manuscript and added text regarding metformin to the Discussion section.

Page 11, lines 12-13: While these medications generally provide adequate control of blood sugar levels, some of them fail to significantly reduce the risk of HCC occurrence.

Page 23, lines 1-8: The biguanide metformin is an oral anti-hyperglycemic agent which has been identified as a potential hepatoprotective drug against HCC development [21]. Metformin activates AMPK and inhibits the downstream mTOR

pathway, which plays a key role in preventing hepatocarcinogenesis [22]. Indeed, previous studies have reported a metformin treatment group with a 43-76% reduced risk of HCC and reduced overall mortality risk [8, 9]; however, our subgroup analysis showed no difference between biguanides users and non-users (adjusted HR= 1.16; 95% CI=0.84, 1.61; p-value=0.368).

3. The references of "studies have demonstrated an elevated risk of HCC incidence in diabetic patients treated with insulin" (line 14-15, page 11) should be added.

Reply:

Thank you for the observation. We have added the reference as below.

Page 32, lines 32-35: 9 Bosetti C, Franchi M, Nicotra F, Asciutto R, Merlino L, La Vecchia C, Corrao G. Insulin and other antidiabetic drugs and hepatocellular carcinoma risk: a nested case-control study based on Italian healthcare utilization databases. *Pharmacoepidemiology & Drug Safety* 2015; **24**(7): 771-778 [PMID: 26013675 DOI: 10.1002/pds.3801]

4. Some baseline variables were not similar between the two groups, please provide the statistic p values in table 1.

Reply:

In this large-scale cohort study, we used standard mean difference (SMD) to identify the differences between the two groups. A significant difference was shown for SMD >0.1. We have further noted the SMD mean in the Results section.

Page 17, lines 4-5: After propensity matching, the baseline variables were similar between the herb users and non-users (SMD < 0.1) (Table 1).

5. In the results of table 2, the authors stated that "After adjustment for the variables noted in Table 1, the adjusted HR of HCC was 0.88 (95%CI=0.80, 0.97) for herb users compared to the control group". All the variables in table 1 were adjusted or the different distributed variables were adjusted?

Reply:

We appreciate your query. We have modified that sentence as below.

Page 17, lines 15-17: After adjustment for the variables noted in Table 1, the adjusted HR of HCC was 0.88 (95%CI=0.80, 0.97; p-value=0.001) for herb users compared to the control group.

6. In the "Chinese herbal medicine decreased HCC risk among DM patients with insulin management" part of the Results section, please provide the p value and the results reference in the text.

Reply:

Thank you for the suggestion. Accordingly, we have provided the p value in Tables 2, 3 and 4 and added it throughout our Results and Discussion sections, and highlighted them in the revised manuscript.

7. In table 3, some bias existed. Patients had longer survival time had more chance to receive herbal treatment.

Reply:

We agree with your insightful comment. We have listed this possibility in our Discussion as a limitation.

Page 26, line 19 to Page 27 lines 1-4: Fourth, due to the nature of retrospective cohort studies, bias may exist in our results. More specifically, it is reasonable to suggest that patients with a longer survival time may have received herbal treatment for a correspondingly longer period, thus warranting a prospective study to confirm our results.

8. The results in this report indicated that HBV and HCV infections as major risk factors of developing HCC. Confusingly, the results also demonstrated that the HBV treatment and HCV treatment were risk factors for HCC incidence.

Reply:

We appreciate your insight, and have amended the manuscript to be clearer in this regard.

Page 21, lines 12-19: In our study, HBV and HCV treatments were adopted in accordance with patient preference, and following NHI payment guidelines. HBV treatments were covered for patients with HBV DNA≧2,000IU/mL with 5-fold elevated liver enzymes or decompensated liver status, while HCV treatments were covered for patients positive for anti-HCV for >6 months with cirrhosis ≧F2 or positive for HCV RNA. We hypothesize that the liver status of patients in the HBV and HCV treatment groups were generally more severe, thus causing the elevated risk of HCC incidence noted in our study.