Dear Dr. Ma,

Thank you very much for your letter and advice. We revised the manuscript and would like to re-submit it for your consideration. We addressed the comments raised by the reviewers, and the revisions are highlighted in red in the revised manuscript. The point-by-point responses to the reviewers' comments are listed below.

We hope that the revised version of the manuscript is currently acceptable for publication in your journal.

We look forward to hearing from you soon.

Best regards,

Yours sincerely,

Wen-Fu Tang

Replies to reviewer #1 (ID: 02566971)

Comment:

"1. 'SJP is able to prevent AP ... ... due to alleviated oxidative stress in organs.' The data is insufficient to support this conclusion proposed by the authors. 2. The manuscript needs to be carefully edited in English, as there are some grammar errors and bad sentences. 3. The authors explain the reason why detect MDA et al. oxidative stress associated factors and analyze the results only in the Discussion. Those should be extended and included in the main body of the manuscript as they will make the manuscript a more complete story."

Answer:

We appreciate the constructive comments made by reviewer #1.

1. We admit that according to the current data, the conclusion that "SJP is able to prevent AP...due to alleviated oxidative stress in organs" is not very accurate. Hence, we revised our manuscript, especially the title and the conclusions in the Abstract and Discussion.

2. We apologize for the grammar errors and poorly written sentences and have made revisions as suggested. Moreover, we sought the assistance of a native English speaker from a language editing company to polish the language usage throughout this manuscript.

3. In the Introduction, we added a comment about oxidative stress in obese AP rats to present a more complete story in this manuscript.

Replies to reviewer #2 (ID: 02510721)

Comment:

"The hypothetical clinical scenario that this study could assess can be difficult to demonstrate. The experimental setting is very different from usual clinical data and consequently it's very difficult to draw therapeutic conclusions even in hypothesis. The sentence 'the obesity can worsen the evolution of AP' is true but is generic and imprecise, because there are various clinical evolutions of AP in obese patients and these cannot be overlappable about the severity. Moreover the causes of AP are numerous and based on different pathological mechanisms. Overall the severe forms of AP with SIRS and MOF can develop in few cases. The Sheng-jiang powder should ameliorate the inflammatory response in obese subjects (the complete demonstration needs), which can suffer a serious evolution of AP, but I do not see the action of SJP on prevention of AP. In conclusion the study adds a valuable contribution to understand the biological effects of SJP, that now can be evaluated as generic; in this moment can't be hypothesized the specific therapeutic use of SJP. I suggest to insert in the Introduction and in the Discussion more clear definition and presentation of the aim and content related to actual clinical perspective in the therapeutic applications of SJP. In particular it's need to eliminate in the Title the words 'SJP prevents acute pancreatitis from causing multiple-organ……'"

Answer:

We appreciate the constructive comments made by reviewer #2.

We agree with the reviewer's opinion that the experimental setting is very different from the usual clinical setting, and currently, the therapeutic efficacy of SJP is difficult to prove. However, SJP has shown some positive effects in the high-fat-diet-induced obese rats with AP; thus, according to the reviewer's suggestion, we clarified the aim in the Introduction and Discussion. In addition, we changed the title.

Replies to reviewer #3 (ID: 01714826)

Comment:

"1. The pathogenesis is far from clear. This seems to be the result of understanding of the exact composition of the powder. 2. Additionally, the mechanism of the observed decreased in SOD and MOD is not well studied. 3. For those who are unfamiliar with this powder (its chemical composition) information such as active ingredients is essential. In that case the blood levels and the pharmacodynamics can also be studied making it more scientific. Further, the rate of absorption of the active ingredients and other details are needed."

Answer:

We appreciate the constructive comments made by reviewer #3.

1. We agree with the reviewer that the pathogenesis of the increased severity of obesity-related AP is unclear. In the Introduction of our previously submitted draft, we mentioned that the most common mechanisms were visceral fat-induced acute lipotoxicity, the inflammatory response, and oxidative stress and that this experiment was designed to capture these three main mechanisms, but the underlying mechanism needs further study.

2. We admit that in this manuscript, we only reported the decreases in SOD and MOD, but in another study, which is still under investigation (the data have not been published), we found that the changes in oxidative stress in obese AP rats might be related to the TNF- α -ROS-RIP3 pathway.

3. We have already measured the active ingredients of the Sheng-jiang decoction in rats with acute pancreatitis[1], and these comments were addressed in the Discussion of the re-submitted draft. In addition, the determination of the active components in obesity-induced pancreatic inflammatory injury is underway.

[1] Zhu L, Li JY, Zhang YM, Kang HX, Chen H, Su H, Li J, Tang WF. Pharmacokinetics and pharmacodynamics of Shengjiang decoction in rats with acute pancreatitis for

protecting against multiple organ injury. World J Gastroenterol 2017; 23: 8169-8181

Replies to reviewer #4 (ID: 02954661)

Comment:

"**Major concerns:** 1. The sheng-jiang powder is not known to western readers. Please introduce in Discussion a table with high-quality studies proving its effects in human diseases. 2. Please add in Abstract P values. 3. The tile and the conclusion in abstract are too strong to come from only 8 rats in each group. 4. In abstract: the conclusion does not come from results. Why there are three groups? For me is not clear if we compare the powder effects in obese rats, or the effects of obesity in AP. Please be clearer in abstract and introduction. 5. Please detail in Methods the usefulness of Lee s index? What is the reason to calculate this index? 6. The authors used too strong words for effects of traditional chines medicine, for example in Discussion, citations 42 - 44, 45, 46. However the quality of the studies is very low. Please discuss as a limitation of the study the Cochrane systematic reviews about the Chinese medicine. Although well-respected, the evidence is quite inconclusive.

Minor concerns: Too many abbreviations and is difficult to follow. Not all of them explained at their first appearance in the text. MDA is not clear for me what means. M for control group, ML for obese group in MS for SJP group are not very intuitive. I suggest to modify them (CG -control group, OG - obese group, PG - powder group for example)."

Answer:

We appreciate the constructive comments made by reviewer #4.

Major concerns:

1. We added a table listing high-quality studies introducing the effects of Sheng-jiang powder in human diseases in the Supplementary material and cited the table in the Discussion.

2. Because of the limitation of the number of words allowed in the Abstract (less than 350 characters) according to *World Journal of Gastroenterology*, we did

not include the P-value in the Abstract, but each P-value is presented in the Results and figures and tables.

3. We changed the title to "Effect of Sheng-jiang powder on multiple-organ inflammatory injury in acute pancreatitis in rats fed a high-fat diet"; currently. the conclusion is as follows: "SJP may alleviate multiple-organ inflammatory injury in AP in rats fed a high-fat diet".

4. This study had the following two objectives: to explore how obesity may contribute to aggravating inflammatory organ injury in AP in rats and observe the effect of SJP on multiple-organ inflammatory injury in AP in rats fed a high-fat diet. In the Abstract of our previously submitted draft, we omitted some content, which led to confusion. Thus, we clarified the aim and conclusion in the Abstract and Introduction.

5. We added details to the Methods regarding the usefulness of Lee's index and explained why we calculated this index.

6. We removed some low-quality citations and discussed the limitation of studies using Cochrane systematic reviews to investigate SJP.

Minor concerns:

According to the reviewer's suggestions, we checked the abbreviations and ensured that all abbreviations were explained at their first appearance in the manuscript. In addition, we modified the abbreviations of the three groups (CG -control group, OG - obese group, and SG – Sheng-jiang powder treatment group).

Replies to reviewer #5 (ID: 01714826)

Comment:

"**Obesity model**

- Animals. When Sprague-Dawley rats are placed on a high-energy diet, only subsets of them overeat and develop diet-induced obesity (DIO) whereas others remain lean (diet resistant) (1). **Did the authors have to face this problem? If so, they should include in the manuscript a comment about it.**

According to the initial rat weight (although weight is not an accurate surrogate marker for age), the animals were juvenile when they started the 10-wk feeding period. There is controversy on this point. Young animals have different metabolism that results in greater gain in lean mass, so some authors advise that older animals are submitted to DIO (2). However, there have been studies in which young animals fed a high-calorie diet for a long period of time increased body weight in comparison with the control group (3). It has even been reported that it is most effective to start high fat diet feeding at a young age (4).

- Diets. The choice of experimental diets has been the best possible. While some researchers add the fat to a chow (this leading to an unbalanced diet composition and dilution of other nutrients like protein, vitamins, etc), the authors of this manuscript have used purified ingredient diets. In purified diets, each nutrient is represented by one ingredient and can therefore be varied individually while all other ingredients remain unchanged. Further, this approach avoids some problems of the chows, such as the presence of interfering compounds.

As a control, authors have used have used a diet that is basically the AIN93G one (**this should be mentioned in the manuscript**), very well-known and referenced (5), and designed for growing rodents. A high-fat (HF) formula with a closely matched composition has been chosen to induce obesity. Calories from fat in the HF diet (around 33%) and type of fat added (lard) are appropriate to induce obesity in this model (6). Importantly, care has been

taken that both diets have a similar nutrient to calorie ratio, which is important, given that animals eat for calories, not weight of food.

Experimental design

I am not sure that the experimental design allows the second objective of the work to be achieved. This objective was "to evaluate the preventive effect of SJP in improving multiple-organ injuries of AP outcomes in rats with obesity".

The authors did not give SJP treatment to obese rats, but administered SJP at the same time as obesity was induced. In fact, one of the surprising results obtained (and that would deserve to be explored by itself) is that the SJP seems to largely avoid the development of obesity. That is, those animals that receive SJP at the same time as a high-fat diet do not develop obesity, or develop it to a lesser degree (I am not sure if body weight and Lee obesity index in group MS are statistically similar to values in group M, see below). Therefore, the question is, is it possible that the improvement in AP outcomes after SJP is simply due to the fact that the MS rats are less obese?

Because of this, the writing of the work (including the second conclusion) should be reformulated. The authors make continuous reference throughout the manuscript to the fact that the administration of SJP to obese rats achieved this or that, but, I insist on the fact that SJP was not administered to rats in which obesity had previously been induced. It would have been different if obesity had first been induced in two groups of rats and from here on, one group was treated with saline and another with SJP for a certain time. With this type of design (more realistic, on the other hand, in terms of medical applicability), one could possibly say that the SJP alleviates the harmful effects of obesity in AP, but not with the current design. In addition to reformulating the writing, I think it would be desirable to include some comment in this sense when the authors mention the limitations of the study (a study that, on the other hand, I find very interesting).

Results

In my opinion, the existence (or not) of significant differences between the three study groups should be better expressed. The authors only show the differences between group ML vs. M, and, on the other hand, MS versus ML. However, the information provided would be much more complete if the results of the comparison between MS and M were also indicated in the tables and graphs. In other words, we see that in some cases, obesity aggravates different parameters of pancreatitis in comparison with the lean rats. We also see how SJP treatment improves the values of these parameters, getting close to those obtained in lean rats, but we do not know if indeed those values are statistically similar to those of lean rats or if they remain statistically different. It would be preferable if the results were expressed through another system, for example, by using letters in columns (table) or bars (bar chart) in such a way that the existence of common letters indicates the absence of significant differences.

References

(1) Farley C et al. Obes Res 11: 845-851, 2003.

(2) Tschöp M and Heiman ML. Exp Clin Endocrinol Diabetes 109:307-319, 2001.

(3) Nascimento AF et al. Arq Bras Endocrinol Metabol 52:968-974, 2008.

(4) Reuter TY. Drug Discovery Today: Disease Models 4: 3-8, 2007.

(5) Reeves PG et al. J Nutr 123: 1939-1951, 1993.

(6) Wang H et al. Am J Physiol Endocrinol Metab 282: E1352-E1359, 2002. ”

Answer:

We appreciate the constructive comments made by reviewer #5.

Obesity model

After carefully reading all references proposed by reviewer #5, we have a better understanding of the diet-induced obesity rat model and AIN93G

growth diet. The paper about the AIN93G diet provided support for the control diet choice. We certainly cited these references as needed. Actually, we did not experience a problem with diet resistance in our study. After 12 weeks of the high-fat diet, the 8 rats in ML all became obviously obese. We appreciate the generalized information provided by reviewer #5 and will consider this possibility in our future experimental designs. In this study, we started the 12-week feeding period when the rats were 4-5 weeks of age.

Experimental design

We admit that using our experimental design, achieving the aim "to evaluate the preventive effect of SJP in improving multiple-organ injuries of AP outcomes in rats with obesity" is difficult. Hence, we revised our manuscript. According to the results, we provided the more reasonable conclusion that SJP has some positive effects on multiple-organ inflammatory injury in AP in rats fed a high-fat diet. In addition, we added some comments regarding the limitations of our study.

Results

Reviewer #5 suggested that the comparisons between the control group (M) and the Sheng-jiang powder treatment group (MS) should be indicated in the tables and graphs. However, there are two factors (control diet/high-fat diet and normal saline/SJP) between M and MS; thus, we think that this suggestion may not be proper. Hence, we did not change any statistical methods in our manuscript.

Although we think comparisons between M and MS may not be proper, we used Student's t-test to compare the difference in body weight and Lee's index. The body weights in MS were significantly higher than those in M ($P= 0.0255$), but Lee's index showed no significant difference between the two groups ($P= 0.5894$). These findings are very interesting and require further investigation.