Dear Editor,

Title: Effects of ω-3 Fatty Acids on Toll-Like Receptor 4 and Nuclear Factor-kBp56 of lungs in Rats with Severe Acute Pancreatitis

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1. Having induced AP in rats, I am surprised that the authors confined their evaluation to the effects of ω-3FA on the lungs only and not on the pancreas and other end organs also.

Because Acute lung injury (ALI) is one of the most common complications in SAP(severe acute pancreatitis) and often occurs at the early stage of the disease, and may progress into adult respiratory distress syndrome (ARDS). ARDS is a primary cause of patient death during the early stages of SAP. The effects of ω-3FA on the pancreas and other end organs will be studied in the follow-up rearch.

2. The study was on "severe " AP. How did the authors ensure that that all the study animals had severe AP?

In this study, the amylase(AMY) levels in serum was measured, and after 5% sodium taurocholate in distilled water (1 ml/kg body weight) was injected into the bilio-pancreatic duct, we could see obvious hemorrhage and necrosis of pancreas.The picture below is the model of SAP in rat of our study, meanwhile ,AP complicated with other organs injury such as ALI,we could range it to be SAP. 

3. The material and methods section needs thorough revision; it is lengthy but a specific details are left vague (a few detailed below) and not a single reference is provided for the methods used.

Related reference has been added in revised manuscript

4. How long after the induction of AP was the drug intervention done?

   After SAP model induction, intravenous injection of intervention drug was done immediately .

5. How was the lung tissue processed? Were all analyses done on formalin fixed smears? All samples were tested in duplicate and averaged. *How was ELISA done on solid tissue for TNF-a and IL-6*

   The entire lungs was removed, and a sample was frozen immediately at -80℃ for biochemical analysis. The others was fixed in 10% formalin in anatomic orientation for histological analysis.

   For ELISA, the lungs were homogenized in 5 volumes of buffer composed of 10 mM HEPES, 10 mM KCl, 0.5 M sucrose, 1 mM EGTA, 1 mM DTT. Homogenates were then centrifuged at 750 xg for 10 minutes to isolate the nuclei. The supernatants which contained the cytosolic fraction were stored at –70 degrees C and used for MIP-2 protein determination.

6. What scale was used for grading the histopathological changes in the lung? Was it based on a subjective impression? What is the validity of this method? Any reference?

Sections of lungs were cut, fixed in 10% formalin, and HE-stained to obtain lung histological score by using a standardized scoring system. Reference: Lichtenstein A, Milani RJ, Fernezlian SM, Leme AS, Capelozzi VL,Martins MA. Acute lung injury in two experimental models of acutepancreatitis: infusion of saline or sodium taurocholate into the pancreatic duct. Crit Care Med 2000;28:1497e502.

7. Lung changes may be patchy in this situation. What was done to take care of this?

Pulmonary alterations were scored using a grading system developed by Lichtenstein et al. The grading involved measurements of inflammatory infiltration, pulmonary edema and alveolar collapse, each on a scale of 0-3. After fixation, 5-mm sections of lung tissue samples were stained with haematoxylin/eosin, and subsequently examined by an experienced morphologist who was not aware of their identity. For the morphological examination, 10 microscopic fields (6100) were randomly chosen for each tissue sample, and the extent of acinar cell injury or necrosis in each sample was expressed as a percent of the total lung tissue.

8. Was the person scoring the histology and IHC blinded to the group the animals belonged to?

Yes, experienced morphologist who was not aware of the animals' identity.

9. Results: " TNF-α and IL-6 levels of lungs in the SAP-ω-3FA group could be seen as higher than that in the SAP- soybean oil group at each time point *(P <0.05)"* The corresponding figures in table 2 shows the results to be the other way round.

   Sorry, it should be "lower", not "higher"

10. Different terms are used in the different sections of the report to refer to the same histological change. This is confusing. Uniform terminology will help .

    It has been revised in the revised manuscript

11. Table 2 can be simplified to read better by placing the most important results compared side by side. It is likely to read better if the columns and rows are interchanged. Column 3 is redundant. The foot notes on the groups compared is especially confusing.

    It has been revised in the revised manuscript

12. Table 3 can be similarly modified. The foot note suggests that a group has been compared to itself to get a statically significant difference!!! E. g. ("versus SAP-ω-3FA 12h group, g P <0.05, h P <0.05, i P <0.05, j P <0.05;versus SAP-ω-3FA 24h group, gg P <0.05, hhP <0.05, ii P <0.05, jjP <0.05.")

    It has been revised in the revised manuscript

13. "Several studies have confirmed that the expression and activation of TLR4 and NF-κBp56 were upregulated, and a large amount of inflammatory cytokines were detected in the SAP rat model induced through various ways [19]." The authors mention several studies, but only 1 quoted.

    Related reference has been added in the revised manuscript

14. The paper needs attention to language and punctuations. 1 e. g.: "The injuries would further induce SIRS or even MODS[7,8] .it was found that TLR4 plays"

    English language editing services that specialize in scientific and medical manuscript were used for grammatical clarity and appropriate vocabulary