

Reviewer #1: Since 2013, nab-paclitaxel in combination with gemcitabine has been approved in many countries as a first-line treatment for patients with metastatic adenocarcinoma of the pancreas. The safety and efficacy of the combination of nab-paclitaxel followed by gemcitabine therapy has been established. Please described the advantage of Orthosiphon stamineum over nab-paclitaxel in discussion section.

In 2013, the MPACT phase III trial, which included over 800 patients, showed a significant overall survival benefit with the combination of nanomolecular albumin-bound (nab)-paclitaxel and gemcitabine (nabPGem) over gemcitabine monotherapy, with acceptable toxicity. The overall toxicity of nabPGem is lower than in the triple chemotherapy 5-FU, leucovorin, irinotecan and oxaliplatin, especially haematotoxicity and rates of neutropenic fever (1,2). However, the current study and our previous toxicity study revealed no reduction in white blood cells and platelets (Table 4) (3). In fact, the combination of C5EOSEW5050ESA and gemcitabine at the higher tested doses enhanced its efficacy in reducing tumour growth without causing any side effects, at least in this short term study.

- 1- Vogl, Ursula M., et al. "Nab-paclitaxel and gemcitabine or FOLFIRINOX as first-line treatment in patients with unresectable adenocarcinoma of the pancreas: does sequence matter?." *BMC cancer* 19.1 (2019): 1-8.
- 2- Von Hoff, Daniel D., et al. "Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine." *New England Journal of Medicine* 369.18 (2013): 1691-1703.
- 3- Yehya, Ashwaq HS, et al. "Toxicological studies of Orthosiphon stamineus (Misai Kucing) standardized ethanol extract in combination with gemcitabine in athymic nude mice model." *Journal of advanced research* 15 (2019): 59-68.

In previous reports, Orthosiphon stamineum monotherapy was effective against HCT116 xenograft tumors (Reference #9, J Biochem Tech (2012) 3(5): S170-176). However, in this report using Panc-1 xenograft models, no anticancer effect was observed with Orthosiphon stamineum monotherapy. The authors should investigate why Orthosiphon stamineum monotherapy was not effective against Panc-1 xenograft tumors.

In our study, we have used two doses of *Orthosiphon stamineus* 200mg/kg and 400mg/kg monotherapy. The monotherapy of *Orthosiphon stamineus* (200mg/kg and 400mg/kg) significantly inhibited the tumour growth 25% and 55% compared to negative control, highlighting anti-cancer activities. The monotherapy of *Orthosiphon stamineus* 400mg/kg reduced the tumour growth more than the combination treatment of gemcitabine and low dose of O.s. In our previous study, Al-Suede et al (2014), we have used orthotopic colon cancer model where the *Orthosiphon stamineus* treatment was more effective compared to our ectopic pancreatic cancer model. We would like to highlight that the difference in test models and cancer type may likely influence the degree of anti-cancer activities and cannot be directly compared, as such.

Discussion is too long with a lot of textbook knowledge and can be made shorter.

Please refer to the amended discussion.

Reviewer #2: Is there any reason why the route of administration of gemcitabine was subcutaneous injection (IP) instead of intravenous injection (IV)?

Although IV injection is another commonly used route for gemcitabine administration, IP injection has also been used by other researchers as the route of administration of gemcitabine in nude mice (1, 2, and 3). The important outcome for this proof-of-concept study is the observed treatment combination effect, although a pharmacokinetic study is key to determine optimal gemcitabine and plant extract absorption in the future.

1. Selvanesan, B. C., Meena, K., Beck, A., Meheus, L., Lara, O., Rooman, I., & Gravekamp, C. (2020). Nicotinamide combined with gemcitabine is an immunomodulatory therapy that restrains pancreatic cancer in mice. *Journal for immunotherapy of cancer*, 8(2).
2. Sugisawa, N., Miyake, K., Higuchi, T., Oshiro, H., Zhang, Z., Park, J. H., ... & Hoffman, R. M. (2019). Induction of metastasis by low-dose gemcitabine in a pancreatic cancer orthotopic mouse model: an opposite effect of chemotherapy. *Anticancer research*, 39(10), 5339-5344.
3. Chen, L., Zhou, D. A., Liu, Z., Huang, X., Liu, Q., Kang, Y., ... & Sun, C. (2018). Combination of gemcitabine and erlotinib inhibits recurrent pancreatic cancer growth in mice via the JAK-STAT pathway. *Oncology reports*, 39(3), 1081-1089.