

Dear reviewers,

Thanks for reviewing this manuscript and providing those great suggestions. We've revised our manuscript, which were highlighted in red in the updated version. Here are our responses to your comments:

Reviewer #1: This is a very interesting and well-written study. The authors proved the BJOE has anti-cancer effect on esophageal cancer, and can be used as a radiosensitizer to promote the apoptosis in esophageal cancerous cells. These findings may provide a novel way for the therapy of esophageal cancer. The structure of this research is reasonable, and the result well highlighted and clear. Overall, this manuscript can be accepted after some minor language revision.

**Response:** Thank you for your suggestion! We have checked and revised the manuscript, sentence by sentence.

Reviewer #2: The brucea javanica (Title, Abstract, Text) - you should write Brucea javanica (with capitalized generic name). Over 450, 000 people suffered with esophageal cancer all around the world, and its incidence keep increasing (Abstract) - this sentence is excessive in Abstract. To be removed. BJOE (brucea javanica oil emulsion) is a widely used drug, which is against various cancers (Abstract) - "Brucea javanica oil emulsion (BJOE) is a widely used drug against various cancers" is better. potential using as BJOE a radiosensitizer (Abstract) - "using BJOE as radiosensitizer" is better. BJOE (brucea javanica oil emulsion) is a widely used drug, which is against various cancers (Core Tip) - "Brucea javanica oil emulsion (BJOE) is a widely used drug against various cancers" is better. One of the most severe areas is China (Introduction) - is not a good-looking phrase. To be corrected. major type of esophageal cancer is ESCC (esophageal squamous cell carcinoma) - "esophageal squamous cell carcinoma (ESCC)..." is better. And Inhibition of CDK4/6 is resistant - wrong capitalization of the second word. "And Inhibition of CDK4/6 is resistant...", "And the expression of p21 is positively related..." - it is not good idea, to begin the sentence with a word "and". "BJOE (brucea javanica oil emulsion) is the fatty oil that extracted" (Introduction) - see above. Full term first, abbreviation later. "in various cancers, such as liver cancer, colon cancer and ovarian cancer" - "in various malignancies, such as liver, colon and ovarian cancers" is better and shorter. cell lines and cell culture (Material and Methods) - please capitalize the first letter. "cell lines and cell culture", "Cell viability assay" and so on - subheadings should be in BOLD!!! 90%DMEM-H - space error 37 C - wrong symbol "Different amount of brucea javanica oil emulsion" - please use abbreviation here. shenyang, China - capitalization is needed. "Afer 24 h, the cells that grew" - maybe after??? "Datas were analysed" - data is right. The killing effect of BJOE on esophageal carcinoma cells (Results) - here and below - subheadings should be in BOLD!!! "BJOE had same poisoning effect" - maybe some??? "Radiotherapy is one of the frequent methods to treat" - here, a word "frequent" is bad. To be corrected. "BJOE is promising in the treat of esophageal cancer" (Discussion) - "in the treatment" is better. References should be constructed with strict accordance!!! to Instructions for Authors. I have found errors in Refs. 2, 9, 21, 27, 29, 31, 33. Cologenic assays (Fig 3 Legend) - maybe clonogenic??? All the Above dates are presented (Fig 4 Legend) - Above will be decapitalized.

**Response:** Thank you for your reminding! The issues above have been revised according to your advice.

Reviewer #3: This is very interesting paper. Author concluded that BJOE has strong anti-cancer effect on esophageal cancer and can be used as a radiosensitizer to promote the apoptosis in esophageal cancerous cells via cyclin D1-CDK4/6 axis. Brucea javanica oil emulsion (BJOE) has been used clinically to treat esophageal cancer combined with radiotherapy for numerous years in China. However, the detailed mechanism remains unclear. Tumor hypoxia is a well-recognized characteristic associated with resistance to radiotherapy and chemotherapy. Tumor cells adapt to hypoxic microenvironments by activating associated signaling pathways. HIF-1 $\alpha$  plays a crucial role in tumor growth and metastasis. BJOE may increase the radiosensitivity of ESCC cells by suppressing the activation of HIF-1 $\alpha$ . (Brucea javanica seed oil enhances the radiosensitivity of esophageal cancer by inhibiting hypoxia-inducible factor 1 $\alpha$ , in vitro and in vivo Oncol Lett. 2018 Mar; 15(3): 3870–3875.) Please comment the mechanism which BJOE is good effect for radiotherapy of esophageal cancer from the point of HIF-1 $\alpha$  in discussion.

**Response:** The study you mentioned is very interesting. BJOE could inhibit the growth of ESCC via suppressing the expression of HIF-1 $\alpha$ . The discussion of HIF-1 $\alpha$  has been added into our manuscript. Thank you for your suggestion!

Reviewer #4: 1- this medication was tried before in other cancers like gastric cancer and malignant pleural effusion, what is the new in your work 2-some grammar mistakes and sentence style are highlighted with red in the uploaded file.

**Response 1:** Thank you for your suggestion! Considering various mechanisms in different types of cancers, we believe there is still a requirement for concrete evidence to evaluate BJOE's therapeutic effect on esophageal cancer. Our results showed that BJOE significantly inhibit the growth and the ability of metastasis and invasion of esophageal cancerous cells. Besides, our study revealed a new molecular pathway of BJOE against esophageal cancer. It was shown that BJOE inhibit esophageal cancerous cell via Cyclin D1/CDK4/6 axis. To make the highlights clearer, we have revised the manuscript. Again, thanks for your advice.

**Response 2:** Thank you for your suggestion! The grammar mistakes and sentence style have been revised in the updated version according to your mentions.