SHORT COMMUNICATION

Cytotoxic constituents of *Lasiosphaera fenzlii* on different cell lines and the synergistic effects with paclitaxel

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**ABSTRACT**

The fruit body of *Lasiosphaera fenzlii* was found to show cytotoxicity on cancer cells during a preliminary screening. Repeated column chromatography of the fungal methanol extract resulted in the isolation of six compounds identified as $5\alpha,8\alpha$-epidioxy-ergosta-6,22-dien-3$\beta$-ol (1), $5\alpha,8\alpha$-epidioxy-ergosta-6,9(11),22-trien-3$\beta$-ol (2), $5\alpha$-ergosta-7,22-dien-3$\beta$-ol (3), $5\alpha$-ergosta-7,22-dien-3-one (4), ergosta-7,22-dien-3$\beta$,5$\alpha$,6$\beta$-triol (5) and 6-dihydroxy-2,3-dihydro-1H-isindol-1-one (6). The two peroxide compounds, 1 and 2, showed cytotoxic activity and compound 1 was selectively cytotoxic to cancer cells. Furthermore, compound 1 synergised the cytotoxicity of paclitaxel on Hela cells by increasing intracellular accumulation of paclitaxel in cancer cells but not in normal cells.

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**1. Introduction**

*Lasiosphaera fenzlii* Reich (Lycoperdaceae), also known as puffball, is a medicinal fungus recorded in Chinese pharmacopoeia for haemostatic and other disorders (Chinese Pharmacopoeia Commission 2010). Isoindolones (Lue et al. 2013) and sterols (Takaishi et al. 1992; Wang & Sun 2007) have been reported from *Lasiosphaera* genus. In a preliminary screening, the extract of this fungus showed cytotoxicity on cancer cells. The present research was...
carried out to isolate, purify and identify the bioactive constituents and to investigate the synergistic effect of the active compound with clinically used anti-cancer agent, paclitaxel (PTX).

2. Results and discussion

2.1. Compounds isolated and identified from *L. fenzlii*

Six compounds were isolated from a methanol extract of the fruit body of *L. fenzlii*. By analysis of their NMR and MS spectra and by comparing the data with those reported, these compounds were identified as: 5α,8α-epidioxy-ergosta-6,22-dien-3β-ol (ergosterol peroxide, EP) (1) (Wang & Sun 2007), 5α,8α-epidioxy-ergosta-6,9(11),22-trien-3β-ol (9(11)-DHEP) (2), 5α-ergosta-7,22-dien-3β-ol (3) (Wang & Sun 2007), 5α-ergosta-7,22-dien-3-one (4) (Wang & Sun 2007), ergosta-7,22-dien-3β,5α,6β-triol (5) (Piccialli & Sica 1987) and 4,6-dihydroxy-2,3-dihydro-1H-isoindol-1-one (6) (Lue et al. 2013). Compounds 1 and 2 are peroxide compounds with two signals between δ 78 and δ 83 in their 13C NMR spectra for C-5 and C-8, the carbons connected with the peroxide bond. Compound 2 is herein reported from genus *Lasiosphaera* for the first time (Figure S1 in supplemental material).

2.2. The cytotoxicity of compounds 1–6 on cancer cell lines

We selected three kinds of human cancer cell lines and a human normal somatic cell line to assess the cytotoxicity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay according to the reported method (Marrelli et al. 2013) with some modifications and the details are described in supplemental material. As shown in Table S1 in supplemental material, the two sterol compounds with a peroxide group in their structures, EP (1) and 9(11)-DHEP (2), showed cytotoxicity on cancer cell lines, while the other three sterol compounds were not cytotoxic, suggesting that the peroxide group is a functional group for cytotoxicity. The 9(11)-DHEP was more cytotoxic than EP on all of the cancer cells and normal cells tested, while EP selectively killed cancer cells (IC50: 13.6–17.2 μg/mL) with lower toxic to normal somatic cells at a concentration as high as 50 μg/mL (Figure S2).

2.3. Synergistic effect of EP with PTX on the cytotoxicity against Hela cells

Combination of PTX and EP resulted in stronger inhibition on Hela cells growth than did PTX or EP alone (Figure 1). Single paclitaxel (0.025 μg/mL) or EP (6.25 μg/mL) exhibited marginal cytotoxicity on Hela cells (11.7 and 12.9% inhibition, respectively). Combination of PTX and EP at the same concentration enhanced the inhibition rate to 41.9%. Combined PTX (0.05 μg/mL) and EP (12.5 μg/mL) could inhibit 80% of Hela cell growth, an activity that needed 0.5 μg/mL of PTX alone, i.e. to achieve the same inhibitory efficacy, the combination strategy could reduce PTX dose to 1/10 of that when PTX was used alone.

2.4. EP increased the intracellular accumulation of PTX on Hela cells

As shown in Figure 2, the cellular PTX concentration was remarkably higher in Hela cells after co-treated with EP for 2.5 h (p < 0.01). However, PTX content in GES-1 cells was not obviously changed after combined with EP. The result suggested that combination with EP could remarkably reduce PTX dosage to achieve satisfied therapeutic effect.
3. Conclusion

Five phytosterols and one isoindolone compound were isolated from *L. fenzlii*. Among these compounds, EP showed selective cytotoxicity on cancer cells. EP could significantly enhance the PTX-mediated cytotoxicity through increasing intracellular PTX content in cancer cells but not in normal cells. The broad-spectrum anti-cancer agent, PTX, is a natural product produced from yew bark, and recently from cell culture through efficient isolation and purification method (Liang et al. 2015). However, the side effects and drug resistance are the
big challenges for clinical utilisation of PTX. The significant synergistic effect of EP and PTX rendered possibility to use this combination as a new effective strategy to reduce the side effect and drug resistance of PTX.

**Supplemental data and research materials**

Experimental details relating to this paper are available online.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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