



<b>Title</b>	<b>Investigation of Phellinus linteus mushroom extract on cell cycle of colon cancer cell LS 174T under oxidative stress challenge</b>
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## **Investigation of *Phellinus linteus* mushroom extract on cell cycle of colon cancer cell LS 174T under oxidative stress challenge**

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*Phellinus linteus* (PL) has traditionally been used as a medicinal mushroom in some countries such as China, Japan and Korea. It was found the fruiting body of PL is rich in bioactive phytochemicals. The antioxidant property of PL has been associated in preventing cancer progression. It is also suggested that the polysaccharides component of PL is responsible for antitumor and immuno-modulatory effect *in vivo* and *in vitro*. However, the mechanism of the inhibition effect and invasive behavior of PL on cancer such as LS 174T colonic carcinoma is not well elucidated. PL is suggested to be able to inhibit cancer cell adhesion and invasion of the extracellular matrix. Therefore, it is believed that PL can exert antitumor effect by initiating apoptosis and cell cycle blockade in cancerous cells.

Colon cancer is an emerging health problem in Asia due to change in dietary habits. In this study, the effect of PL on cell cycle and its association with antioxidant activity of LS 174T cells were investigated. Two different doses (50 µg/ml and 300 µg/ml) of PL extract that has high antioxidant activity (unit of 3 TEAC) were added to LS 174T cells with or without 200 µM H<sub>2</sub>O<sub>2</sub> challenge. Catalase activity, cell viability and cell cycle of the cells were determined at stationary phase of cell growth.

It was found that treatment of PL alone showed decreased ( $p < 0.01$ ) catalase activity when compared to control. Oxidative stress challenge (200 µM H<sub>2</sub>O<sub>2</sub>) increased catalase activity ( $p < 0.01$ ) compared to control. However, addition of PL reduced the catalase activity ( $p < 0.01$ ) but not to control level. This effect was not dose-dependent in which both PL doses showed similar catalase activity.

Cells treated with PL alone or 50 µg/ml PL + 200 µM H<sub>2</sub>O<sub>2</sub> or 300 µg/ml PL + 200 µM H<sub>2</sub>O<sub>2</sub> showed comparable cell viability as control and higher ( $p < 0.01$ ) cell viability than 200 µM H<sub>2</sub>O<sub>2</sub> treated cells. However, the difference was not significant between two PL doses.

Treatment of 50 µg/ml and 300 µg/ml PL showed increased ( $p < 0.05$ ) cell apoptosis when compared to control. However, the difference was not significant between the two PL doses. It was noted G2/M phase elevated ( $p < 0.05$ ) when the cells were treated with 300 µg/ml PL only.

Cells challenged with 200 µM H<sub>2</sub>O<sub>2</sub> increased apoptosis compared to control. However addition 50 µg/ml or 300 µg/ml PL showed reduced ( $p < 0.05$ ) cell apoptosis but not to control level. Also, a decrease ( $p < 0.05$ ) in G2/M phase for cells treated with 300 µg/ml PL + 200 µM H<sub>2</sub>O<sub>2</sub> was recorded compared to 200 µM H<sub>2</sub>O<sub>2</sub> treated cells. Our findings indicate PL alone could induce cell apoptosis. It is plausible higher dose of PL can effectively reduce cell apoptosis and cell cycle arrest at G2/M phase by its antioxidant effect. Thus, PL interacts with LS 174T cells effectively under oxidative stress to maintain homeostasis: a possible mechanism of hormesis.