A cold methanolic extract of *Ganoderma lucidum* (Curtis) P. Karst induces autophagy in a gastric cancer cell line

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Beneficial effects have been attributed to *Ganoderma lucidum* including antioxidant, antitumor and antibacterial properties [1-3]. Previous work from our group has identified a methanolic extract (prepared at 25 °C) of this mushroom as being a potent modulator of autophagy in a gastric cancer cell line (AGS) [4]. In the present work, the antitumor potential and autophagy modulatory activity of a methanolic extract of *G. lucidum*, obtained under cold extraction (-20°C), was further investigated. The chemical characterization of the extract was previously published by some of the present authors [1]. Tumour cell growth screening was carried out in four human tumor cell lines: AGS (gastric adenocarcinoma), MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and HCT-15 (colorectal adenocarcinoma). The effect of the extract was further studied in the most sensitive cells (AGS), particularly regarding effect in autophagy. The presence of autophagosomes was observed following transfection of cells with a mCherry-LC3 expression vector and the levels of some autophagic proteins was analysed by Western Blot. To confirm if the increase in LC3-II levels was a result from autophagy induction or from a decrease in autophagic flux, cells were treated with the extract and with lysosomal protease inhibitors (E-64d/pepstatin, to prevent formation of the autophagolysosome), and the expression levels of autophagic proteins was analysed. We verified that this extract provided a GI50 of 66.59 μg/mL in the AGS cell line. In addition, treatment with this extract (GI50 concentration) caused an increase in the expression of LC3-II. Additionally, the extract increased the formation of autophagosomes and when cells were treated with the extract together with E-64d/pepstatin a further increase in the LC3-II levels was verified, indicating that the extract caused an induction of autophagy.

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References: