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MO₃₄₆NEPHROPROTECTIVE EFFECT OF A METHANOLIC EXTRACT OF TWO GANODERMA SPECIES AND THEIR ASSOCIATION IN AN IN VITRO MODEL OF CISPLATIN INDUCED TUBULOTOXICITY

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NEPHROPROTECTIVE EFFECT OF A METHANOLIC EXTRACT OF TWO *GANODERMA* SPECIES AND THEIR ASSOCIATION IN AN *IN VITRO* MODEL OF CISPLATIN INDUCED TUBULOTOXICITYSébastien Sinaeve¹, Cécile Husson², Marie-Hélène Antoine², Stéphane Welti³, Cony Decock⁴, Caroline Stevigny¹, Joelle Nortier²¹Université libre de Bruxelles-Faculty of Pharmacy, RD3-Pharmacognosy, Bioanalysis and Drug Discovery Unit, Brussels, Belgium, ²Université libre de Bruxelles-Faculty of Medicine, Laboratory of Experimental Nephrology, Brussels, Belgium, ³Université Lille 2- UFR Pharmacie, Laboratoire des Sciences Végétales et Fongiques, Lille, France and ⁴Université Catholique de Louvain, BCCM/MUCL, Louvain-la-Neuve, Belgium

BACKGROUND AND AIMS: Cisplatin is currently used as a first-line cancer treatment, such as testicular, ovarian or pulmonary cancers. Their nephrotoxicity remains a real problem. Acute kidney injury induced by cisplatin is located on proximal tubular cells, causing necrosis and possibly subsequent interstitial fibrosis and chronic dysfunction. These severe side effects can lead to a cessation of the patient's treatment. Currently, there is no effective prophylactic action to reduce cisplatin nephrotoxicity, beside hyperhydration of the patient [1].

The aim of the present work is therefore to identify new prophylactic therapy. For this, natural products can be studied, in this case, the interest of potential new medicinal mushrooms extracts. Among 13 mushroom extracts, the methanolic extracts of *Ganoderma parvigiibosum* Welti & Courtecuisse, *Ganoderma tuberculosum* Murrill and their association were selected to study their effects on human proximal tubular cells (HK-2) intoxicated with cisplatin.

METHOD: HK-2 cells are grown in 75cm² sterile flasks using DMEM low glucose (1mg/mL), supplemented with FBS (10%), L-Glutamin and a mix of Penicillin/Streptomycin. Dried mushrooms were grounded and extracted 3 times by methanol, evaporated extracts are stored at -20°C. A viability assay allowed to determine the work concentration of extracts range have been done. After that, tests were performed after a pretreatment of 1h with the extracts before adding cisplatin at a concentration of 20 µM. Viability assays (CCK-8) and antioxidant activity (DPPH) were done in 96-well. The intracellular concentration of β-catenin and calcium, Caspase-3, p53, cytochrome C, IL-6, NFκB, the membranal expression of KIM-1 and finally the ROS production (H2DCFDA) were studied by flow cytometry.

RESULTS: Tests have shown that methanolic extracts of *G. parvigiibosum* and *G. tuberculosum* (10 µg/mL) and their association (5 + 5 µg/mL) prevented the loss of viability after a 24h incubation. They also have prevented the increase of cytochrome C and p53 after 24h. *G. parvigiibosum* and the association of the two mushrooms extracts have also prevented the increase of caspase-3 and intracellular β-catenin. Finally, *G. parvigiibosum* was the only to prevent the ROS overproduction. None of them showed a scavenger activity, nor a prevention in the increase of IL-6 and NFκB or the membrane expression of KIM-1.

CONCLUSION: *Ganoderma parvigiibosum* appears to be therefore more beneficial than *Ganoderma tuberculosum* and the association of the two mushrooms extracts by acting also on the ROS overproduction. In conclusion, in this study, the extracts have shown a significant activity on the prevention of the pro-apoptosis pathway rather than a pro-inflammatory prevention. Further investigation will be performed to identify the precise activity and chemical content of these extracts.

Reference

[1]. Bunel V. et al, Pharm Biol. 2015