The *Withania Somnifera* root extract, that modifies the morphine analgesic profile, differently affects MOP and NOP gene expression regulation in neuroblastoma SH-SY5Y cells

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Withania somnifera is a Solanaceae family shrub, known as having a wide range of activities including the antioxidant one which may contribute, at least in part, to the reported antistress, immunomodulatory, cognition facilitating and anti-inflammatory effects (1). Recently, it has been also demonstrated that WSE prolongs the analgesic effect elicited by morphine (2). It is known that the development of tolerance and physical dependence is a characteristic feature of the opioids and causes the major limitations in their clinical use. Although behavioral studies demonstrated the ability of WSE to prolong morphine analgesic effect (2) and to reduce the development of tolerance to the opiate analgesic effect (3), little is known about its molecular mechanism. Therefore, based on these premises the aim of the study was to evaluate the effects induced by the Withania somnifera root extract (WSE) on the μ opioid receptor (MOP) and nociceptin opioid receptor (NOP) gene expression in the human neuroblastoma SH-SY5Y cells. To this end, concentrations of WSE devoid of cell toxicity were preliminarily assessed by the MTT assay; then, different experimental set up have been made to test the effects of 5 h exposure of SH-SY5Y cell cultures to morphine, naloxone and WSE, alone or in combination, on the opioid receptor gene expression. WSE was used at 0.25, 0.50 and 1 mg/ml, whereas morphine and naloxone at 10 μM and 100 μM concentrations, respectively. In combined treatment experiments, WSE and/or naloxone were added to cell culture medium thirty minutes before morphine. The gene expression analysis was assessed by Real-Time qPCR, and evaluated by the Delta-Delta Ct (DDCt) method.

Results showed a significant decrease of MOP (0.50±0.08 *vs.* control 1.0±0.13; $p < 0.05$) and NOP (0.69±0.11 *vs.* control 1.0±0.03; $p < 0.05$) gene expression induced by morphine. In contrast, naloxone elicited an up-regulation of MOP and NOP mRNA levels (2.67±0.29 *vs.* control 1.0±0.16; $p < 0.01$; 3.36±0.39 *vs.* control 1.0 ± 0.14; $p < 0.01$, respectively). The co-exposure to morphine and naloxone prevented the morphine-elicited gene expression alteration of MOP and NOP mRNA levels (1.0±0.04 *vs.* control 1.0±0.13; 1.04±0.14 *vs.* control 1.17±0.11 *vs.* control 1.0±0.14, respectively). WSE alone induced a marked down-regulation of MOP mRNA level at all concentrations (0.20±0.01, 0.10±0.01, 0.43±0.01 respectively *vs.* control 1.0±0.04; $p < 0.01$), showing a U-shaped effect; no alterations were observed in NOP gene expression analysis. The presence of the highest concentration of WSE (1 mg/ml, added 30 min before morphine) in the medium reverted the MOP down-regulation caused by the opiate alkaloid. The simultaneous presence of WSE and naloxone prevented morphine-induced MOP down-regulation at 0.50 mg/ml and 1.0 mg/ml, whereas at 0.25 mg/ml WSE a significant decrease of MOP mRNA levels was still observed. These two observations may suggest that different WSE concentrations could induce diverse effects depending on the presence of multiple components with different activities.

Finally, WSE pretreatment prevented NOP down-regulation elicited by morphine at all concentrations, and potentiates naloxone effect at 1 mg/ml. Taken together, these data indicate that WSE is able to alter MOP gene expression and to prevent, at its higher concentration, morphine-induced MOP down-regulation. Our data also reveal a potential antagonism of WSE against the naloxone ability to revert the morphine-induced MOP down-regulation. Furthermore, we observed that WSE pretreatment obstructs the down-regulation of receptor NOP induced by morphine; this result could be related to a less rapid onset of tolerance effect (3, 4).

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