Preclinical evaluation of *Withania somnifera's* efficacy: a natural therapeutic perspective for pain relief

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Pain has negative consequences on health status and life quality, and its relief is considered one of the major and complex medical needs. To date, opioid analgesics remain the most efficacious pharmacological agents for the treatment of moderate to severe pain, but their therapeutic benefit is hampered by the development of analgesic tolerance and opioid-induced hyperalgesia (OIH). A considerable effort has then been expended to develop novel therapeutic strategies that may maintain an adequate opioid-induced analgesia and, at the same time, mitigate the appearance of these side effects. Previous studies demonstrated that the mechanisms regulating the development of analgesic tolerance share some similarities with those involved in OIH. Furthermore, pre-clinical analyses indicated that several compounds, such as NMDA antagonists, calcium channel blockers, gabapentin and α_2 adrenergic agonists, prevented the development of both antinociceptive tolerance and OIH. The same compounds have also been found to prolong or increase the acute analgesic effect of opioids, justifying their clinical use as adjuvant pharmacological therapies.
Medicinal plants have also been proposed as putative adjuvant agents that may reduce analgesic tolerance and OIH. Particularly, *Withania somnifera* Dunal (WS, family: Solanaceae), a safe medicinal plant commonly used in Ayurvedic medicine to treat several diseases, has been found to prevent the development of tolerance to the analgesic effect of morphine in mice.
Starting from these considerations, the aims of the present study were to investigate whether a WS extract (WSE) may modulate the analgesic effect induced by acute morphine administration, and whether it may prevent the development of rebound hyperalgesia induced by a low morphine dose. Further, competition receptor binding assays [opioid (μ, δ, k), cannabinoid (CB_1, CB_2), glutamatergic (NMDA), GABAergic ($GABA_A$, $GABA_B$), serotoninergic ($5HT_{2A}$) and adrenergic (α_2)] have been carried out to better characterize the receptor binding profile of WSE.
Male CD1 mice were used for both behavioural and receptor binding studies. The effects of WSE pre-treatment (100 mg/Kg, i.p.) on morphine-induced analgesia was evaluated in the tail-flick test (mean pre-drug basal latency: 2-3 s; cut-off time: 12 s) and the hot plate test (plate temperature: 55°C; cut-off time: 15 s). The behavioural testing were assessed 30, 60, 120, 240 and 360 min after morphine (2.5, 5, 10 mg/Kg, s.c.). The ability of WSE (100 mg/Kg, i.p.) to inhibit the development of morphine-induced hyperalgesia was evaluated using the low-intensity tail-flick test (mean pre-drug basal latency of 5-7 s, and cut-off time of 10 s). The behavioural testing was performed 30, 60, 120, 240, 300, 360 and 420 min after morphine (2.5 mg/Kg, s.c.). WSE or vehicle were injected 30 min before morphine administration.
The results of the present study demonstrated that i) WSE alone was devoid of analgesic activity in both tests, ii) WSE pre-treatment significantly protracted the analgesic effect induced by 5 and 10 mg/Kg of morphine in the tail-flick test, iii) WSE pre-treatment prevented the development of morphine-induced hyperalgesia in the low intensity tail-flick test, and iiii) WSE exhibited a high affinity for the $GABA_A$ and moderate affinity for $GABA_B$, NMDA and δ opioid receptors.
In conclusion, the concurrent action of WSE on $GABA_A$, $GABA_B$, NMDA and δ opioid receptors could be responsible for the behavioural effects observed in the present study. Overall, the results suggest that WSE may posses potential therapeutic properties as a valuable adjuvant agent in opioid-sparing therapies.