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Title: Antinociceptive activity of petroleum ether fraction obtained from methanolic extract of Clinacanthus nutans leaves involves the activation of opioid receptors and NO-mediated/cGMP-independent pathway

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Abstract: BackgroundMethanol extract (MECN) of Clinacanthus nutans Lindau leaves (family Acanthaceae) demonstrated peripherally and centrally mediated antinociceptive activity via the modulation of opioid/NO-mediated, but cGMP-independentpathway. In the present study, MECN was sequentially partitioned to obtain petroleum ether extract of C. nutans (PECN), which was subjected to antinociceptive study with aims of establishing its antinociceptive potential and determining the role of opioid receptors and l-arginine/nitric oxide/cyclic-guanosine monophosphate (l-arg/NO/cGMP) pathway in the observed antinociceptive activity.MethodsThe antinociceptive potential of orally administered PECN (100, 250, 500mg/kg) was studied using the abdominal constriction-, hot plate- and formalin-induced paw licking-test in mice (n=6). The effect of PECN on locomotor activity was also evaluated using the rota rod assay. The role of opioid receptors was determined by pre-challenging 500mg/kg PECN (p.o.) with antagonist of opioid receptor subtypes, namely -funaltrexamine (-FNA; 10mg/kg; a -opioid antagonist), naltrindole (NALT; 1mg/kg; a -opioid antagonist) or nor-binaltorphimine (nor-BNI; 1mg/kg; a -opioid antagonist) followed by subjection to the abdominal constriction test. In addition, the role of l-arg/NO/cGMP pathway was determined by prechallenging 500mg/kg PECN (p.o.) with l-arg (20mg/kg; a NO precursor), 1H-[1, 2, 4] oxadiazolo [4,3-a]quinoxalin-1-one (ODQ; 2mg/kg; a specific soluble guanylyl cyclase inhibitor), or the combinations thereof (l-arg+ODQ) for 5 mins before subjection to the abdominal constriction test. PECN was also subjected to phytoconstituents analyses.ResultsPECN significantly ($p<0.05$) inhibited nociceptive effect in all models in a dose-dependent manner. The highest dose of PECN (500mg/kg) also did not significantly ($p>0.05$) affect the locomotor activity of treated mice. The antinociceptive activity of PECN was significantly ($p<0.05$) inhibited by all antagonists of -, -, and -opioid receptors. In addition, the antinociceptive activity of PECN was significantly ($p<0.05$) reversed by l-arg, but insignificantly ($p>0.05$) affected by ODQ. HPLC analysis revealed the presence of at least cinnamic acid in PECN.ConclusionPECN exerted antinocicpetive activity at peripheral and central levels possibly via the activation of non-selective opioid receptors and modulation of the NO-mediated/cGMP-independent pathway partly via the synergistic action of phenolic compounds.

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