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Bioassay-directed isolation of hypotensive alkaloids from *Holarrhena pubescens*

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Holarrhena pubescens belongs to the family Apocynacea, commonly known as "kurchi" is highly reputed in traditional medicine as a remedy for amoebic dysentery and other intestinal ailment. Bioassay-directed fractionation of the ethanolic extract of *Holarrhena pubescens* resulted in the isolation of steroidal alkaloids i.e., Holamide and Pubscinine. Holamide showed a three proton doublet at 1.45 (J=6.56 Hz) and two AB doubles at 3.17 and 3.00 each for on proton (J=12.06 Hz) in the 1H NMR spectrum suggested that it belongs to conanine series of alkaloid (A class of compound with the steroid nucleus and a five members heterocyclic ring with nitrogen). In contrast Pubscinine showed one methyl at 1.28 while the doublet is missing a three proton singlet was observed at 2.28 due to a vinylic methyl indicated a double bond in the 18,20-epimino ring of the conanine series of alkaloids. In anaesthetized rats, the Holamide and Pubscinine caused a fall in blood pressure in a dose-dependent manner. Pretreatment of animals with atropine completely abolished the hypotensive response of acetylcholine; whereas hypotensive effect of Holamide and Pubscinine were not modified by atropine. Similarly acetylcholine produced contractile effect in guinea-pig ileum, which was antagonized by atropine; however both (Holamide and Pubscinine) failed to produce any stimulant response on guinea-pig ileum. These data indicate that the steroidal alkaloids i.e., Holamide and Pubscinine from *Holarrhena pubescens* mediated hypotensive response through a mechanism different to that of acetylcholine.

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