

## Therapeutic Activity of HPLC Purified Pure Compound Lantadene D from *Lantana camara* Leaves against *Trypanosoma evansi*

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Trypanosomiasis, a zoonotic disease is on the increase in recent years. Current limited classes of drugs in used are facing lot of problems. As a result of this *Lantana camara* leaves, a poisonous leaves were subjected to different stages of purification such as methanolic extraction, column chromatography, rechromatography, thin layer chromatography, preparative thin layer chromatography, High performance liquid chromatography (HPLC) and structure elucidation and lantadene D was obtained. Lantadene D at concentrations (250-1000 µg ml<sup>-1</sup>) was screened against *Trypanosoma evansi* on Vero cell line grown in Dulbecco's Modified Eagle Medium (DMEM) (Sigma) in 96-well flat bottom micro culture plates (Nunc, Denmark). Each well received 100 µl of DMEM containing 5x10<sup>5</sup> cells/ml. The plates were incubated at 37°C under 5% CO<sub>2</sub> for 48 hr to complete development of monolayer. After the formation of confluent monolayer, the medium (DMEM) was discarded and replaced with a fresh DMEM. And the medium was supplemented with 20-40% fetal calf serum (FCS), Gibco USA and antibiotics (100 unit's penicillin, 100 µg streptomycin and 40 µg gentamycin). A high parasitaemic blood from mouse was diluted with DMEM to obtain final trypanosomes of 1x10<sup>6</sup> trypanosomes/ml. The suspension (100 ml of medium with trypanosomes) was added at rate of 1:1 to pure compound lantadene D from *Lantana camara* and the plate was incubated under the same conditions mentioned above. The test was repeated at least thrice. *In vitro* cytotoxicity was performed on the same medium at concentrations (1.56-100 µg/ml) but without supplement of fetal calf serum in triplicate and incubated under the same conditions described previously. In this research, pure compound lantadene D from *Lantana camara* showed marked trypanocidal activity with reduction of trypanosomes in corresponding ELISA plate wells at concentration of 250 µg/ml, (40.±0.0 to 4.667±0.3), after 9 hr of incubation. However at 500 µg/ml of lantadene D, trypanosomes were not detectable in ELISA plate wells at 9 hr of incubation. Lantadene D and diminazine aceturate were cytotoxic to Vero cells in all concentrations except at 6.25 µg/ml, respectively. Pure compound Lantadene D from *L. camara* at the same concentration showed the same level of cytotoxic effects with diminazine aceturate, the reference drug despite toxic nature of the former to animals. Lantadene D displayed significant trypanocidal activity. This may pave way for a nascent drug discovery.