

Cytotoxic effect of Melittin peptide; major pain producing substance in European honeybee (*Apis mellifera*) venom on leishmaniasis parasite *Leishmania donovani*

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Melittin is a peptide in the venom of the European honeybee (*Apis mellifera*). It has indicated various therapeutic effects including parasitic infections. Leishmaniasis is a parasitic disease caused by a protozoan parasite under the genus *Leishmania*. In Sri Lanka, *Leishmania donovani* is the parasite causing leishmaniasis of cutaneous origin. This study evaluates the anti-leishmanial property of melittin peptide against the *L. donovani* parasite. *L. donovani* promastigote cultures were established in M119 medium (HIMEDIA, Cat # AL189) supplemented with 20% fetal bovine serum (FBS, Sigma, Cat # F4135) at room temperature (26-30°C). A standard series of Melittin peptide was prepared (30, 45, 60, 75, 90, 105, 120, 135, 150, 165 and 180 µg/mL). Cytotoxicity for each concentration was assessed using an MTT (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay by introducing stationary phase parasites at a concentration of 2×10^6 cells/mL into each well of a 96-well flat-bottom microtiter plate. The experiment setup was repeated 12 times in each concentration for 24 and 48-hour incubation separately at room temperature (26-30°C). The relative amount of formazan produced by viable cells in each well was quantified by measuring absorbance at 590 nm using an ELISA plate reader. Cell viability was assessed from the concentration-response curve, and the IC₅₀ value was calculated. The highest toxicity was identified at 180 µg/mL at 24 h ($96.28 \pm 3.71\%$) and 48 h ($96.12 \pm 0.44\%$) periods. However, 50% mortality of the parasites were identified at 50.25 µg/mL, and 50.50 µg/mL concentration at 24 h and 48 h periods. The results revealed that the proliferation of *L. donovani* promastigote was inhibited in a dose-dependent manner, and the IC₅₀ value of Melittin was denoted as 50.32 mg/mL, indicating an anti-leishmanial property.

Keywords: Anti-leishmanial, IC₅₀ value, *Leishmania donovani*, Melittin, MTT assay

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