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Drug Susceptibilty of Indian field isolates of *Leishmania donovani* and development of experimental resistance towards Miltefosine and Paromomycin

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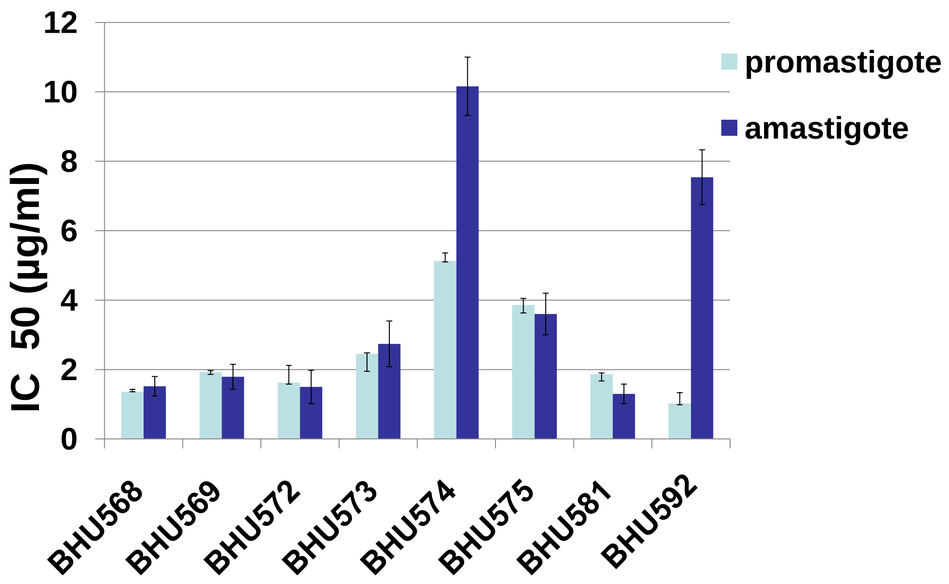
INTRODUCTION

❑ **Control of Visceral Leishmaniasis (VL) relies mainly on chemotherapy. The high toxicity and emergence of resistance towards Sodium antimony gluconate (SAG), has resulted in introduction of alternate drugs Miltefosine (MIL) as the first line drug in endemic parts of Bihar. Anthroponotic VL transmission in India as well as long half-life of MIL poses threat of development of resistance. Paromomycin (PMM) is the new antileishmanial drug in phase IV trials which appears to be safe, affordable and effective. Here, we have investigated the antileishmanial activity of MIL and PMM in cloned field isolates (n=8) from VL patients in hyper endemic areas of Bihar, India. The isolates were initially characterized as Leishmania donovani by ITS - 1 based PCR RFLP. Susceptibility of the isolates was evaluated at promastigote and amastigote stage for both MIL and PMM. Based on the IC_{50} values, we selected two isolates for induction of resistance to MIL and PMM. The parasites were adapted to MIL 30µg/ml and PMM 60µg/ml by stepwise increase of drug concentration.**

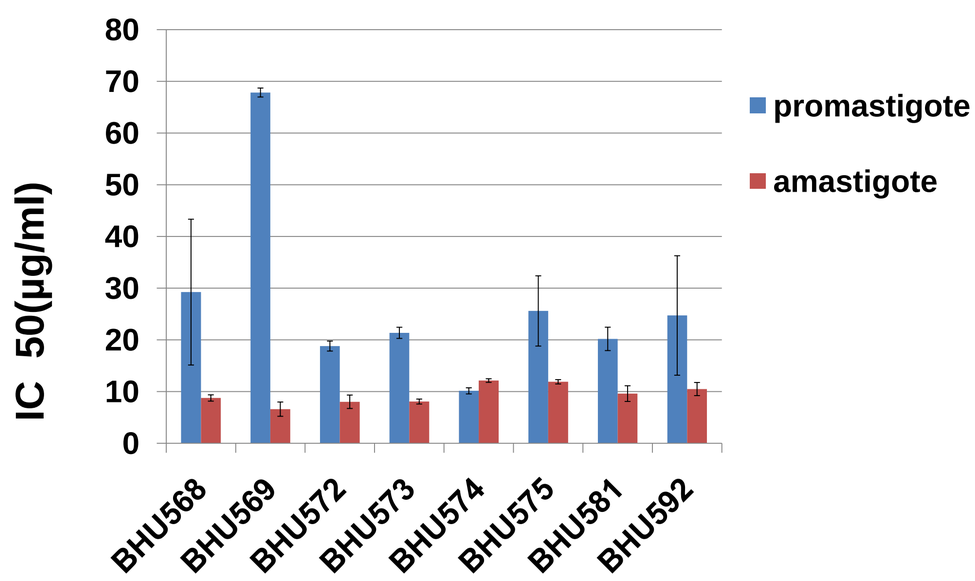
RESULTS:

MIL Sensitivity Profile of Indian Field Isolates



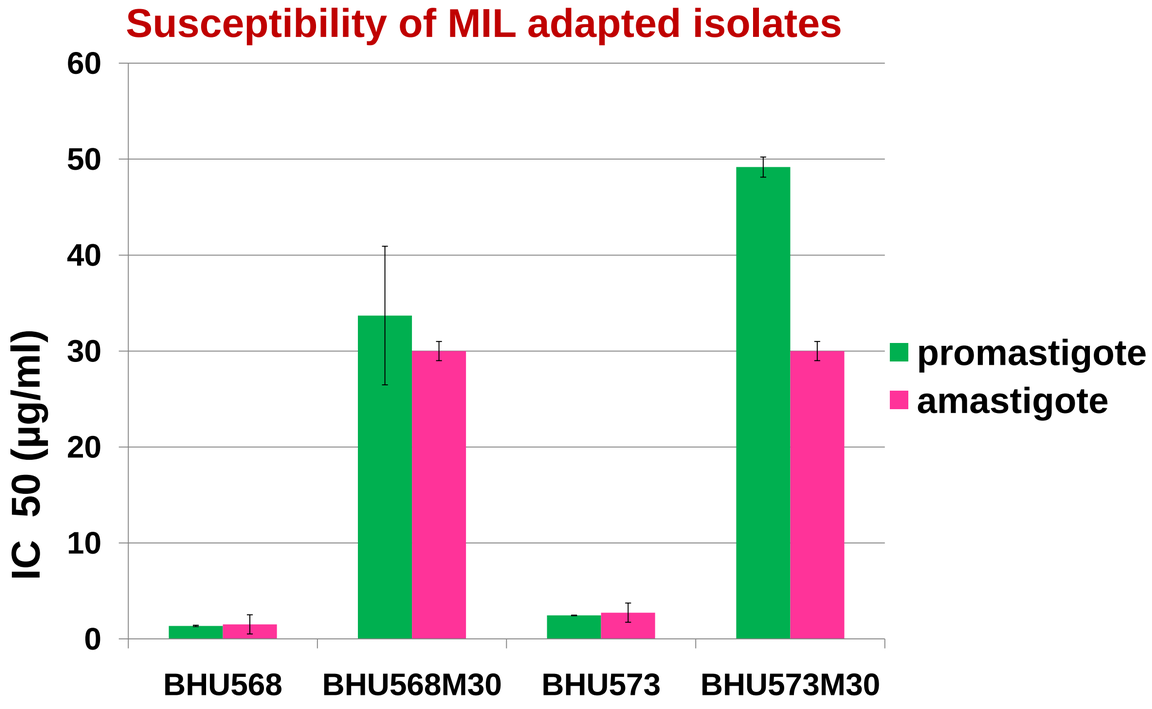
Promastigote IC_{50} ranged from 1.02 to 5.13µg/ml
Amastigote IC_{50} ranged from 1.3 to10.16 µg/ml

PMM Sensitivity Profile of Indian Field Isolates



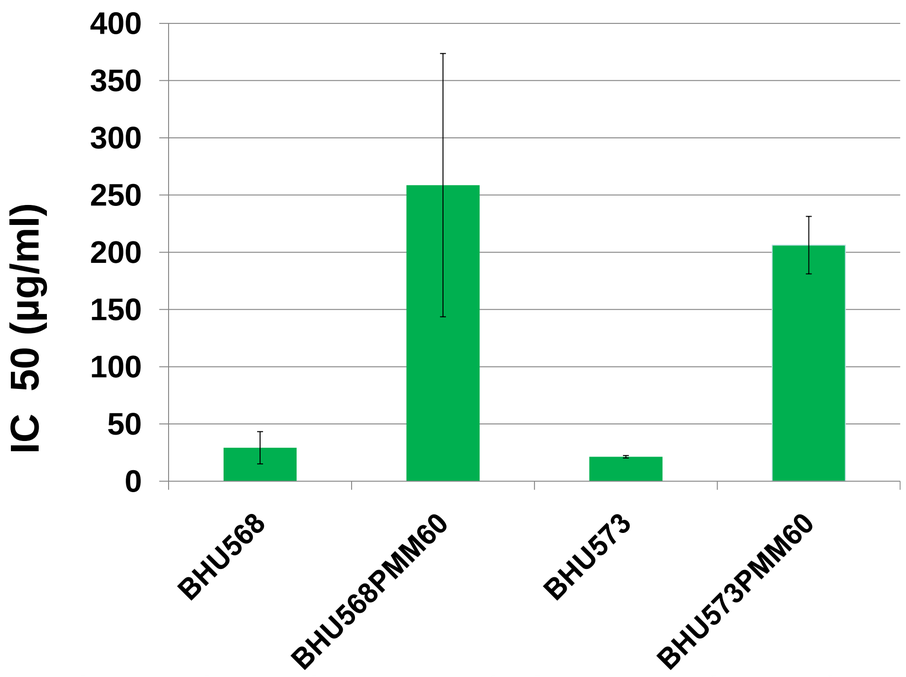
Promastigote IC_{50} ranged from 10.15 to 67.8µg/ml
Amastigote IC_{50} ranged from 6.61 to12.14 µg/ml

Susceptibility of MIL adapted isolates



The parasites adapted at 30µg/ml MIL concentration, showed decreased susceptibility towards MIL at both promastigote and amastigote stage, as compared to the wild type isolate

Susceptibility of PMM adapted isolates



Parasites adapted at 60µg/ml PMM, showed marked increase in IC_{50} for PMM at promastigote level.

Conclusions: The 8 Indian SAG resistant parasites showed that they are not cross-resistant to MIL & PMM. Adapted isolates of MIL & PMM showed growth similar to their wild type.

We propose to explore the mechanism of resistance towards MIL and PMM using drug resistant parasites. Simultaneously verification of experimental MIL resistance markers (LdMT, LdROS3) will be carried out in all field and MIL adapted isolates

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