Conclusions
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Diseases caused by insect-borne trypanosomatid parasites constitute a significant but neglected public health problem worldwide. *Leishmania*, a unicellular trypanosomatid protozoan parasite, is the causative organism of Leishmaniasis and is transmitted by female phlebotamine sandflies. New therapeutic alternatives are desirable for treatment of this life-threatening infection as the treatment mainstay, pentavalent antimonial drugs, have over the years been beset with increasing cases of resistance. The plant kingdom has in the past provided several affordable compounds and it should be noted that due to limited availability of effective pharmaceutical products, people in endemic areas suffering from Leishmaniasis tend to depend upon traditional medicines for alleviation of symptoms. The scientific evaluation of medicinal plants used in the preparation of folk remedies has provided modern medicine with effective pharmaceuticals for the treatment of diseases caused by protozoan pathogens. Taken together, in the ongoing search for better leishmanicidal compounds, plant derived products are emerging as an attractive option. Generally, detection of plant secondary metabolites with leishmanicidal activity has been performed in promastigotes as they are easier to maintain under *in vitro* conditions, although its efficacy should always be complemented by evaluation in intracellular amastigotes and host macrophages to evaluate cytotoxicity. Compounds isolated from plants with potent anti-leishmanial activity include phenolics like Aurones, Lignans, Chalcones, Flavonoids, Isoflavonoids, Saponins, Quinones, Alkaloids, Tannins, Terpenoids, Iridoids, Terpenes, Oxylipins and many other plant secondary metabolites.

Our study with different indigenous plant derived compounds included Rampatri, *Piper betle, Artemisia* sp. and its phytoconstituent Artemisinin along with its analogs. Malabaricones or Rampatri derived compounds showed efficacy in promastigotes but because of its high toxicity in host cells, we could not screen its efficacy in amastigotes. Artemisinin proved to be a potent anti-leishmanial agent, exhibiting efficacy both in promastigotes and amastigotes while remaining non toxic to mouse macrophages even at concentrations up to >500 μM. Some Artemisinin analogs were effective and demonstrated better antileishmanial activity than Artemisinin e.g. GC003, GC012, GC009 and ML86-1. In our study, the safety indices of Artemisinin and its analogs ranged between 45 to 166 and the most promising Artemisinin analog was GC012 with a safety level >166. An ethanolic
extract of PB was equally effective as it showed potent anti-promastigote and anti-amastigote activity with a safety index >12.

The mitochondrion of kinetoplastid protozoa can be considered as potentially its most valuable organelle because of its unique structure and function. *Leishmania* has a unique but relatively weak anti-oxidant system as it has no catalase and uses a trypanothione dependent system that targets deleterious hydroperoxides. In our study, we established Artemisinin had potent anti-leishmanial activity, mediated by increased generation of ROS with a concomitant decrease in level of non protein thiols. In malaria, the proposed mechanism of action of Artemisinin is that cleavage of its endoperoxide bridge in presence of free iron or heme leads to generation of free radicals. Programmed cell death pathway or PCD is an attractive chemotherapeutic target for any pathogen and in *Leishmania*, PCD is triggered via oxidative stress signals from antileishmanial compounds. We found that Artemisinin also triggers oxidative stress to parasites, culminating in a caspase-independent apoptosis-like death in promastigotes evidenced by increased calcium influx, altered mitochondrial membrane potential, phosphatidylserine exposure, *in situ* DNA fragmentation and an increase in the sub G_0//G_1 population.

Leishmaniasis is associated with immunological dysfunction of T cells and macrophages in particular. Murine models of Leishmaniasis have demonstrated that host defense mechanisms include increase in IL-12 induced Th1 cytokines such as IL-2, TNF-α, and IFN-γ, which by activating macrophages ultimately eliminate the parasite through enhanced release of nitric oxide, along with down regulation of Th2 cytokines including IL-4, IL-10 and TGF-β. We demonstrated Artemisinin increased generation of NO and upregulated mRNA expression of iNOS and concurrently decreased mRNA expression of IL-10 in parasitized macrophages.

Several promising antileishmanial leads have been reported over the past few years in various test systems. To identify the compounds that are the most promising, candidate molecules should be compared to one or more standard antileishmanial drugs, preferably using standard *in vivo* models. In our BALB/c mouse model of experimental VL, we found that treatment with Artemisinin decreased splenic weight, IgM and IgG levels and expression of PMNs in infected mice; it also increased proportions of IFN-γ and IL-2 expressing T
lymphocytes to levels comparable with normal untreated mice. Similarly, Artemisinin analogs also showed encouraging effectiveness in a murine model of Leishmaniasis.

Collectively, our studies have revealed the efficacy of plant derived products in triggering generation of reactive oxygen species (ROS) that in turn served as critical effector molecules, responsible for programmed cell death or caspase-independent apoptosis-like cell death of parasites. In *Leishmania*-infected BALB/c mice, Artemisinin and its analogs were effective via restoration of Th1 response along with low toxicity. As vaccine strategies have failed to enter clinical trials, potential plant derived compounds could therefore be a useful treatment strategy to screen new drugs and advance biomedical research against deadly diseases like Leishmaniasis.

Conclusions