The process of programmed cell death (PCD) that occurs in unicellular parasites is being put forth as a phenomenon that can be exploited clinically to devise therapeutic agents against them. Over the years, it has been discovered that Leishmania parasites commit suicide by a version of the PCD that appears different from the conventionally known apoptosis, yet resembling it in some parts. In a search for molecules involved in Leishmania PCD, metacaspases are currently considered as the front-runners. Discovered with the help of Bioinformatics a decade ago in plants, fungi and protozoa but found missing in humans, and the fact that they have been implicated in processes having a bearing on the cellular survival like cell cycle progression, secretory processes, protein aggregation and cell death, researchers are closely examining various facets of this important cysteine peptidase. Heat-shock response is a ubiquitously conserved adaptive response to cellular stresses. The heat-shock proteins perform a role in proteostasis by regulating protein folding and protein turnover, and can be found in all organisms from protozoa to man. Leishmania, which shuttles between two hosts for the completion of its life cycle, faces heat-shock as an indispensable part of its biology. The parasites experience an enormous jump in temperature from 22°C in the insect vector to 37°C in the human host on invasion of mammalian reticuloendothelial cells, which causes differentiation from promastigote to amastigote stage, as well as a concomitant upregulation of various heat-shock proteins, including Hsp70. Thus. Hsp70 too occupies an important place in the biology of Leishmania parasites.

Realizing the need for understanding PCD and its exclusive pathways in Leishmania in detail, the role of Leishmania donovani metacaspase, LdMC1 and the heat-shock protein, Hsp70 in Leishmania programmed cell death was investigated in this study. Proteasome inhibitor MG132 [N-(benzyloxycarbonyl)leucinyl-leucinylleucinal; Z-Leu-Leu-Leu-al] was used to induce PCD in the in vitro culture of Leishmania donovani, which was confirmed by morphological and molecular markers, namely giemsa staining, DNA fragmentation assay, cell cycle analysis, decreased expression of PARP protein and measurement of mitochondrial membrane potential. Exposure to MG132 led to a nominal increase in the expression
of LdMC1 as well as Hsp70 in both promastigotes and axenic ALFs.

To probe the role of LdMC1 and Hsp70, gene silencing by antisense oligonucleotides (ASOs) was carried out, for which ASOs with partially modified phosphorothioate (PS) backbone, were designed against the protein coding regions of these genes. The selection of a suitable target sequence was done after the analysis of mRNA secondary structures of the genes. The designed ASOs were 20 bases in length having 3 PS modifications on 5’ end and 4 PS modifications on 3’ end, leaving a core of 13 bases with normal phosphodiester bonds. Promastigotes and axenic ALFs were exposed to ASOs complexed with the transfection reagent under in vitro culture conditions, and gene knockdown was confirmed by RT-PCR. Exposure to MG132 and ASOs led to morphological defects (like incomplete cytokinesis, arrested cell division, biflagellate promastigotes, binucleate forms etc.), DNA fragmentation, delay in progressing through the S-phase of the cell-cycle, decrease in the mitochondrial membrane potential ($\Delta \Psi_m$) and decreased expression of PARP protein. Antisense knockdown of both these genes, individually as well as together, caused phenotypic and molecular characteristics of PCD. Simultaneous knockdown of both LdMC1 and Hsp70 led to exacerbation in these features of PCD. Parasites co-exposed to MG132 along with ASOs suffered the maximum damage.

Together, these data suggest that both LdMC1 and Hsp70 have a vital role in the cell cycle of Leishmania donovani. The abrogation of the expression of these genes aggravates the features of PCD induced due to proteasome inhibition by MG132, in promastigotes as well as in axenic ALFs. The products of these genes are important for its survival and therefore could be used as potential drug targets to control the infection of Leishmania donovani in human host. The present study further demonstrates that the unicellular protozoan Leishmania uses conserved mechanisms like DNA damage and decrease in mitochondrial membrane potential as intrinsic triggers for the execution of PCD in response to perturbation of genes controlling essential cellular processes. This work, therefore, sheds some light as well on the mechanisms of PCD retained by the unicellular organisms, using Leishmania as a model.