Chapter 1

Introduction
The primary aim of this body of work is to characterise the physiology of heat shock in *Leishmania donovani* in order to obtain a better insight into the host parasite interactions involved in *Leishmaniasis*.

*Leishmania* spp. form a large complex of protozoan parasites with a wide range of clinical manifestations - ranging from the self-healing but chronic cutaneous and severely disfiguring muco-cutaneous infections endemic to parts of South and Central America, the Middle East and Southern Asia; to the acute visceral infections or kala azar endemic to certain parts of Africa, pockets of South and Central America, Southern Europe and Southern Asia. Mortality rates of the disease vary widely. However, untreated cases of visceral Leishmaniasis, the typical clinical presentation of *Leishmania donovani*, can result in a fatality of above 95%. In addition to being one of the most common infectious diseases in the world, the problem of Leishmaniasis is compounded by the lack of any effective vaccines, the emergence of treatment resistant strains, and most significantly, the ability of the parasite to establish co-infections, especially in AIDS patients. In this background, studies that increase our understanding of the biology of the parasite within their mammalian hosts become especially significant.

In the milieu of such studies, very few focus on the nature of the initial interactions between the parasite and their mammalian hosts, before a successful infection is established. This aspect of parasite biology is centred on its ability to cycle between two hosts – arthropod sand fly vectors and mammals. During its transmission between these two hosts, *Leishmania* exists in two forms that are morphologically and physiologically different from each other, with each form adapted to survival within a unique environment. The promastigote forms of the parasite are elongated in shape, flagellate and motile; and physiologically capable of surviving within their insect vectors at an ambient temperature of 26°C and pH of 7.4. In contrast, amastigote forms are rounded in shape, much smaller in size, non-flagellate and non-motile; and physiologically suited for survival as obligate intracellular parasites that reside and propagate within the phagolysosomes of macrophages at an ambient temperature of 37°C and pH of 5.5. Since amastigotes are the infective forms of the parasite, a study of the factors and
processes that lead to differentiation of Leishmania into these forms is essential in attempting to control or cure its pathogenesis.

Since one of the main differences between the conditions for growth between the promastigote form and the amastigote form of the parasite is the difference in their ambient temperature, it has seemed natural to hypothesize that it may be one, if not the main, factor in parasite differentiation. In view of this hypothesis, the change in temperature that the parasite is exposed to during its transfer from its insect hosts to mammals is equivalent to heat shock in most other systems. Several studies have shown that this heat shock is associated with the two forms that the parasite displays during its in vivo life cycle. One of the most compelling arguments that prove this association is that promastigotes exposed to heat shock at 37°C in combination with or followed by pH shock at 5.5 in a host free in vitro system differentiate into forms that display the biochemistry and physiology typical of amastigotes obtained from macrophages of infected animals.

Heat shock, being one of the most common environmental changes that living organisms are exposed to, is associated with cellular responses that are highly conserved across evolution. The functional effects of this phenomenon are mediated by a wide variety of proteins that are up-regulated in response to it. The key players in this process are proteins that belong to a family collectively called the heat shock proteins or HSPs. An increasing body of work has revealed that in addition to their primary role as molecular chaperones, these proteins are associated with diverse roles in a wide variety of organisms, some of which are connected to development, differentiation and pathogenesis.

Available literature shows that even short exposures (1-2 hours) to heat shock at 37°C, induces the increased synthesis of heat shock proteins in the promastigote forms of Leishmania. Some studies indicate that inhibition of certain heat shock proteins, like Hsp90 and Hsp100, have important effects on their morphological transformation at 37°C. Others establish the fact that certain parasite heat shock proteins, like Hsp70, have important roles in modulating host immune responses, e. g., by acting as immuno-dominant antigens or B-cell mitogens. In this context, it is significant that an increase in expression of parasite heat shock proteins is known to occur both during infection and in response to their in vitro treatment with hydrogen peroxide or TNF-alpha. Apart from being classical inducers of the cellular stress
response, these signals are doubly relevant for the parasite with respect to their ability to establish infections.

However, several aspects of this stage of the *Leishmania* life cycle are still unclear. The physiology of changes in promastigote forms of the parasite exposed to chronic heat shock at 37°C, prior to their uptake by macrophages, is poorly understood. In addition, though an important role for the Hsp70 family of parasite heat shock proteins in the immunology of Leishmaniasis is hinted at in several reports, there are almost no studies characterizing the cellular expression levels or roles of this family of proteins in the survival and infectivity of the parasite during its normal life cycle. Thus, the work presented in this thesis can be broadly classified under three objectives:

1. Characterisation of the effects of chronic heat shock at 37°C on the viability, phenotype, biochemistry and infectivity of *Leishmania donovani* promastigotes in a host free *in vitro* system.

2. Identification of heat shock proteins, especially those from the parasite Hsp70 family, that show maximal changes in response to heat shock.

3. Effects of modulation of levels of these heat shock proteins on the *in vitro* survival and infectivity parameters of *Leishmania donovani* promastigotes exposed to heat shock.

The current study is therefore an attempt to shed light on these aspects of *Leishmania* biology with the ultimate objective of laying the foundation for vaccines or treatment regimens centred around the role(s) of parasite Hsp70 proteins and effects of heat shock on parasite physiology.