Aims

and

Objectives
Visceral leishmaniasis (VL) or kala azar, caused by the protozoan parasite *Leishmania donovani*, is the most severe form of Leishmaniasis and is associated with irregular fever, weight loss, enlargement of liver, spleen and anaemia (Murray et al., 2005). The disease is of increasing concern due to worldwide occurrence of 0.5 million new cases per annum and is magnified due to HIV *Leishmania* co-infection (Murray, 2001). Current first line treatment of leishmaniasis includes pentavalent antimonials or conventional amphotericin B. However, treatment with these drugs suffers from several limitations as specific toxicities, parental administration, emergence of drug resistance and elevating costs (Murray, 2001). Miltefosine is a new addition into the collation of antileishmanial drugs, but teratogenicity, development of resistance and low therapeutic window pose limitations on its use (Croft et al., 2006). Thus, identification of new, safer and cheaper drugs that can be effective against both antimony-susceptible and -resistant strains of *Leishmania* is of tremendous economic and medical importance. Fucoidan an immunomodulatory, sulfated polysaccharide mainly composed of L-Fucose, is extracted from marine brown algae (Nakamura et al., 2006). As a ligand for the Scavenger Receptor A (SR-A), fucoidan, increased the level and activity of PKC, interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α) and IL-12, in macrophages. (Nakamura et al., 2006; Hsu, 2001 #205). Moreover fucoidan is known to increase NO production from macrophages by modulating the transcription and expression of nitric oxide synthase (iNOS). Identification of exact signaling events and network of signaling molecules activated by fucoidan is of worth investigating to achieve an optimum effector response with minimum side effects. This study is also beneficial deriving from the fact that fucoidan is already approved for human consumption (Irhimeh et al., 2007), so the cumbersome and time taking process of clinical trials can be minimized, which ultimately will benefit the patients from poor countries, where leishmaniasis is most prevalent. The aim of this work is to elucidate the intricate signaling network by which fucoidan triggers macrophage microbicidal functions, to kill both antimony-susceptible and-resistant strains of VL which would help in the development of immunomodulators useful for visceral leismaniasis.
Chapter I

The efficacy of fucoidan, a polyanionic sulfated polysaccharide from brown algae, was evaluated in experimental infections of BALB/c mice with antimony-sensitive (AG83) and -resistant (GE18FR) Leishmania donovani. In addition to having appreciable inhibitory effect on amastigote multiplication within macrophages (>93% inhibition at 50 μg/ml), complete elimination of liver and spleen parasite burden was achieved by fucoidan at a dose of 200 mg/kg/day given orally, 3 times weekly, in a 6-week mouse model of both antimony-sensitive and -resistant strains. This curative effect was associated with switching of CD4+ T cell differentiation from disease-progressive Th2 to disease-resolving Th1 mode. Further, splenocytes of fucoidan-treated infected (AG83 and GE18FR) mice generated significantly enhanced levels of superoxide and nitric oxide. Not only was this treatment curative when administered orally 15 days post-infection, but it also imparted resistance to reinfection. These results suggest superior efficacy of fucoidan as potent immunomodulator for controlling non-healing visceral leishmaniasis.

Specific Objectives:

i. To determine the effect of fucoidan on generation of Th1 cytokines and NO production against both antimony-susceptible and-resistant strains.

ii. To evaluate in vitro and in vivo antileishmanial effect of fucoidan.

iii. To explore the possible involvement of proliferation of splenocytes and T cell immune response in vivo.

iv. To assess the requirement of NO and ROS in vivo for leishmanicidal effect of fucoidan.

Chapter II

Fucoidan could cure both antimony-sensitive and -resistant visceral leishmaniasis through immune activation. Signaling events underlying this cellular response was studied in macrophages and in BALB/c mouse model of visceral leishmaniasis. Fucoidan induces activation of both p38 and ERK1/2 as well as NF-κB DNA binding activity in both normal and L. donovani-infected macrophages. Pharmacological inhibition of p38, ERK1/2 or NF-κB pathway markedly attenuated fucoidan-induced proinflammatory cytokine synthesis and
iNOS gene transcription. Since impairment of protein kinase C (PKC) signaling is one of the adaptive strategies for successful propagation of Leishmania parasites, the effect of fucoidan on PKC isotypes was studied. Fucoidan elicited an increase in expression and activity of PKC-α, βI and βII in infected macrophages, while level and activity of both PKC-ε and ζ were significantly abrogated. Functional knockdown of PKC-α and β resulted in down regulation of p38 and ERK1/2 along with marked reduction of IL-12 and TNF-α production in fucoidan-treated infected macrophages. Fucoidan treatment of Leishmania-infected mice enhanced NF-κB DNA binding activity, inhibition of which resulted in marked attenuation of Th1 cytokines and reduced parasite clearance. Collectively, these results suggest that curative effect of fucoidan is mediated by MAPK/NF-κB pathway through modulation of specific PKC isotypes.

**Specific Objectives:**

i. To investigate the therapeutic effects of fucoidan.

ii. To ascertain the involvement of MAPK in fucoidan-mediated immune response.

iii. To study the effect of differential modulation of PKC isoforms on fucoidan-mediated generation of Th1 cytokines.

iv. To investigate the role of NF-κB induced regulation of Th1 cytokines and iNOS by fucoidan.

v. To study the role of modulation of NF-κB in vivo by fucoidan.

**Chapter III**

We have demonstrated that fucoidan as a novel antileishmanial agent can cure both antimony-susceptible and-resistant strains of *L. donovani* by shifting the Th1/Th2 paradigm in favour of host along with generation of NO and ROS. Immune activation achieved by fucoidan was dependent on p38 and ERK1/2 MAPK, which in turn activated NF-κB. Moreover our previous study also shed light on the differential activation of PKC isoforms after fucoidan treatment. As kinase/phosphatase balance has a critical role in modulation of infection, the effect of fucoidan in general phosphatase activity and in particular MAPK phosphatase was studied. Fucoidan treatment could significantly downregulate MKP1, MKP3 and PP2A along with decrease in phosphatase activity. However administration of ROS quencher, N-acetyl-L-cysteine (NAC), abrogated PTP inhibition by fucoidan, implying a role
of ROS in inactivating PTP. Moreover increased kinase activity after treatment of fucoidan in infected cells was also abrogated after treatment with NAC. NAC treatment also abrogated fucoidan mediated increase in Th1 cytokines and the phosphorylation levels of ERK1/2 and p38. In infected mice fucoidan mediated immune activation and parasite clearance was also abrogated by ROS quencher NAC, thereby pointing a role of ROS in fucoidan mediated protection.

Specific Objectives:

i. To investigate the role of fucoidan in modulating PTP and MAPK phosphatase.

ii. To ascertain the involvement of ROS in fucoidan mediated protective immunity.

iii. To study the role of ROS in fucoidan-treated mice in vivo.