SUPPLEMENTARY INFORMATION

Supplementary Methods:

Culture, differentiation and infection of U937 cells:

The human promonocytic cell line U937 was obtained from ATTC (American Type Culture Collection). Cells were cultured in suspension in stationary cell-culture flasks in RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% FCS, penicillin and streptomycin at 37^0C in a humidified atmosphere of 5% CO_2 prior to infection with *L. donovani***,** U937 cells were cultured for 18 h in the presence of PMA (10 ng/ml; sigma), rendering the cells adherent and capable of phagocytosis of *L. donovani* promastigotes (Nandan et al., 1995). PMA-differentiated U937 cells were infected with freshly transformed promastigotes of *L.donovani* at a cell to parasite ratio of 1:10. After incubation at 37^0C for the 18 h, noningested parasites were removed by vigorous washing with RPMI-1640 medium three times. The infected macrophages (U937) were treated with different concentrations of DIM for 12 h. At least 400 macrophages were examined for each cover slip by Giemsa staining. Anti-leishmanial activity was determined by calculating the number of amastigotes per 100 macrophages.

Determination of *In vivo* parasites burden inhibition:

Female golden hamsters (4 wk, 60-80 gm/hamster) with 2 weeks after established infection were administered 10 mg and 20 mg/kg body weight of DIM by intramuscularly for 4 weeks before they were sacrificed. Control group received phosphate buffer saline (PBS). Liver and spleen parasitic loads were determined from impression smears after Giemsa staining. Experiments were carried out according to adequate ethical regulations. The results are expressed as the number of amastigotes/100 nuclei and the total parasitic load per organ, using the formula (Mittra et al., 2000):

Organ weight in mg X the number of amastigotes per nuclei X (2×10^5)

Supplementary Results:

DIM reduces the parasite burden in Liver and Spleen:

The inhibition of catalytic activities of DNA topoisomerases, growth of parasites and induction of apoptosis indicate that DIM is a highly potent anti-leishmanial agent. The therapeutic potential of DIM was further substantiated by an *in vivo* experiment. To carry out this experiment golden hamsters were infected with *L. donovani* AG83 promastigotes. Two weeks after infection the hamsters were treated with DIM (10 mg and 20 mg/kg body weight) for 4 weeks. Compared with the infected and untreated hamsters, which showed a progressive and fatal visceral leishmaniasis, when DIM at 20 mg/kg body weight was administered intramuscularly to the infected animals there was a reduction of 93% and 96% parasitic burden in liver and spleen respectively (Supplementary Fig. 1C and D). Giemsa-stained micrographs at liver and spleen smears for intramuscularly DIM-treated hamsters also show this profound effect and nearly complete reduction of parasitic burden (Supplementary Fig. 1B). DIM was found to be nontoxic as evident from the analysis of SGOT and SGPT of DIM treated animals. The weight of infected, untreated hamsters reduced drastically, compared with the weight of normal and DIM-treated infected hamsters, indicating healthy recovery. Infected organ (Liver and Spleen) weight of animals reduced at normal weight by DIM treatment (Supplementary Table 1 and 2).

Supplementary References:

Mittra B, Saha A, Chowdhury AR, Pal C, Mandal S, Mukhopadhyay S, Bandyopadhyay S and Majumder HK. (2000) Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. *Mol Med.* **6:** 527-541.

Nandan D and Reiner NE. (1995) Attenuation of gamma interferon-induced tyrosine phosphorylation in mononuclear phagocytes infected with *Leishmania donovani*: selective inhibition of signaling through Janus kinases and Stat1. *Infect. Immun.* **63**: 4495-4500

Supplementary Legends for Figures:

Figure S1: DIM reduces parasites burden in macrophages (U937) and in animals.
(**A**). Micrographs of Giemsa-stained of the infected macrophages (U937) with *L. donovani* AG83 promastigotes and incubated with 5 and 10 µM of DIM and 10 µM of SAG as positive control as indicated in Figure. (**B**). Micrographs of Giemsa-stained hepatic and splenic smears of *L. donovani*-infected golden hamsters after treatment with DIM and SAG as a positive control. (**C** and **D**). DIM reduces the both hepatic and splenic parasites burden in infected golden hamsters. Experiments with intramuscular administration of DIM and SAG were performed in triplicate and representative data from one set of these experiments for liver and spleen are expressed as mean ± SD.

Figure S2: Characterization of mtDDC.
Confocal microscopy of kDNA loss in *L.donovani* wild type parasites and mtDDC. The parasites were stained with EtBr (0.1 µg/ml) in PBS (1X) containing 10% glycerol. Right column indicates the fluorescence-labeled cells viewed with a Leica confocal microscope. Left column indicates the merged pictures of fluorescence and phase contrast microscope. Magnification X100. Pictures are representative from one of three similar studies. N indicates to nucleus and K indicates to kinetoplast.

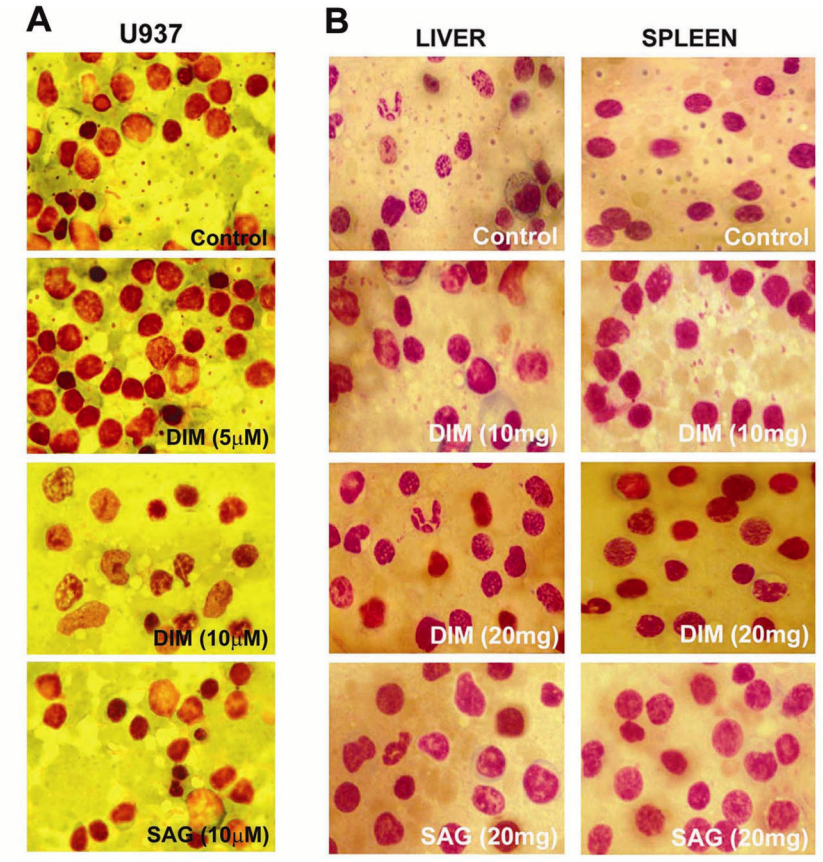
Supplementary Table 1

Groups	Organ weight (LIVER)	No. Of Amastigotes/ 100 Nuclei	Total parasites loading [*Organ wt. in mg X No. Of Amastigotes/Nucleus* X *(2X10^5)*]	Total % Of Inhibition
Infected control	5950 mg	185	5950x1.85x*(2X10^5)* **=22.02 x10^8**	00
SAG Control (20 mg/kg)	4530 mg	15	4530x0.15x*(2X10^5)* **=1.36 x10^8**	**94%**
DIM (10 mg/kg)	5024 mg	59	5024x0.59x*(2X10^5)* **=5.93 x10^8**	73%
DIM (20 mg/kg)	4668 mg	16	4668x0.16x*(2X10^5)* **=1.49 x10^8**	**93%**

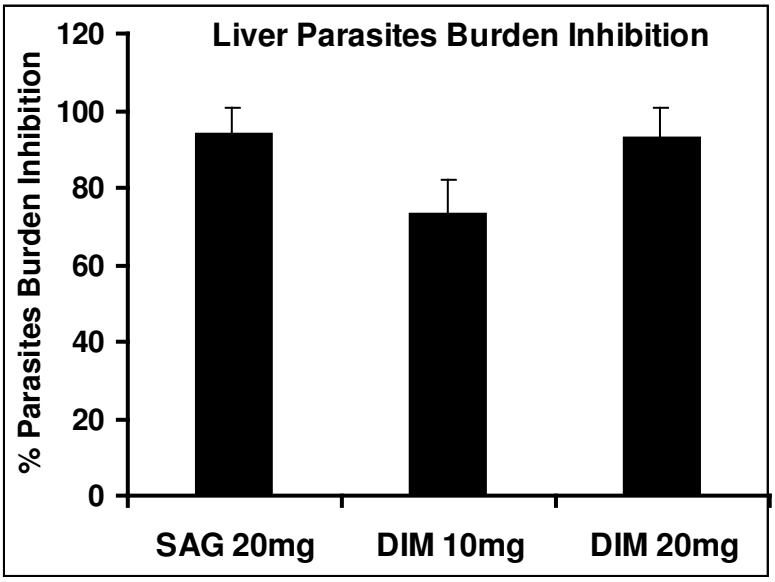
Supplementary Table 2

Groups	Organ weight (SPLEEN)	No. Of Amastigotes/ 100 Nuclei	Total parasites loading [*Organ wt. in mg X No. Of Amastigotes/Nucleus* X *(2X10^5)*]	Total % Of Inhibition
Infected control	730 mg	240	730x2.40x*(2X10^5)* **=3.50 x10^8**	00
SAG Control (20 mg/kg)	420 mg	14	420x0.14x*(2X10^5)* **=0.12x10^8**	**97%**
DIM (10 mg/kg)	572 mg	56	572x0.56x*(2X10^5)* **=0.64 x10^8**	82%
DIM (20 mg/kg)	424 mg	18	424x0.18x*(2X10^5)* **=0.15 x10^8**	**96%**

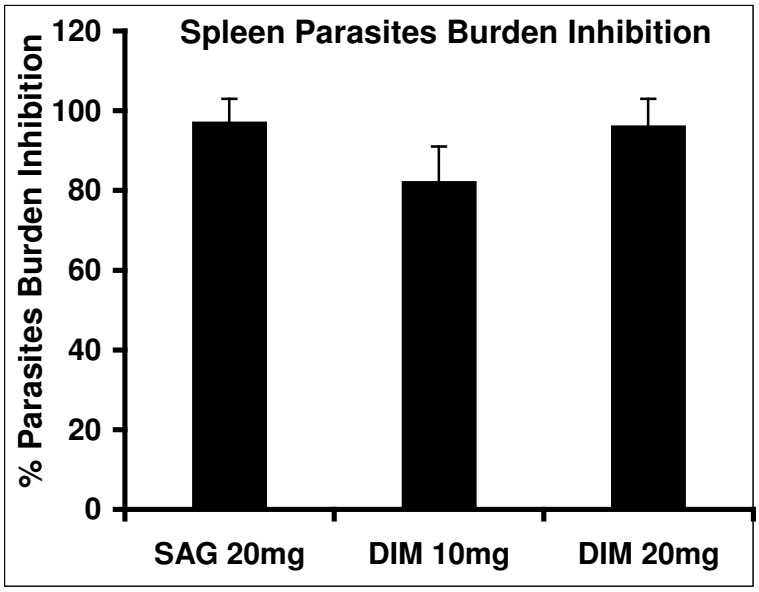
Supplementary Figure 1:



C


Liver Parasites Burden Inhibition
% Parasites Burden Inhibition
120
100
80
60
40
20
0
SAG 20mg
DIM 10mg
DIM 20mg

D


Spleen Parasites Burden Inhibition
% Parasites Burden Inhibition
120
100
80
60
40
20
0
SAG 20mg
DIM 10mg
DIM 20mg

Supplementary Figure 2: