In the present study we have evaluated the antiviral activity of a flavonoid-rich plant extract and its isolated compound luteolin against *in vitro* and *in vivo* HSV infection, along with the molecular mechanism of action. The flavonoid rich extract was isolated from an ethnomedicinal shrub *Pedilanthus tithymaloides* L. Poit of the family Euphorbiaceae, commonly used for antiviral, antibacterial, anti-inflammatory and related ailments (Bunyapraphatsara & Chokchaichareonporn, 2000; Ghosh *et al*., 2013). Previously we have reported that the methanol extract of *P. tithymaloides* leaves had promising wound healing (Ghosh *et al*., 2012), anti-inflammatory (Ghosh *et al*., 2013) and moderate antiviral activity and in the present study we aimed to validate its antiviral potential against HSV. Following a bioactivity guided study of the flavonoid-rich extract we have isolated two compounds out of which luteolin, a bioflavonoid, showed promising antiviral activity against the wild type and some clinically isolated strains of HSV-1 and HSV-2 at 43.6-52.6 µg/ml of the extract and 19.2-26.8µg/ml of luteolin, nearly ten times less than their 50% cytotoxic concentrations (436.5 and 278.6 µg/ml). The CPE reduction, MTT and Plaque reduction assay revealed that the isolated luteolin have better activity than its parent extract and even inhibit a thymidine kinase deficient (TK⁻) acyclovir resistance HSV-2 isolate, with SI value of 10-14, indicated that this compound needs further study for its potential antiviral nature. To find out the possible stage of viral life cycle affected by the test agents, we conducted time kinetic assays and observed that the test agents had significant activity at 2 h prior to 4 h post-infection, correlating with the preinfection, viral entry and immediate early replication.

To study the antiviral mode of action of the test agents we conducted attachment and penetration assay of the uninfected and virus-infected Vero cells, as well as the host cell immune modulations. Our results showed that luteolin might interact with the viral glycoprotein and/or cellular receptor during its attachment to the Vero cell at higher dose, along with modulation of host defense. The immunoblotting assay of virus-infected Vero cells detect p65 at relatively low level whereas p65 activation was significantly induced in drug-treated cells, indicating that the test drugs abated HSV-induced p65 nuclear translocation, and might blocked HSV induced NF-κB activation, with significantly down-regulated expression of viral ICP0 and ICP27 leading to the inhibition of HSV replication along with the inhibition of IκB-α degradation. Further, to address whether this inhibition is due to the interference on viral replication or not we analyze the cDNA synthesis and its amplification by RT-PCR from extracted cellular RNA of HSV-2 infected drug-treated or
untreated Vero cells upto 12 h post-infection, and observed significantly down-regulated expression of ICP0 and ICP27 in the drug-treated, indicating that the test agents interact with the immediate early replication of HSV. Moreover, we observed that HSV infection of murine macrophage leads to significantly increased production of TNF-α, IL-1β, IL-6, IFN-γ, NO and iNOS expression, which was significantly down-regulated in drug-treated macrophages, along with reduced expression of NO and iNOS, compared with infected and untreated cell. Our results collectively revealed that the test agents can inhibit replication of the wild-type, clinical isolates and TK-deficient ACV-resistant strains, accompanied with NF-κB, but not MAPK activation; which also support the earlier observation with some other natural compounds.

The toxicity studies of the test agents in Balb/C mice revealed its non-toxic nature at the antiviral dose upto 28 days with almost normal Pathophysiology, hematology, biochemistry and histopathology of the treated mice at 250-1000 mg/kg dose.

Further, the efficacy study showed that the virus yields in the skin and brain of infected mice on 2-8 days after infection were significantly reduced by the test extract (200 mg/kg) and luteolin (50 mg/kg) and the histopathology of skin and brain tissues of treated animals demonstrated limited infection, compared to the infection control. Therapeutic efficacy study on genital herpes model also demonstrated significantly reduced virus yields in the vaginal mucosa and brain tissue of infected mice on day 2-8 after infection, compared to infection and ACV control groups.

Further, our study with HSV-1 infected drug-treated Trigeminal Ganglia showed significantly down-regulated level of pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and IFN-γ compared to infection control, indicating the immunomodulatory potential of the test agents to clear the virus. Moreover, the HSV-1 infected mice, untreated or treated, showed abated HSV-induced p65 nuclear translocation, suggesting that the test agents might block HSV induced NF-κB activation and thereby prevent HSV-1 infection. Moreover, the test agents can able to reduce the significantly high level of TLR3 mRNA expression in infected mice, compared to the controls; and western blot analysis illustrated a luteolin-mediated inhibition of TRIF expression in HSV-infected mice, indicating that the flavonoid-rich extract or luteolin could reduce TLR3 mRNA levels, and inhibit TRIF expression following HSV-infection.