Malaria even today is a major public health problem of human beings in tropical and subtropical countries. The emergence of drug resistance in malaria parasite and insecticide resistance in mosquito vector make difficult the control of malaria by existing malaria control measures. Development of an effective vaccine against malaria would provide a cost effective adjunct to control malaria. Several antigens of \textit{P. falciparum} from different life cycle stages have been identified and characterized for the development of a subunit malaria vaccine. However, several vaccines have been entered in phase I, II and III field trials but they have shown low efficacy during the field trials. Presence of antigenic polymorphism in vaccine candidate antigens may be one of the reasons for low efficacy of these vaccines. Peptide based vaccine can also be utilized to control malaria but HLA polymorphism is a major obstacle in development of peptide based vaccine. However promiscuous peptides which have potential to bind with more than one HLA alleles may be utilized to develop vaccine against malaria. Identification of an appropriate T-Cell epitope for activation of immune system is of great importance before a strategy for development or trial of malaria vaccine is formulated. Keeping these points in view the present study was aimed to predict promiscuous peptides by analyzing the published gene sequences of strong vaccine candidate antigens, MSP-1, MSP-2, CSP, S-antigen, GLURP and EBA-175 of \textit{P. falciparum}. The immune response of predicted promiscuous peptide was quantified by using immune-proliferation analysis.

The \textit{Plasmodium falciparum} vaccine candidate antigens, MSP-1, MSP-2, CSP, GLURP, EBA-175 and S-antigen of \textit{P. falciparum} were screened to predict conserved promiscuous peptides by using NCBI database. Seven different freely available servers were used to predict promiscuous peptides. The peptides were predicted on the basis of 50% binding criteria of peptide with HLA alleles. The predicted peptides were characterized as strong binders, weak binders and non-binders on the basis of IC$_{50}$ value. To reduce the number of promiscuous peptides and to cover maximum HLA alleles, the peptides were further classified on the basis of 100% binding criteria. The best promiscuous peptides were selected for 3D homology modeling. The 3D homology modeling of promiscuous peptides and HLA-alleles was performed with Chimera1.8.1 and Modler9V7 program respectively. Further the docking of Promiscuous peptides with HLA alleles was performed by using Autodock1.5.4 program to select best docked peptide for \textit{in-vitro} immunogenic study. The best docked promiscuous peptide with minimum free binding energy EBA-9 obtained from F2 region of EBA-175 antigen was synthesized for the study of \textit{in-vitro} immune response. A
total of 10 blood samples were collected from the healthy individuals those have earlier exposure to *P. falciparum* infection and 10 blood samples were collected from healthy normal volunteers not having the history of *P. falciparum* infection. Peripheral Blood Mononuclear Cells (PBMCs) were collected and cultured then sensitized with different doses of promiscuous peptide to generate immune response. HLA typing was performed by sequence specific primer based assay. Lymphoproliferative response was measured by flowcytometric analysis. The production of cytokine IFN-γ and IL-4 by stimulated PBMCs was measured by ELISA.

On the basis of 50% binding affinity of predicted peptides with HLA alleles, a total of 39 highly conserved promiscuous peptides were identified for MSP-1, 32 for MSP-2, 10 for CSP, 25 for EBA-175, 01 for S-antigen. Further on the basis of 100% binding affinity selection criteria, 14 promiscuous peptides were found for MSP-2, 9 for EBA-175 and 05 for CSP antigen for HLA Class-II. Not a single peptide was found promiscuous for MSP-1, GLURP and S-antigen.

The docking interaction of promiscuous peptides with different HLA-Alleles was performed and the best docked peptide EBA-9 from F2 region of EBA-175 antigen with different HLA-alleles was found suitable for immunological response.

The peptide showed an encouraging immunological response and T cell proliferation was observed highest with 100µg/ml while 50µg/ml concentration showed moderate proliferation and percentage immune response was 100% for exposed population. The PBMCs obtained from unexposed population showed very low proliferation of lymphocytes. The measurement of cytokines IFN-γ and IL-4 production in supernatant was measured by ELISA. We observed the expression of IFN-γ significantly high in comparison to IL-4.

The study revealed that *in-silico* tools are time reducing and efficient tools for prediction of promiscuous peptides. In addition to this 3D homology modeling and docking study is a proficient way to identify an appropriate binder with different HLA-alleles in spite of testing a large number of peptides in a laboratory. The immunological study of synthetic peptide revealed that the predicted synthetic peptide is able to sensitized PBMCs and enhance the proliferation of CD4⁺T significantly among the PBMCs obtained from earlier *P. falciparum* exposed individuals as compared to unexposed individuals. It also determined that the memory cells (B Cells and T cells) are present in the isolated PBMCs and recognized the peptide of EBA-175 antigen in response to this sensitized PBMCs secreted the cytokines IFN-γ and IL-4. However the significantly low proliferation of lymphocytes obtained from
healthy individuals and absence of cytokines in supernatant shows the absence of memory cells in unexposed population. The present study also suggested that the in-silico technique is a powerful tool to develop a vaccine based on promiscuous peptides.