Summary
Malaria is a major killer disease in most part of the African and South East Asian countries including India. The drugs that are used to treat acute infection more often also results in mild to severe undesirable side effects in target organs. Liver and kidney being the major organs involved in drug metabolism, and its excretion are more susceptible to antimalarial drug toxicity. In spite of several reports of antimalarial induced drug toxicity in these organs, the molecular mechanism of this drug induced toxicity is not very clear. Present endeavor is thus an attempt to elucidate the mechanism of antimalarial drug toxicity and to analyze the underlying global gene expression changes following antimalarial drug treatment. Swiss albino mice of 7 to 10 weeks of age weighing 25 -30 g were used as animal models in this study.

Healthy Swiss mice were dosed with varying concentration of Chloroquine (CQ), the widely used antimalarial drug used against acute infection and disease prophylaxis. Although human therapeutic equivalent dose of CQ (360 mg/kg) in mice did not cause any toxicity neither in liver nor in kidney, its higher doses 1000mg/kg and 2000mg/kg did cause damage in liver and kidney tissue. The reason for this dose specific CQ toxicity in mice is investigated in present study and found that CQ at its highest dose causes generation of reactive oxygen species resulting in oxidative stress. Furthermore, noted was decrease in the activity and gene expression of antioxidant enzymes i.e. SOD, CAT, GPx and GR. These results are further supported by the histological examination of liver and kidney section of mice treated with CQ. To substantiate these findings, Quercetin (QN) a natural antioxidant is utilized and showed that CQ induced oxidative stress and perturbation in activity and gene expression of antioxidant enzymes can be restored by the prior treatment of quercetin.

Amodiaquine (AQ) and Sulfadoxine-Pyrimethamine (SP) are WHO recommended antimalarial drugs for the regions where CQ resistance is common. However these drugs and their combinations too are reported to be responsible for several cases of drug induced toxicity. Presently, Swiss mice were treated with the human therapeutic equivalent dose of AQ, SP and their combinations (AQ+SP) and followed its effects on activity and gene expression of antioxidant enzymes, and markers of toxicity in liver and kidney. Result shows that combination of AQ+SP is detrimental and causes significant oxidative stress both in liver and in kidney. However, histological
investigations of the concerned tissues fail to support this finding. These in conclusive and quite confounding results required further investigations. High throughput global gene expression profiling of liver and kidney tissue through DNA microarray is then conducted to reveal further mechanistic details. It is believed that high throughput expression profiling facilitates prediction of toxicity and interpretation of mechanism of toxicity based on distinct gene expression changes. The salient findings of global gene expression profiling of mice treated with AQ, SP and their combination AQ+SP were (i) more pronounced gene expression alterations were present in kidney than that of liver, (ii) in murine liver more number of genes were perturbed following combination therapy AQ+SP than that of individual treatment with AQ or SP, and (iii) both in murine liver and kidney the number of down regulated genes clearly outnumber the number of up regulated genes following exposure to AQ and SP. Moreover different pathway finding and clustering algorithms, indicates involvement of EGFR1 signaling pathway, electron transport chain, TCA cycle, apoptosis, DNA replication, inflammatory response, MAPK signaling, mRNA processing, cell adhesion, DNA replication, ion transport, T cell activation, transcription regulation pathway, and TGF-β signaling pathway to be the most affected one following antimalarial drug toxicity in murine liver and kidney. Diverse nature of these perturbed pathways following antimalarial drug treatment shows an overall disturbance in gross physiology and homeostasis in murine liver and kidney.

Malaria infection as such causes severe organ failure and tissue damage leading to morbidity and death. After analyzing gene expression changes and toxicity in healthy mice tissues exposed to antimalarial drugs, attempts were also made to elucidate the mechanism of toxicity and global gene expression changes in mice tissues infected with malaria. After infection with rodent malarial parasite Plasmodium vinckei in mice, its infected liver and kidney tissues were extracted and analyzed for abnormalities, biochemical markers of toxicity and global gene expression profile. Mice were grouped as per the rising level of percent parasitemia. It is observed that as the level of percent parasitemia increases the intensity of hepatic injury as measured in terms of levels of hepatic biomarkers (ALT, AST and total bilirubin) increases. Liver histology also shows plasmodium infiltration inside and around hepatocytes. However, plasmodium infection
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do not cause elevation in the level of serum creatinine or BUN the markers of nephrotoxicity. Further, no histological defects were seen in infected murine kidney.

Presently, global gene expression profiling in *Plasmodium vinckeii* infected swiss mice using microarray lead to conclusion that malarial infection at its early stage when it shows low parasitemia perturbs more genes in liver than that in kidney. Furthermore, the no. of genes which are down regulated are much more than those that are up regulated both in kidney as well as in liver. Another key finding of this study was large degree of similarity (approximately 80%, both in liver as well as in kidney) among the expression profiling of intermediate and high level of percent parasitemia while the expression profile in the group with low percentage parasitemia is quite distinct from rest of groups. This shows that intermediate and later stages of parasitic infection were more similar in terms of number of genes deregulated than that of initial stages of infection. Further, parasitemia as low as 2% is not sufficient enough to cause robust changes in liver and kidney as that exhibited by high and intermediate level of parasitemia. The major biochemical pathways that were perturbed by malarial infection as revealed by using clustering and pathway finder software for gene expression dataset include cell adhesion, antigen processing and presentation, natural killer cell mediated cytotoxicity, MAPK signaling pathway, JAK-STAT signaling pathway, T cell receptor signaling pathway, cytokine-cytokine receptor interaction pathway and complement and coagulation cascade.

This study is a first comprehensive analysis at genomic scale to elucidate gene expression signatures of antimalarial drug toxicity and malarial pathogenesis. The study further establishes microarray as a useful prognostic tool to detect toxicity following drug administration or pathogen infection at earliest stage.