Discussion
Malaria continues to be a menace for most of the third world tropical countries affecting lives of nearly half a billion people. As we are still far away from obtaining a reasonably good antimalarial vaccine, efforts were made to judiciously utilize the currently available antimalarial drugs. However, most of the currently available antimalarials possess mild to severe side effect for vital body organs including liver and kidney. It is therefore earnestly required to understand the molecular mechanism of antimalarial drug toxicity in liver and kidney. High throughput expression profiling facilitates prediction of toxicity and interpretation of mechanism of toxicity based on distinct gene expression changes. The simplest approach to identify genes of potential interest through several related experiments is to search for those that are consistently either up or down-regulated. Present study is an attempt to understand the mechanism of antimalarial toxicity using conventional and high throughput microarray techniques. Onset of malarial infection has also resulted in severe damage to many body organs and therefore, in this study I also look to delineate the major affected pathways following malarial infection in mouse model of disease. This study is therefore expected to provide insight about the relative safety of selected antimalarials and their combinations, unearth gene expression signatures related to hepatotoxicity and nephrotoxicity, if any, and establish microarray technology as a predictive tool to understand tissue responses at an early stage following treatment with antimalarials.

It has been previously reported that Chloroquine (CQ) imparts hepatotoxicity in animal models but its mechanism was not very clear. Present study evaluates dose specific effect of CQ on mice liver and kidney and its possible protection by quercetin a natural herbal flavonoid in Swiss Albino mice. Serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST) are established biomarkers of liver damage. It is believed that the increase in the level of these enzymes in the serum is indicative of tissue injury with subsequent release of the enzymes into the circulation from the damaged liver tissues (Ozer et al., 2008). Statistically significant (P<0.05) elevation of ALT & AST in animals treated with 1000 and 2000 mg/kg of CQ respectively is observed in present study. Animals treated with therapeutic equivalent dosage of 360 mg/kg of CQ did not show any elevation of ALT & AST level when compared with untreated controls. Similarly, serum creatinine and blood urea nitrogen are established
biomarkers for kidney damage. A significant rise in level of Creatinine and BUN indicating nephrotoxicity is observed in groups treated with 2000 mg/kg of CQ. However lower doses of CQ including therapeutic equivalent dose of 360 mg/kg did not show any liver damage. Moreover, quercetin pretreatment to chloroquine administration was found to be very effective in attenuation of nephrotoxicity as levels of creatinine and BUN in these groups of animals were near to controls. My investigation hence revealed that CQ is safer at its therapeutic dose but its aggravated doses do causes hepatotoxicity and nephrotoxicity in mice. Histological observations of liver and kidney shows eosinophilic bodies and lymphocyte infiltration an indicator of stress in mice treated with 2000mg/kg of CQ. However, there are no alterations in liver histology in other groups, reinforcing dose dependency of CQ hepatotoxicity. Interestingly, no appreciable histological changes were observed for kidney tissue in any of the treatment groups. This might be due to acute dosing of CQ and sacrificing the animals just after 24 h which fails to elicit any histological changes.

Previous studies have indicated generation of reactive oxygen species (ROS) and oxidative stress following CQ intoxication (Dass et al., 2000; Pari et al., 2004). I have used flow cytometry method to measure intracellular ROS generation and found an increase in blood ROS level as measured in terms of mean fluorescence intensity for animals treated with 2000 mg/kg of CQ when compared with controls. This CQ mediated ROS generation might be the factor responsible for liver toxicity and oxidative damage observed in group treated with 2000 mg/kg of CQ. I further observed in the present study that quercetin pretreatment significantly decreases ROS generation in CQ treated animals establishing free radical scavenging property of this natural flavonoid. Membrane lipids are among the first to succumb to deleterious effects of ROS and measurement of lipid peroxidation is a commonly used index of increased oxidative stress and subsequent cytotoxicity. It is believed that lipid peroxidation is initiated by the attack of a free radical on fatty acid or fatty acyl side chain of any chemical entity (de Zwart et al., 1999) and is regarded as one of the basic mechanisms of tissue damage caused by free radicals. In present study a significant increase in the concentration of thiobarbituric acid reactive substances in liver of mice treated with 1000 and 2000 mg/kg of Chloroquine respectively was observed. In kidney tissue however, I could observe a statistical
decrease in TBARS level of only 2000 mg/kg CQ treated mice. Quercetin pretreatment in 2000 mg/kg mice significantly decreases the membrane lipid damage as the concentration of thiobarbituric acid reactive substances in both liver and kidney tissues were brought to near normal. These findings not only establishes antioxidant and free radical quenching property of quercetin in CQ treated mice but also suggests a dose specific oxidative stress production in mice liver and kidney treated with chloroquin.

Reduced glutathione i.e. GSH, a non enzymatic antioxidant pool in the body that is synthesized in the cytoplasm of the liver cells and then distributed through circulatory system into different organs and subcellular compartments is considered as first line of defense against oxidative insult (Meister et al., 1983). GSH plays a crucial role in both scavenging ROS and detoxification of drugs and chemicals. GSH reacts directly with ROS and electrophilic metabolites, protects essential thiol groups from oxidation. Therefore, perturbation in the redox status of GSH can not only impair cell defense against toxic compounds, but also result in enhanced oxidative stress and tissue injury (Meister et al., 1983). I observed a significant decrease in the level of GSH both in liver and kidney of animals treated with 2000mg/kg CQ in comparison to controls. Furthermore, when supplemented with 50 mg /kg body wt. of quercetin, it was able to recoup the loss of GSH content to almost normal. This further establishes the potential of CQ to impose oxidative stress in mice liver and kidney.

Having established the dose specific potential of CQ in imparting oxidative stress and subsequent toxicity in murine liver and kidney it was imperative to study the status of antioxidant enzymes in these tissues upon CQ treatment. GR and GPx are glutathione dependent major phase –II drug metabolizing enzymes. I found a decrease in levels of both these enzymes in animals treated with 2000mg/kg CQ in comparison to controls in mice liver. Interestingly, the profile of these two enzymes in murine kidney seems to differ from their liver counterparts as the GR and GST levels in murine kidney is quite unaffected by CQ treatment. This discrepancy might be a reflection of difference in anatomy and biochemical physiology of liver and kidney.

SOD accelerates the conversion of superoxide radical (O\textsubscript{2}\textsuperscript{-}) to H\textsubscript{2}O\textsubscript{2} while CAT and GPx scavenge H\textsubscript{2}O\textsubscript{2} and convert it to water (Mate's et al., 1999; Jenkins, 1993). In this study, CQ at the dose of 2000mg/kg showed significant decrease in the activity of
Discussion

SOD and CAT both in murine liver and kidney when compared with untreated controls. Previous researchers have reported that decreased SOD activity reflects oxidative stress (Jenkins, 1993). Moreover, decrease in the activity of CAT in these group of mice may be due to the excess of superoxide anion radical as a consequence of a reduction in the activity of SOD. Previous reports have also indicated that high production of superoxide anion radical inhibits CAT activity (Kono & Fridovich, 1982). Further in this study it is observed that human therapeutic equivalent dose group treated with 360 mg/kg CQ did not show any appreciable ROS generation or decrease in the level of antioxidant enzymes, suggesting that chloroquine at therapeutic dose is quite safe at both liver and kidney and did not causes oxidative damage. Furthermore, I also look for the mRNA transcript change of SOD, CAT, GR and GPx in murine liver and kidney after dose specific treatment of CQ. A gain or loss of mRNA expression of a particular gene is a profound indicator of accompanying cellular environment of that gene. My speculation was that decrease in the activity of antioxidant enzymes in mice treated with 2000mg/kg CQ, may be a consequence of either decreased de novo synthesis of enzyme proteins characterized by reduction in mRNA transcript level or irreversible inactivation of enzyme proteins from increased free radical production resulting from chloroquine metabolism.

To test this hypothesis a real time qPCR analysis of mRNA expression of these antioxidant enzymes in liver and kidney tissue is carried out in order to check their abundance at transcript level. My investigations revealed decrease in liver mRNA transcript of major antioxidant enzymes (SOD, CAT, GPX and GR) in group treated with 2000mg/kg CQ with respect to that of controls. This decrease in expression level might be the effect of generation of ROS and/or development of oxidative stress resulting finally in the decline in enzyme synthesis (Limaye et al., 2003). Contrary to liver, kidney mRNA expression profile shows not only decrease in expression of SOD and CAT but also a slight increase in the expression of GPx and GR. However, some processes such as post transcriptional modification, makes it possible that mRNA expression does not fall in line with protein abundance and activity in spite of obvious fact that protein turn out is dependent on its mRNA transcript synthesis. Quercetin pretreatment improves the decreased mRNA expression of these antioxidant enzymes in CQ treated animals.
Altogether, these expression studies establishes that in spite of some differences in liver and kidney transcripts, after effects of oxidative stress and free radical damage can be seen at transcript levels of both these organs.

Although, CQ was the drug of choice against malaria for several decades, rapid emergence of resistance to CQ in plasmodium species (mainly Plasmodium falciparum) forced health professional to search for other suitable drug or drug combinations that could treat malaria. Another 4-Aminoquinoline derivative, namely Amodiaquine (AQ) was successfully utilized against malaria since last 10-15 years. AQ is used both either as single monotherapy or in combination with Sulfadoxine-Pyrimethamine (SP)- an antifolate drug. WHO guidelines (2006) have also advocated the use of AQ and SP as antimalarials in the areas where CQ resistance is seen. However, the use of AQ and SP as antimalarials is also under scanner as these drugs have resulted in some untoward side effects resulting in organ and tissue toxicity. Hence, present study also looks into the AQ and SP induced toxicity/changes in liver and kidney so as to further our knowledge about antimalarial drug toxicity.

Dosages and duration of AQ and SP treatment in swiss mice was as per the human therapeutic equivalent dose and malaria treatment regime as according to recent WHO guidelines (WHO guidelines for malaria treatment 2006). It is observed that AQ treatment alone do not produce toxicity or oxidative stress be it liver or kidney. Although administration of SP too, does not cause any elevation in level of ALT, AST, creatinine and BUN and does not produce oxidative stress in kidney, it does causes appreciable oxidative stress in liver. However, co treatment of AQ and SP (AQ+SP) as per the recommended combination therapy regime does produce toxicity and oxidative stress in liver. Furthermore, AQ+SP treatment causes oxidative stress in kidney but do not result in nephrotoxicity. None of these drugs or drug combinations results in alteraions in normal liver and kidney histology as no histopathological damage was observed in any sections of these two tissues.

It is further observed in the case of liver that activity of SOD is not affected either by the treatment of AQ or SP or their combination (AQ+SP). However, the level of GPx is significantly reduced in all three treatment groups and catalase activity is reduced in SP and AQ+SP group in murine liver fraction. Interestingly, GR activity was observed to
increase in AQ and AQ+SP. In kidney tissue, although AQ treatment does not induces any alteration in the activity of antioxidant enzymes studied, SP and AQ+SP treatment do causes decrease in activity of SOD, CAT, and GPx. As stated above in the case of CQ toxicity, the alterations in activities of antioxidant enzymes of liver and kidney observed here are also the outcomes of oxidative insult to the cells and tissues brought by the AQ and SP dosing. Furthermore, I observe in liver tissue an increase in mRNA transcript level of SOD, CAT and GPx in case of AQ treatment while for SP and AQ+SP treatment, transcript levels of SOD, CAT and GR decreases. The mRNA level of GPx is however increasing in all the treatment groups. In kidney tissue, I observed very vague kind of mRNA expression pattern for SOD, CAT and GR in all three treatment groups. It seems that transcript of these enzymes do not change in response to drug treatment. However, in AQ+SP group the mRNA expression of GPx is significantly decreased reestablishing the oxidative stress potential of AQ+SP combination therapy in murine kidney. I observe that mRNA expressions of antioxidant enzymes both in kidney and liver do not fall in complete agreement with the pattern of changes observed in their protein activity. Again, I speculate that this may be an affect of inherent biochemical and molecular machinery within cell interior that disturbs the harmony between the transcription and translated products. Our results so far gave us the notion that it is the combination AQ+SP which is more detrimental both for liver and kidney than the individual monotherapy of AQ and SP. However, it was clearly that biochemical, histopathology and antioxidant gene expression are not able to provide a concrete picture of mechanism of AQ and SP induced changes in murine liver and kidney. Therefore I supplemented present findings with mouse whole genome expression profiling for liver and kidney following exposure to AQ, SP and their combination AQ+SP.

High throughput expression profiling facilitates prediction of toxicity and interpretation of mechanism of toxicity based on distinct gene expression changes. The simplest approach to identify genes of potential interest through several related experiments is to search for those that are consistently either up or down-regulated. I have studied differential expression in murine liver and kidney at high statistical stringency (i.e. \( P < 0.01 \) and expression fold change >2) and at a dose and duration of exposure, which is therapeutic equivalent to that used in human. This increase the
statistical confidence in the detection of important genes and cellular processes with a probable role in the initiation and propagation of toxicity as well as those possibly involved in regeneration during the early phase of tissue response. Furthermore, inherent biological variations among the members of the same group were reduced by pooling RNA samples. Technical means of error were corrected by performing dye swap experiments and normalizing the microarray data. This ensured detection of probes corresponding to significantly deregulated genes with a high statistical confidence following drug treatment. Furthermore, validation of microarray findings using qRT-PCR further substantiates these results, which is the most sensitive and accurate method of validating microarray based differential expression of genes (Chuaqui et al., 2002). Therefore, identification of genes with similar expression changes, at such a high stringency, both in direction and magnitude indicate important biological functions under these circumstances.

In murine liver exposed to AQ results in differential regulation of 133 probes of which 60 are up regulated and 73 are down regulated. The same dose and exposure schedule of AQ however results in more robust changes in murine kidney as the number of differentially expressed probes increases to 359 in kidney almost 2.8 fold to that observed in liver. More interestingly, of the 359 probes differentially expressed in kidney, 235 is down regulated, greater than two fold to the number of up regulated probes which is only 124. Similarly in the case of SP treated animals, number of differentially expressed probes in kidney which is 347 clearly outnumbers their counterparts in liver which are just 156. However, in SP treated groups, number of up and down regulated probes are fairly balanced both in liver (90 upregulated and 66 down regulated) and kidney (160 up regulated and 187 down regulated). The pattern of differential expression of genes in combination therapy i.e. AQ+SP treated groups in murine liver were on expected line, showing more robust expression pattern than either of the drug given alone. Here the number of differentially expressed probes is 231 far ahead of differentially expressed genes in AQ (133) or SP (156). Of the 231 differentially expressed genes in murine liver after AQ+SP treatment, number of up regulated (118) and number of down regulated (113) probes are almost similar. However, the pattern of differential expression of AQ+SP in murine kidney is different from their liver
counterparts. Here in kidney, total no. differentially expressed probes is 360 a figure close to the number of differentially expressed probes in AQ (359) and SP (347). Moreover, of the 360 differentially expressed probes in murine kidney after exposure to AQ+SP, 170 were up regulated and 190 were down regulated, numbers which are fairly similar.

One of the gene that is consistently up regulated in murine kidney after exposure to all treatment regime in the study i.e. AQ, SP and AQ+SP is adapter protein Mal also known as TIRAP. Mal mediates downstream signaling of TLR2 and TLR4. These Toll-like receptors (TLRs) are members of the signaling pathway and are important in the initiation of the innate immune response to a wide variety of pathogens. Fitzgerald et al. (2001) showed that overexpression of MAL activates NFKB, JNK1, ERK1, and ERK2 - important genes in signaling cascade. More recently, Khor et al. (2007) found that heterozygous carriage of a leucine substitution at ser180 of the TIRAP gene (rs8177374) conferred protection against malaria, invasive pneumococcal disease, bacteremia, and tuberculosis independently in 6,106 individuals from the UK, Vietnam, and several African countries. I interpret that TIRAP overexpression in murine kidney in response to the antimalarial drug is a kind of preventive measure of the body against stress development by conferring initiation of immune cascade. It is also supported by findings of Yamamoto et al. (2002) which show that splenocytes and macrophages from Tirap-deficient mice are unable to proliferate or produce cytokines in response to lipopolysaccharide (LPS).

Another important genes showing up regulation in murine kidney following exposure to antimalarials is BIRC4 also known as XIAP (X-linked inhibitor of apoptotic protein). It belongs to a multigene family whose members show extensive homology to baculovirus IAPs (inhibitor of apoptosis proteins) and encode proteins that demonstrate significant inhibition of apoptosis. Deveraux et al. (1997) showed that X-linked IAP directly inhibits at least 2 members of the caspase family of cell-death proteases, caspase-3 and caspase-7. As the caspases are highly conserved throughout the animal kingdom and are the principal effectors of apoptosis, these findings suggest how IAPs might inhibit cell death, providing evidence for a mechanism of action for these mammalian cell-death suppressors. Our finding of up regulation of XIAP in antimalarial treated mice
is important in this context that it shows suppression of apoptotic pathway in murine kidney after drug treatment. Similarly, it was found that one gene called RNF6 (Ring finger protein 6) was down regulated in murine kidney following exposure to antimalarials. Lo et al. (2002) found RNF6 to be a tumor suppressor gene. The down regulation of this tumor suppressor gene following antimalarial drug treatment may suggest rapid cell proliferation and intracellular stress. In murine liver, I found up regulation of DUSP3 (Dual-Specific Phosphatase 3) which constitute a large heterogeneous subgroup of the type I cysteine-based protein-tyrosine phosphatase superfamily. DUSPs are characterized by their ability to dephosphorylate both tyrosine and serine/threonine residues. They have been implicated as major modulators of critical signaling pathways (Patterson et al., 2009). Alonso et al., (2003) noted that DUSPs suppresses T-cell receptor (TCR)-induced activation of ERK2 and JNK. They extended this finding by showing that DUSP3 expression reduced activation of an NFAT1-AP1 driven reporter and TCR signaling to interleukin-2. Their up regulation in murine liver following antimalarial drug exposure is yet another indication of modulation of immune surveillance in murine liver by treatment with antimalarial drugs.

One of the many genes that are up regulated in murine liver following exposure to AQ,SP and their co-treatment i.e. AQ+SP includes EPRS (Glutamyl-prolyl tRNA synthetase). EPRS is a multifunctional aminoacyl-tRNA synthetase that catalyzes the aminoacylation of glutamic acid and proline tRNA species (Cerini et al., 1991). Sampath et al. (2004) showed that EPRS has a regulated, noncanonical activity that blocks synthesis of ceruloplasmin. They identified EPRS as a component of the gamma-interferon activated inhibitor of translation (GAIT) complex by RNA affinity chromatography using the GAIT element in the 3-prime UTR of ceruloplasmin as ligand. Sampath et al. (2004) further demonstrated that, in response to IFNG, EPRS is phosphorylated and released from the multisynthetase complex. It subsequently binds the 3-prime UTR of ceruloplasmin in an mRNA ribonucleoprotein complex containing GAPDH, NSAP1 (SYNCRIP), and L13a and silences ceruloplasmin mRNA translation. Fall in the level of ceruloplasmin which is the major copper carrier protein, is an indication of hepatic stress (Scheinberg et al., 1951), so the elevation in the level of EPRS following antimalarial drug treatment can be the reason for observed hepatic
stress. Supervilin (SVIL) is another gene that is consistently up regulated in murine liver following exposure to AQ, SP and their combination. This gene codes for a protein which is tightly associated with both actin filaments and plasma membranes, suggesting that it forms a link between the actin cytoskeleton and the membrane. Ting et al. (2002) cloned and characterized supervillin as an androgen receptor coregulator from a skeletal muscle cDNA library. An upregulated SVIL (which is required for membrane integrity) following drug treatment may be an explanation for the rise in lipid peroxidation level observed in the present study. It appears that membrane damage following antimalarial drug treatment is an inducing factor for up regulation of supervillin.

One of the several genes that were down regulated following antimalarial exposure in murine liver is MTMR2 (Myotubularin related protein 2). The MTMR2 gene encodes a protein that belongs to the myotubularin family, which is characterized by the presence of a phosphatase domain. Berger et al. (2002) determined that mouse Mtmr2 dephosphorylates phosphatidylinositol 3-phosphate (PI3P) and phosphatidylinositol 3,5-bisphosphate (PI3,5P2) with high efficiency and peak activity at neutral pH. A perturbation in phoshatidyl inositol pathway resulting from down regulated MTMR2 expression is an indication of disturbances of signaling pathways following antimalarial treatment.

DNA microarray analysis also has the potential of delineating toxic signatures of different tissues with respect to drug/chemical exposure. These signatures are obvious representation of tissue specific responses induced by the drug or chemical or any other external stimulus. Using SAM (Significance analysis of Microarray), I was able to obtain 22 distinct probes that could distinctly differentiate the gene expression patterns of liver and kidney observed in the study. These probes includes HSP90ab1 (Heat Shock Protein 90KD, Alpha Class B member 1), SLC43a3 (Solute Carrier Family 43 member 1), PAWR (PRKC, apoptosis, WT1, Regulator), and IKbRb (Inhibitor of Kappa light chain gene enhancer in B cells, kinases of Beta) among others. HSP90 proteins are highly conserved molecular chaperones that have key roles in signal transduction, protein folding, protein degradation, and morphologic evolution. They are often considered as cytosolic markes of stress physiology (Chen et al., 2005). An upregulated HSP90ab1 observed in the present study in the murine liver emphasize that antimalarial drug
exposure has resulted in the development of hepatic stress. SLC43A1 belongs to the system L family of plasma membrane carrier proteins that transports large neutral amino acids (Babu et al., 2003). Chuaqui et al. (1997) found that prostate carcinoma samples from 5 of 10 patients showed substantial overexpression of SLC43a3. This report should be visualized with the observed intracellular stress following antimalarial treatment in the present study. PAWR genes which are also called PAR4- the transcriptional repressors are found to be transcriptionally induced by apoptotic signals in the rat ventral prostate (Johnstone et al. (1996). Through functional analyses they were able to show that PAR4 inhibit transcription activated by WT1(Wilms tumor suppressor protein) and augmented WT1-mediated transcriptional repression. Woronicz et al. (1997) observed that IKbRb activates NF-kappa-B when overexpressed and phosphorylate serine residues 32 and 36 of I-kappa-B-alpha and 19 and 23 of I-kappa-B-beta. The activity of IKbRb was stimulated by TNF and IL1. Overexpression of catalytically inactive IKK-beta blocked cytokine-induced NF-kappa-B activation (Ozes et al., 1999). I believe that upregulated PAWR and IKbRb in murine liver is an indication of cellular toxicity and inflammatory responses within liver hepatocytes following antimalarial exposure.

Having established the distinct gene expression signature of antimalarial drug exposure in liver and kidney of healthy Swiss mice, I was also interested in knowing the similar expression signatures in the aforesaid tissues in malaria infected Swiss mice. These expression signatures will add in our understanding of malaria pathogenesis and can give new insight into the mechanism of malaria driven toxicity in concerned tissues. I have utilized the Plasmodium vinckei petrii infected murine malaria model for my further study. Plasmodium vinckei petrii infected mice exhibit symptoms and physiological characteristics similar to human malaria, hence used extensively as good model for human malaria (Puri et al., 2006). Our strategy was to look for the events inside liver and kidney at different course of malaria infection in terms of number of parasites infecting red blood cells. As per the earlier reports (Guha et al., 2007), I observe the rise in level of ALT, AST and total bilirubin indicating hepatotoxicity following infection with Plasmodium vinckei. I observe 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. My observation of hepatotoxicity and severe malaria
infection is further substantiated by histopathological findings of liver tissue. Liver section of group of mice heavily infected with *Plasmodium vinckei* shows presence of parasite in and around hepatocytes and distorted liver histology. However kidney tissue histology does not seem to change after malaria infection as both the glomerulus and renal corpuscles were seen intact in all the kidney section studied. Furthermore, malaria infection do not elevates the levels of Serum creatinine and serum BUN indicating no apparent kidney damage following malarial infection. As the malaria parasites do not traverse through the kidney, it is expected that histological and serum biomarker changes will not appear following malarial infection. Although, malarial parasite does not enter into renal circulation, the effects of parasitic infection on kidney can not be ruled out. There were reports of gross renal abnormalities during malaria infection (Guha et al., 2007).

In line with our previous microarray experiments with antimalarial drugs, here also I used statistical stringencies of P \(<0.01\), and fold change (FC) >2.0. The gene expression profile which is commonly seen in murine liver at all levels of percent parasitemia in the study (P1, P2, and P3) shows perturbation in gene expression of 429 genes. Of these, 256 are up regulated while 173 are down regulated. As expected, gene expression changes in murine kidney after malarial infection was not very robust as only 56 genes are found that are deregulated at all levels of parasitic burden. Among 56 genes that are differentially expressed in kidney, 32 are upregulated and 24 of them are down regulated. Furthermore, in murine liver infected with malaria, P1 group showing low parasitemia exhibit perturbation in gene expression of 321 genes, of which majority (251),is down regulated. Only 70 genes were upregulated in this category of animals. Moreover, in murine kidney only 20 genes were differentially regulated of which 7 is up regulated and 13 is down regulated. These findings lead me to conclude that malarial infection at its early stage when it shows low parasitemia perturbs more genes in liver than that in kidney. Further, the number of genes which are down regulated are much more than those that are up regulated. Malaria infection at intermediate stage (P2) after 5 days of infection when parasitemia is between 20 to 30 %, 57 and 196 genes were up regulated in murine kidney and liver respectively. In this group I found the down regulation of 64 and 108 genes in kidney and liver respectively. Similarly, in P3 group
where parasitemia percentage is more than 50, 190 and 31 genes were up regulated respectively in murine liver and kidney. The corresponding down regulated no. of genes in this group were 141 and 61 respectively for liver and kidney. These findings further lead me to conclude, that gene expression changes after malarial infection both in initial as well as in later stages of infection were more pronounced in murine liver than that in murine kidney. This finding is on expected line as it is the liver and not kidney where parasite goes through and multiplies, the changes observed in kidney is just after effect of changes in normal physiology and inflammatory responses of the body following malarial infection.

Another key finding of this microarray experiments was large degree of similarity (approximately 80%, both in liver as well as in kidney) among the expression profiling of P2 and P3 while P1 expression profile is quite distinct from rest of groups i.e. P2 and P3 showing less than 20% similarity for the perturbed genes. I believe that 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.

Among several of the genes that were consistently upregulated in murine liver following malarial infection is HMOX1 (Heme oxygenase-1). The HMOX1 gene encodes heme oxygenase, the rate limiting step in the catabolism of heme to form biliverdin, which is subsequently converted to bilirubin by biliverdin reductase, free iron, and carbon monoxide. The basal level of heme oxygenase expression may serve as a first protective environment against acute oxidative and inflammatory insults (Wagener et al. (2003). Using quantitative RT-PCR, Pamplona et al. (2007) earlier showed that Hmox1 was upregulated to a greater extent in BALB/c mice after P. berghei infection. Thus our study falls in line with previous reports and together advocates upregulated HMOX1 expression in liver tissue as an indicator of malaria infection and toxicity. Igfbp1 (Insulin like growth factor–binding protein 1) is another gene which has been upregulated in murine liver following malarial infection. Previously, Leu et al. (2003) reported that Igfbp1 is one of the most rapidly and highly induced genes in regenerating liver following partial hepatectomy. Further, Leu & George (2007) found that p53 activation
led to enhanced expression of IGFBP1 in human hepatoma cells. A portion of intracellular IGFBP1 localized to mitochondria, where it bound the proapoptotic protein BAK. Binding of IGFBP1 to BAK impaired formation of the proapoptotic p53/BAK complex and induction of apoptosis in cultured human and mouse cells and in mouse liver. Thus, overexpression of IGFBP1 observed presently is an indication of apoptosis induction (as also observed by Guha et al., 2006) and underlying hepatic toxicity.

CHN2 (Chimerin2) was one of the gene which has been down regulated following *Plasmodium vinckei* infection in murine liver. It is believed to play a role in membrane/cytoskeletal reorganization events. Caloca et al. (1999) analyzed its properties as a DAG receptor by using a series of conformationally constrained cyclic diacylglycerol (DAG) analogs (DAG lactones) as probes. It appears that downregulation of CHN2 following malarial infection is an indication of membrane damage and impeded signaling cascade following plasmodium infection in liver tissue.

In murine kidney I observed up regulation of TNFS10 (tumor necrosis factor superfamily member 10). Tumor necrosis factor (TNF) family cytokines function as prominent mediators of immune regulation and the inflammatory response. Wiley et al. (1995) showed that TNFS10 rapidly induces apoptosis in a wide variety of transformed cell lines of diverse origin. Hoffmann et al. (2007) concluded that TNFS10 is a modulator of granulocyte-driven inflammation that limits the life span of activated leukocytes through its proapoptotic activities. They also observe that it enhances granulocyte recruitment and may have potential for terminating an acute inflammatory response during invasive infections. These findings suggest that murine kidney might be put into apoptotic changes following malarial infection as like liver. IRF1 (Interferon regulatory factor 1) is another gene which I have found to be upregulated in murine kidney infected with *Plasmodium vinckei*. It has been reported previously that IRF1 functions as a transcriptional activator for the type Interferon(IFN) genes (Harada et al., 1990). Further, Ko et al. (2002) noted that Irf1 -/- mice are deficient in INOS, Il12b, Cd8 -positive T cells, and natural killer (NK) cells. Thus, IRF1 downregulation in kidney following malarial infection suggests perturbations in immunological pathways that may lead to proinflammatory responses. Among several genes that have been downregulated in murine kidney following malarial infection, SLC24a3 (Solute carrier family) is one of
Discussion

them. Plasma membrane sodium/calcium exchangers are an important component of intracellular calcium homeostasis and electrical conduction. Potassium-dependent sodium/calcium exchangers such as SLC24A3 are believed to transport 1 intracellular calcium and 1 potassium ion in exchange for 4 extracellular sodium ions (Kraev et al., 2001). A down regulated SLC24A3 indicates membrane damage or some intracellular stress as disturbances in solute balance in and across may lead to loss of polarisability across the membrane.

Thus, high throughput gene expression profiling both in the case of mice infected with malaria and mice treated with antimalarial drugs was successful in distinctly characterizing the gene expression signatures specific to tissues, the severity of malaria infection and the type of drug administered. Furthermore, present differential gene expression profiling suggests the involvement of multiple pathways ranging from inflammatory pathways to changes in oxidative stress and immune surveillance in determining the toxicological or other changes observed in liver and kidney tissues of swiss mice exposed to antimalarial drug or infected with rodent malarial parasite—Plasmodium vinckeii. Moreover, identification of robust gene expression alterations in the absence of biochemical and histological markers, encourage microarray technology as a useful tool to understand molecular events at an earlier stage. However, assigning precise molecular functions and understanding complete functional significance of each of the differentially regulated genes is difficult at present and requires further studies at different doses both at gene and protein level. Moreover, many of the probes identified in this study have no biological function assigned as yet and may be important in the initiation and progression of disease or may merely represent an initial protective signature which might otherwise have led to more serious effects under chronic exposure of the drug.