5. DISCUSSION

Rodent malaria parasites have been widely used as experimental models due to their adaptability to the laboratory conditions. *P. berghei* (NK-65) is a virulent strain (Waki *et al.*, 1982). BALB/c mice has been found to be susceptible to this strain in present study too. Virulence of *Plasmodium* depends partly on strain of the parasite and partly on host (Cox, 1988). *P. berghei* takes distinctive course of infection, which is marked by rapid increase in number of circulating parasites and fatal termination of infected animal within 7-14 days (Suzuki, 1973). Similar results have been obtained in present investigation. Ager (1984) has reported that *P. berghei* infection in mouse results in high levels of parasitaemia and mortality. *P. berghei* (in rodents) is equivalent to *P. falciparum* (in humans) in causing cerebral malaria resulting in death of their respective hosts (Liew *et al.*, 1991). Migration of parasite into reticulocytes leads to enhancement in survival time of mice. Preference of *P. berghei* for reticulocytes is also established (Galinski *et al.*, 1992). Use of rodents as research model, has helped in the understanding of many aspects of biology of *Plasmodium* (Janse and Waters, 1995).

Chemotherapy is the treatment of disease with chemical compounds that has selective toxicity against the diseased target (Riddler and Jenning, 1983). Chemotherapy has become more complex and challenging because of multidrug resistant strains of *P. falciparum* (Kumar *et al.*, 2003). Mankind has always mined plants for the treatment of various ailments (Jones, 1996). The most important
drugs (Quinine and Artemisinin) have been obtained from the plants. Quinine has been obtained from *Cinchona* sp. (Phillipson and Wright, 1991; Phillipson, 1994), while artemisinin has been derived from *Artemisia annua* (Klayman *et al.*, 1984; Klayman, 1985; Trigg, 1989). A more recent example of natural product derived drug is the antimalarial atovaquone found in South American species of *Bignoniaceae*. The success of these drugs has broadened the search for natural plant products as a source for exploring new potential drugs for malaria. Fortunately, nature also provides the knowhow to help, conquer the disease that it sets loose. Plants are important source of biologically active compounds and have potential for development of novel antimalarial drugs (Hilou *et al.*, 2006).

Traditional medicines have always focused on the power of natural products to treat and care disease. More than 70% of India’s 1.1 billion population, still use alternative system of medicine (Vaidya and Devasagayam, 2007) without having any relevant scientific evidences for their clinical use. The present study has evaluated antiplasmodial efficacy of traditionally used medicinal plants, *Ajuga bracteosa*, *Xanthium strumarium* and *Berberis aristata* both in vitro as well as in vivo. These plants have been selected on the basis of their ethnopharmacological use. The cultivation of both human and non-human *Plasmodium* species in vitro has been a major research success, which has paved the way for the understanding of parasite and rapid screening of antimalarial plants for their effectiveness. The development of candle jar method by Trager and Jensen (1976) has led to the successful culturing of *Plasmodium* species. The first ever long-term in vitro culture of *P. berghei* was maintained by Janse *et al.* (1994). In vitro assay can test several compounds with rapidity, low cost and with small amount of drug. The limitation of in vitro screening includes the lack of information on drug metabolism, pharmacokinetics and drug toxicity.
In vitro screening of ELEXS and ELEAB has pointed to presence of significant antiplasmodial efficacy in these extracts, with an IC\textsubscript{50} of 5 and 10 µg/ml respectively (Chandel \textit{et al.}, 2009). AREBA has been found to possess weak antiplasmodial efficacy with an IC\textsubscript{50} of 40 µg/ml. The standard drug, chloroquine at 10 µM/ml concentration exhibited more than 90% inhibition of schizont maturation \textit{in vitro} in present investigation. The classification of \textit{in vitro} results have been based on the classical studies performed with antiplasmodial extracts by Deharo \textit{et al.} (2001). According to this study, extracts exhibiting IC\textsubscript{50} less than 5 µg/ml are considered as active, extracts with 5-10 µg/ml of IC\textsubscript{50} are considered as moderately active, whereas, extract with more than 10 µg/ml (IC\textsubscript{50}) are considered as inactive. ELEXS can be classified as an active, ELEAB as moderately active and AREBA as an inactive antiplasmodial extract according to this classification.

The phytochemical screening of plant extracts has pointed towards the presence of various secondary plant metabolites. The ethanolic leaves extract of \textit{Ajuga bracteosa} (ELEAB) and \textit{Xanthium strumarium} (ELEXS) have shown the presence of alkaloids, saponins, phenolic compounds, steroids and cardiac glycosides like compounds, while anthroquinones have not been detected in these plant extracts in present study. The phytochemical screening of aqueous root bark extract of \textit{Berberis aristata} (AREBA) has shown the presence of all other compounds except phenolic compounds, and anthroquinones detected in ELEAB and ELEXS. Alkaloids, terpenes and flavonoids have been reported to be involved in providing protection against malaria parasite (Phillipson and Wright, 1991; Christensen and Kharazmi, 2001). Phenolic compounds are well known for their diverse physiological properties, including among others, anticarcinogenic, inflammatory and antiparasitic (Ma and Kinner, 2002). Traditional use of these plants against malaria is based on real antiparasitic activity.
Ergosterol 5, 8 peroxide isolated from \textit{A. remota} has been found to exert significant IC$_{50}$ (8.2 ± 1.1 \textpm{M}) value against chloroquine sensitive (FCA20/GHA) strain of \textit{P. falciparum} (Kuria \textit{et al.}, 2001). This compound has also been found to inhibit the growth of protozoan parasite of Trypanosomatidae family such as \textit{Trypanosoma cruzi} and various \textit{Leishmania} species by interfering with the integrity of cell membrane (Linares \textit{et al.}, 2006). The active involvement of this compound in the plant extract to inhibit plasmodial growth cannot be denied. However, the active mode of action is yet to be elucidated.

The aerial parts of \textit{Xanthium strumarium} has been found to contain tomentosrin and 8-epi-xanthatin 1-\beta-5\beta-epoxide. These compounds have been reported to possess significant \textit{in vitro} antiplasmodial efficacy with an IC$_{50}$ of 7.8 \textmu{g}/ml against \textit{Plasmodium falciparum} (K-1) (Joshi \textit{et al.}, 1997). However, no \textit{in vivo} study has been reported in literature regarding antiplasmodial efficacy of these compounds or extracts of \textit{Xanthium strumarium}. \textit{Berberis aristata} contains a bitter principle berberine with tannins like compounds. Sheng \textit{et al.} (1997) reported that berberine, a chemical found in \textit{B. aristata} is beneficial in treatment of chloroquine resistant malaria when used in combination with pyrimethamine. This combination is more effective in clearing parasites as compared to tetracycline and cotrimaxazole in combination with pyrimethamine. However, neither \textit{in vitro} or \textit{in vivo} antiplasmodial efficacy of this compound has been reported else where in the literature.

The results of \textit{in vitro} culture does not necessary confirm a plant's bioactivity due to many factors. Some \textit{in vitro} studies have pointed weak antiplasmodial efficacy of plant extracts but same extracts have been found very effective \textit{in vivo} and vice versa (Gessler \textit{et al.}, 1995; Sunita and Das, 2007). Thus a plant extract must be tested both \textit{in vitro} and \textit{in vivo} to confirm its antimalarial activity. Plants contain various secondary metabolites, some of them
may exert toxic effects. Thus determination of their toxicity becomes important before evaluation of their medicinal properties \textit{in vivo}. ELEAB and AREBA have been found to be safe up to 5g/kg concentration in naïve mice. The concentration of 5 g/kg is the highest recommended dose, which can be administered orally to rodents for testing toxicity of any extract/drug (Lorke, 1983). The LD$_{50}$ of ELEXS was found to be 1.5 g/kg in naïve mice. The ethanolic leaf extract of \textit{X. strumarium} has been found toxic to the rodents with an LD$_{50}$ of 1000 g/kg (Talakal \textit{et al.}, 1995).

Thus \textit{in vivo} studies were carried out to evaluate suppressive, preventive and curative efficacy of plant extracts in \textit{P. berghei} infected BALB/c mice. Chloroquine (CQ, 5 mg/kg) has been used as standard schizontocidal drug in early and established infection of \textit{P. berghei} in the present study. Chloroquine interrupts the heme polymerization by forming FP-CQ complex in infected red blood cell. This complex is highly toxic to the cell. It disrupts membrane function ultimately leading to parasite cell autodigestion (Chou \textit{et al.}, 1980; Sugioka and Suzuki, 1991; Ginsberg \textit{et al.}, 1999). Plant extracts have been found to exert dose dependent chemosuppression in Peter's 4-day (suppressive) test during early infection of \textit{P. berghei}. ELEXS (500 mg/kg), ELEAB (1000 mg/kg) and AREBA (350 mg/kg) exhibited maximum chemosuppression of 88.6, 86.6 and 67.1\% respectively with significant p value ($< 0.001$), as compared to negative control. However, (ELEAB 1000 mg/kg) and ELEXS (500 mg/kg) exhibited 60\% mortality in experimental groups, which might be due to a mixture of unidentified alkaloids present in aerial parts of plant or due to poisonous water soluble toxic kaurine glucosides (McLeod \textit{et al.}, 1990). It has been reported that antimalarial effect of any drug depends upon its synergism with the vertebrate immune response (Melendez and Kerettli, 1987). Further, it might be due to immunosuppressive activity of plant \textit{in vivo}, as reported by Pereira \textit{et al.} (1999). Therefore, lesser concentrations of these extracts were
used further for the evaluation of preventive and curative activity. 
$ED_{50}$ of ELEXS, ELEAB and AREBA has been found to be 125 mg/kg, 
300 mg/kg and 250 mg/kg respectively during early infection.

For the evaluation of repository activity, the standard drug 
pyrimethamine (1.2 mg/kg) is used as reference drug, as it prevents DNA replication of parasite by binding to dihydrofolate reductase 
(DHFR), which interferes with the folic acid mechanism necessary for DNA and RNA synthesis in parasite leading to parasite death (Foote and Cowman, 1994). ELEXS (350 mg/kg), ELEAB (750 mg/kg) and AREBA (350 mg/kg) exhibited 90.4, 58.8 and 53.9% chemosuppression respectively, which was statistically significant ($p < 0.001$) as compared to negative control group during preventive test. However, standard drug caused around 90% chemosuppression.

The curative activity of ELEXS (150 mg/kg), ELEAB (750 mg/kg) and AREBA (350 mg/kg) has been found to exert 91.1, 85.1 and 30.6% chemosuppression respectively i.e. statistically significant ($p < 0.001$) as compared to negative control on day 7. Mean survival time period of mice has also been enhanced up to 29.8 ± 0.19, 27.4 ± 0.14 and 12.8 ± 0.46 days in respective treated groups, with significant p value (< 0.001) as compared to negative control. The enhanced mean survival time of mice in ELEXS (150 mg/kg) and ELEAB (750 mg/kg) was well comparable to standard drug chloroquine (5 mg/kg). The higher concentration of ELEXS has been found to exhibit lower chemosuppression during established infection, which might be due to the down modulation of immune response of host to cope up with high drug pressure and high parasitaemia.

In vivo antiplasmodial efficacy of ELEAB, ELEXS and AREBA has not been reported earlier in the literature. So the results of present study indicate that all these plant extracts possess significant blood schizontocidal activity, as evident from chemosuppression obtained during early infection in 4-day test. Extracts also exhibited significant repository activity. Significant curative activity ($p < 0.001$)
has also been recorded during established infection of *P. berghei* with these extracts. Antimalarial efficacy of these extracts may be due to alkaloid content or synergistic effect of different components present in the extract. The therapeutic benefits of traditional remedy are often attributed to combination of active constituents (Chindo *et al.*, 2003). Although, the mechanism of action has not been elucidated in the present study. *In vivo* antiplasmodial efficacy of ELEAB, ELEXS and AREBA might be due to the presence of ergosterol 5,8, peroxide, tomentosrin and berberine respectively. These compounds have been reported to possess significant *in vitro* antiplasmodial efficacy. Some extracts are known to exert antiplasmodial action either by causing elevation of red blood cell oxidation (Etkin, 1997) or by inhibiting protein synthesis (Kirby *et al.*, 1989). Either of two mechanisms mentioned above or some other unknown mechanism might be operating in these extracts.

The comparative evaluation of schizontocidal activity of ELEAB (750 mg/kg), ELEXS (250 mg/kg) and AREBA (350 mg/kg) with standard antimalarials (Quinine 10 mg/kg, Artesunate 100 mg/kg) pointed to significant chemosuppression (*p < 0.001*) by extracts, which is comparable to standard antimalarial drugs. Except AREBA (13 day) mean survival time period of extract/drug treated mice was between 28 and 30 days. When compared with homeopathic mother tincture of China and *Eucalyptus*, extract treated mice exhibited significant chemosuppression (*p < 0.001*). However, *Eucalyptus* treated mice exhibited mean survival time period of 19 days only during follow up study. In all other groups, it was between 27-28 days. Artesunate get absorbed faster than the quinine and it is known to cause oxidative membrane damage to RBCs (White, 1994). Quinine involves the inhibition of hemozoin biocrystallization, which facilitates the aggregation of cytotoxic heme. Toxic free heme accumulate in parasite, leading to their death. Quinine causes allergic reactions in many people (Kojouri *et al.*, 2001). Both quinine and artesunate have been observed to exert more than 97%
chemosuppression and enhanced mean survival time period of 30 days without any mortality. These results are in accordance with study of Haroon et al. (2005).

Mortality of mononuclear (MN) and polymorphonuclear (PMN) white blood cells has been assessed by Acridine Orange (AO)/Ethidium Bromide (EB) staining. AO is a vital dye, which stains both live and dead cells, whereas EB stains only those cells, which have lost their membrane integrity (Kashibhatla, 1998). Percentage of live mononuclear cells was between 96-97% in all drug/extract treated groups, except infected (G-2) and ELEXS (G-4) treated groups. Similar observations were observed about live PMN cells of various groups. Mortality of white blood cells does not seem to be affected in presence of various extracts/drugs.

Surface of red and white blood cells display a rapid and varied response to various conditions. Scanning electron micrographs of P. berghei infected red cells, exhibited changes in size density and distribution of knobs on the surface. It might be the result of parasite provoked results inflicted during parasite maturation (Miller et al., 1972; Kijejian et al., 1977; Kutner et al., 1982). Scanning electron micrographs of red blood cells of plant extracts treated groups, did not show much alteration on the surface of red blood cells. Some knobbed cells observed in these groups are infected cells. It points to the safety of these extracts on red blood cells. However, RBCs obtained from quinine (G-8) and artesunate (G-9) treated mice exhibited some depressions on the surface of cells.

In G-9, the irregular shape of RBCs might be due to sesquiterpene like compounds leading to generation of oxygen and superoxide radicals, which are capable of inducing free radicals which might be involved for oxidative damage in the parasite (Olliaro et al., 2001). It even changes the surface of membrane, which has raised the questions regarding the safety of artemisinin and its derivatives because erythrocytes are the primary site of action of this drug.
DISCUSSION

White cells have common function of recognition and defense. Scanning electron microscopy can give better understanding of mechanisms involved in such functions. Various techniques are available to separate WBC for SEM preparation e.g. Ficoll-Hypoque, Histopaque-1077 and 1119. Surface morphology of cells is not distorted after passing through such preparations (Wetzel et al., 1974). Lymphocytes can be identified by smooth/villous surface as reported by Firket and Schaaf-Lafontaine (1976). Microvilli on the surface of lymphocytes plays crucial role in cellular recognition mechanisms (Ewijk et al., 1975). It has been suggested that the development of complex villous surface in the mouse is the general concomitant of lymphocyte activation and their transformation (Criswell et al., 1975). Similar type of morphology (G-1) of MN cells has been reported in the present investigation too. Parasite has been reported to induce cell death in the lymphocytes leading to lymphopenia (Kerr et al., 1987). The surface of MN cells in *P. berghei* infected group exhibits various types of depressions. MN cells of G-3, G-6, G-8 and G-9 exhibited ruffled surface as compared to G-4 and G-5. Membrane of MN cells seems to be activated in presence of various extracts/drugs. PMN cells are activated due to infection. Therefore, more pointed finger-like projections were evident in PMN cells of G-2 group as compared to normal PMN cells (G-1). All the groups exhibited activated PMN cells with pointed finger-like structures on their surfaces.

Histological studies were carried out on normal (G-1), *P. berghei* infected (G-2) and drug/extract treated (G-3 to G-9) groups. A comparative histological study of liver, spleen and kidney of various groups (G-1 to G-9) was performed. Normal mouse liver exhibits repeating hexagonal units, hepatic lobules. Hepatocytes radiating outward central vein were observed. Macrophages (kupffer cells) are visible in the sinusoids. Humans and mice suffer from liver injury after infection with fatal strains of *P. falciparum* and *P. berghei* respectively (Good and Doolan, 1999). In infected mice, hepatomegaly occurs due
to proliferation of reticuloendothelial cells. Significant hepatocyte abnormalities have been reported with infiltration of parasitized red blood cells and haemozoin depositions inside degenerated hepatocytes (Rodriguez-Acosta et al., 1998). Accumulation of pigment has been reported to occur due to protein degradation by parasite (Sullivan et al., 1996). The present finding also indicates accumulation of haemozoin and infiltration of red blood cells in sinusoidal spaces in infected mouse.

General hepatic morphology was not distributed under the influence of various extracts/drugs. Cytoplasm of hepatocytes was slightly basophilic in ELEXS treated group. Increase in sinusoidal space was observed in china treated mouse, whereas, it was decreased in quinine treated mouse. AREBA treated liver did not show enlargement of hepatocytes and sinusoidal spaces. Janbaz and Gilani (2000) have reported preventive and curative effect of berberine, a chemical induced hepatotoxicity in rodents. Basophilic cytoplasm of hepatocytes indicates towards the altered liver functions. Maximum hepatotoxicity has been observed in ELEXS (G-4) and Eucalyptus (G-7) treated mouse.

Splenomegaly is one of the most striking feature of malaria infection. Size of spleen increases several fold after infection, due in part to an influx of lymphocytes in both human (Pitney, 1968) and mouse (Weiss et al., 1989) infection. Malaria associated splenomegaly has been reported due to increased erythrocytes in mice (Tsubata et al., 2005; Nogueira et al., 2006). Lost of marginal zones and general loss of follicle structures in spleen has been reported in P. chabaudi infection (Cadman et al., 2008).

Histology of normal spleen revealed the presence of capsule on the outer side from which trabeculae emanate into splenic parenchyma. Distinct red pulp and white pulp separated by marginal zone are also distinct in the present study. The proliferative change in the spleen has been reported in all human, primates and rodent
malaria parasites (Aikawa et al., 1980). Splenomegaly has been observed in infected mouse in the present study. This enlargement is due to proliferation of reticulo-endothelial system. These changes lead to partial obstruction of blood cells in bird malaria and in complicated cases of *P. falciparum* (Rigdon, 1944; Boonpucknavig et al., 1984). Sophiagena and Frankenburg (1988) also observed proliferative changes in spleen, increased white pulp area and enhanced macrophages during lethal infection. Hyperplasia of red pulp and white pulp along with hypertrophy of macrophages and erythrocytes has been observed in the infected mouse spleen in the present study. Similar observations were made by other investigators. Massive pigment deposition and damaged reticular cells in red pulp observed in the present study has also been reported by Bajpai and Dutta (1987). General histology of spleen has not been distorted in various extract/drug treated groups. G-3, G-8 and G-9 groups revealed normal spleen architecture comparable to G-1 group. Widening of white pulp, haemozoin pigment deposition, lymphatic cell proliferation has been observed in G-4, G-5, G-6 and G-7.

Histology of normal mouse kidney revealed clear outer cortex and inner medullary region. Presence of glomeruli, PCT and DCT in cortex and renal pyramids in medulla are also evident. Histopathological changes in infected mice observed during present study have been similar to those reported in human malaria (Dockrell et al., 1980). Malaria is a major cause of renal complications. Albuminuria and proteinuria are common complications. Glomeruli show proliferative changes, which were similar to those reported in rodents infected with *P. berghei* (Boonpucknavig et al., 1973). Tubular damage and pigmentation has also been observed in infected mice. Accumulation of parasitized and non-parasitized RBCs in Bowman’s capsule is similar to as observed in *P. yoelii* infection in Swiss mice (Bajpai and Dutta, 1987). Group G-3, G-8 and G-9 exhibited normal architecture of kidney comparable to normal mice (G-1). Group G-4,
A COMPARATIVE STUDY OF SOME ANTIMALARIALS ON CLEARANCE OF BLOOD STAGE PLASMODIUM BERGHEI INFECTION

G-5, G-6 and G-7 exhibited mesangial cell proliferation, glomerular construction and little tubular damage.

*Plasmodium* induces multiple organ dysfunctions inflicting liver, spleen, kidney and brain (Prommano *et al.*, 2005). In present study a significant fall (p < 0.0001) in the hepatic protein concentration has been observed in the *P. berghei* infected BALB/c mice. On the contrary, a significant rise (p < 0.0001) in the protein content has been recorded in the spleen. Similar results have been reported by Ahmad and Srivastava (2007). However, Sharma *et al.* (1979) and Saxena *et al.* (1981) have reported significant rise in protein content of both liver and spleen of *P. berghei* infected BALB/c mice. Hepatomegaly and splenomegaly are characteristics of *P. berghei* infection. The increase in size and weight of spleen has been attributed to rise in both dry weight and moisture content (Aikawa *et al.*, 1980). This may provide favorable environment for the proliferation of parasite.

Except G-7, hepatic protein concentration has been observed to increase in G-4, G-6 and G-8 groups with significant p value (< 0.05). ELEAB, Artesunate and AREBA treated groups did not show significant enhancement in hepatic protein concentration in the present study. Spleen of G-3, G-8 and G-9 did not show statistically significant rise in protein concentration. However, all other groups exhibited significant increase in protein concentration as compared to normal. However, this increase is less significant in comparison to infected mice. Net increase in the protein content of tissue might be due to either synthesis of protein or the accumulation of macrophages and infected red cells (Ahmad and Srivastava, 2007). The decrease in the protein concentration of liver might be due to utilization of proteins by the pathogens.

Alkaline phosphatase (ALP) is an enzyme which catalysis the hydrolysis of number of phosphate esters, transferring the phosphate group to an acceptor molecule. ALP is located in a wide variety of
tissues. Significant amount of the enzyme is found in liver, placenta, intestine, kidney, bone and platelets, in decreasing order (Nogochi and Yashita, 1987). In the present study, specific activity of ALP increased in all treated groups (G-3 to G-9) as compared to normal mice. However, this increase in ALP activity was non-significant in G-3 and G-9. The rise in ALP activity in all the treated groups of mice was found to be statistically significant ($p < 0.001$) as compared to negative Control (G-2). In the spleen, maximum ALP activity was recorded in G-7 followed by G-8, G-4, G-9 and G-3 with significant $p$ value ($< 0.001$) as compared to normal mice, while this increase in ALP concentration was significantly less ($p < 0.001$) as compared to G-2. The ALP activity of G-3 and G-9 was well comparable to the normal mice indicating positive effect of ELEAB and artesunate on the liver as observed in the histological studies too. Alkaline phosphatase has been reported to be potential important biomarker for the assessment of integrity of hepatic drainage system in acute *P. falciparum* malaria infection. ALP is a tissue specific membrane bound metalloenzyme, whose activity is increased in the serum after the malaria infection pointing to disturbance of host-hepatocyte drainage pathway (Ibrahim and Gregory, 2005).

Acid phosphatase (ACP) is an enzyme associated with organelles related to the digestion of food as well as the endoplasmic reticulum and golgi complex. In the present study, a significant rise ($p < 0.0001$) in acid phosphatase concentration has been registered in the liver and spleen of *P. berghei* infected mice, as compared to normal mice (G-1). The results corroborated with the study of Garba *et al.* (2006). Among treated mice, G-4 to G-8 showed significant increase in the ACP activity, while in G-3 and G-9 non-significant rise in its activity is observed as compared to normal mice (G-1). In spleen, G-3, G-8 and G-9 showed comparable acid phosphatase activity with normal mice, while G-4 to G-7 have been observed to increase in ACP activity, as compared to normal mice. Increase in acid phosphatase activity is attributed to reticulo-endothelial cell

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proliferations, which contains macrophages and lymphocytes along with haemozoin depositions. These cells have been reported to contain more hydrolytic and oxidative enzymes, which might be responsible for elevating acid phosphatase activity (Garba et al., 2006).

Aspartate transaminase (AST) is also called as serum glutamic oxaloacetic transaminase (SGOT). It is associated with liver parenchyma, thus any degeneration in liver cells is responsible for elevated AST activity in both serum as well as tissue. Among treated groups, G-7 and G-5 showed maximum rise ($p < 0.001$) in hepatic AST activity, as compared to normal mice, while G-9, G-3 and G-8 showed non-significant rise in AST activity. In the spleen of treated mice, AST activity has been found to increase as compared to G-1 but its rise is significantly less ($p < 0.001$) as compared to negative control (G-2). G-4 to G-7 showed maximum rise in AST activity, while G-3 and G-9 showed comparable AST activity to positive control (G-1).

Alanine aminotransaminase (ALT) is a biomarker of liver cell toxicity and is termed as serum glutamic proline transaminase. In the present study specific activity of ALT was found to be significantly enhanced ($p<0.0001$) in the liver and spleen of *P. berghei* infected BALB/c mice as compared to the normal mice (G-1). All the treated groups of mice showed significant rise ($p < 0.001$) in the hepatic and splenic ALT activity as compared to normal mouse (G-1). However, this rise in activity was again reported to be significantly less ($p < 0.001$) as compared to G-2. G-7 exhibited maximum rise in the ALT activity, which was found to be comparable to negative control group, while G-9 did not show any significant rise in the ALT activity and was found to be comparable to the normal mice (G-1). Similar results have been observed in the spleen.

Invasion of liver cells by *Plasmodium* causes organ congestion, sinusoidal blockage and cellular inflammation (Jarike et al., 2002).
After this, parenchymal, transaminase (ALT and AST) and membranous (ALP) enzymes of liver leak out and find their way into circulation leading to increased enzyme activity (Burtis et al., 2001). Rise in the level of enzymatic activity vary with causative factors (Nsirim, 1999).

Lactate dehydrogenase (LDH) is an intracellular enzyme, which catalysis the readily reversible reaction, involving oxidation of lactate to pyruvate with NAD serving as coenzyme. The concentration of LDH is high in heart, liver, red blood cells, skeletal muscles and kidney (Calbreath, 1992). Elevated LDH activity found during P. berghei infection in the present study, corroborates with the earlier finding of Garba and Ubom (2005). The enhanced LDH activity in the tissue showed a phenomenon of synergy between the two pathophysiological processes i.e. the hepatic cellular damage and red blood cell destruction. Being rich sources of LDH, the acute liver and spleen injury along with red blood cell destruction leads to the elevation of LDH activity.

The specific activity of LDH was found to be enhanced both in liver and spleen of all treated groups (G-3 to G-9) with statistical significant p value (< 0.001) as compared to G-1. However, this rise in LDH activity was significantly less (p < 0.001) as compared to G-2. G-3, G-8 and G-9 also showed significantly enhanced (< 0.005) LDH activity as compared to normal mice (G-1). The maximum enhanced activity was again recorded in the liver and spleen of G-7, which showed the toxic effects of the drug on liver and spleen.

These findings lend pharmacological support to the reported folkloric use of these plants as malaria remedy. Present study points to the safety of ELEAB which is evident from histological, biochemical and SEM studies. ELEXS has been found to be toxic, however, its purified components might show better efficacy and reduced toxicity. Further work is required to isolate, identify and characterize the active principle from these plants. Enrichment of pure compounds
might be responsible for their better antiplasmodial efficacy as AREBA exhibits weak antiplasmodial efficacy, however, active component berberine has shown better antimalarial efficacy when used in combination with other antimalarial drugs. China and Eucalyptus can be used in combination therapy with artesunate. Artesunate is readily hydrolyzed to dihydroartemisinin so duration of its antimalarial activity is short (Burk et al., 2005). Combination of artesunate (100 mg/kg) with china (1 : 2 i) cleared *P. berghei* infection without disturbing normal function of liver and kidney which was evident from normal activity of ALP, bilirubin, urea and creatinine in the serum of mice treated with this combination, whereas artesunate combination therapy with other antimalarials caused renal impairment (Bagai et al., 2008). In another study (Bagai and Rajan, 2008) has reported that 7 day treatment course of artesunate + *Eucalyptus* (1 : 2 i) inhibits blood stage rodent infection preventing recrudescence in BALB/c mice.