5. DISCUSSION

5.1. Phytochemical studies

Plants have been prized as our oldest medicines, for their pain relieving and healing abilities. From the beginning, combating disease has been an important aspect of interactions between humans and the natural environment and plants have forever been a catalyst for our healing. Plant biodiversity is an outward manifestation of chemical diversity, often with very attractive bioactivities. They are described as chemical factories that are capable of synthesizing unlimited numbers of highly complex and unusual chemical substances whose structures could escape the imagination of synthetic chemists. These phytochemicals are indeed chemically complex and may contain one or many structurally related active compounds that produce a synergistic effect. Therefore medicinal plants are perhaps the most valuable source of new bioactive chemical entities to benefit mankind against various ailments. Many drugs currently available in western medicine, originally isolated from plants, or are derived from templates of compounds isolated from plants were made available/effective based on these scientific validation.

Plants contain a bewildering diversity of secondary metabolites with pharmacological properties. They continue to provide us new chemical entities (lead molecules) for the development of drugs against various pharmacological targets, including cancer, HIV/AIDS, malaria, Alzheimer’s disease and pain. However, their products cannot be simply advertised as effective and applied for human without any scientific and clinical data base. Therefore it is essential to symbolize the efficacy, safety and toxicity dosage of the drug when used on certain animal models. It helps to improve the role of functional ingredients that are intended to produce positive health effects in medical services equivalent to modern medicine.

There are several ways of extracting these valuable organic compounds including steam distillation or hydro-distillation, solvent extraction and maceration. In the present study, the leaves, stem bark and fruits of Swietenia mahagoni were successively extracted with different solvent using soxhlet apparatus. The preliminary phytochemical screening reveals the presence of alkaloids, flavonoids,
steroids and sterols, saponin, tannins, phenolic compounds, glycosides, carbohydrate, protein and amino acids. The methanol extraction was more efficient where most of the said phytochemicals were present (Table 7). Methanol, owing to the higher solubilizing capacity, is already known to be one of the best solvents for extracting bioactive compounds from plant materials (Kapasakalidis et al., 2006). Preliminary phytochemical screening of the plant has served two purposes: chemical identity of the drug and presence or absence of the essential bioactive compounds. The preliminary phytochemical results in the present study provide correlative data for the diagnosis, purity and quality of the drug. The preliminary data collected in the present study, helped in the preparation of an authentic preliminary phytochemical profile. Even though these data are termed preliminary, it may prove to be an authentic reference standard for the characterization of raw drug materials.

5.2. In vitro antioxidant studies

Oxidative stress has been implicated in etiology of a number of human ailments (Thomas and Kalyanaraman, 1997; Payne et al., 1999). Hence, compounds especially from natural sources capable of protecting against reactive oxygen species mediated damage may have potential application in the prevention and/or curing of diseases (Sies, 1996; Rao and Aggarwal, 2000; Cuzzocrea et al., 2001). Naturally occurring biochemical compounds such as flavonoids, catechins, lignans, phenolic acids, vitamins, terpenoids, and some other endogenous metabolites are rich in antioxidant activity (Zheng and Wang, 2001; Cai et al., 2003). It is considered that the administration of natural antioxidants with multiple components could offer protection and combat oxidative stress-induced physiological malfunctions (Nair et al., 2007; Ningappa et al., 2008). The antioxidant and health-promoting capacity of these compounds are thought to arise from their protective effects by counteracting and neutralizing the ROS (Wong et al., 2006). Therefore, search for medicinal plants with high antioxidant content for nutritional purposes is currently of major interest. Thus there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts or isolated products of plant origin rather than looking for synthetic ones (McClements and Decker, 2000). Antioxidant activity of the multiple plant extracts cannot be evaluated by a unidimensional method due to complex nature of the phytochemicals present in them (Frankel and Meyer, 2000). Therefore,
the antioxidant activity is generally evaluated by a number of methods to explain the different mechanism of antioxidant function. In view of this, the antioxidant potential of different solvent extracts of Swietenia mahagoni was evaluated by employing different chemical assays and in vitro methods.

5.2.1. Non-enzymatic antioxidants

Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and plant products. Many of the phenolics have been shown to contain high levels of antioxidant activities, which include tocopherols, ascorbic acid, carotenoids, flavonoids, and tannins (Larson, 1988; Rice Evans et al., 1996). This information has led to the determination of the total phenolic content of the samples under study (Table 1). As antioxidants, the polyphenols may protect cell constituents against oxidative damage and therefore, limit the risk of various degenerative diseases associated to oxidative stress, by acting directly on reactive oxygen species or by stimulating endogenous defense systems (Scalbert et al., 2005). Many of the polyphenols have been identified as anticancer (Lambert and Yang, 2003), cardioprotective (Bagchi et al., 2003), antithrombotic and antihypersensitive (Cheng et al., 1993). Tannins are naturally occurring phenolic compounds which precipitate protein. In general, tannins are high molecular weight (Mr > 500) and have many phenolic groups (Hagerman et al., 1997). The ability of the phenolic substances including flavonoids, phenolic acids, tannins and lignans to act as potential antioxidants has been extensively investigated (Rice-Evans et al., 1996; Suja et al., 2005). In the current study, the total phenolic content and tannin content of different solvent extracts of leaves stem bark and fruits of S. mahagoni were estimated and expressed as tannic acid equivalent. . The total phenolic contents ranged between 57 and 544 mg TAE/g extract, the tannins were in the range of 17 – 322 mg TAE/g extract. The total phenolics content was maximum in methanol extract of stem bark (543.53 ± 3.46 mg TAE/g extract) followed by stem bark acetone extract (525.79 ± 7.48 mg TAE/g extract). Similarly, the tannin content was also registered maximum in the methanol extract of stem bark (321.96 ± 0.01 mg TAE/g extract). Of all the three parts used, the maximum total phenolic contents were recorded in different solvent extracts of bark followed by the leaf extracts and the minimum phenolic contents were observed in the fruit extracts. Tannins were
also observed in a same scenario as that of the total phenolics. The observed variation might be due to the marked difference in the qualitative and quantitative composition of phenolic antioxidants and their conjugates present in these extracts (Macheix et al., 1990). Liu et al., (2007) also demonstrated varying phenolic contents in extracts of Xylaria sp. obtained using different solvents. Methanol was found to be the most efficient solvent to extract antioxidant phenolics and tannins. This is in agreement with the reports of Hertog et al., (1993) and Yen et al., (1996) that methanol is an effective solvent for extraction of antioxidants.

Flavonoids as one of the most diverse and widespread group of natural compounds, are likely to be the most important natural phenolics (Agrawal, 1989). They basically consist of a fused aromatic ring (A-ring) and a heterocyclic ring (C-ring) connected through a carbon-carbon bridge to an aromatic B-ring (Shimoj et al., 1996). It is well-recognized that the structural features and nature of substitutions on rings B and C determine the antioxidant activity of flavonoids (Bors et al., 1990; Rice-Evans et al., 1996; Raj and Shalini, 1999; Silva et al., 2002b; Badami et al., 2003; Balasundram et al., 2006). These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties (Kumar and Karunakaran, 2007). In the present study, flavonoid content of different solvent extracts of leaves, stem bark and fruits of S. mahagoni were estimated and expressed as rutin equivalent (Table 8). The total flavonoid contents ranged between 5.5 and 22 mg RE/g extract. The highest concentration of flavonoids was present in the methanol and water extract of fruit powder (22.27 ± 3.41 mg RE/g extract & 22.27 ± 1.75 mg RE/g extract respectively). On the whole, the fruit powder extracts showed the highest concentration of flavonoids followed by the stem bark extracts and finally the leaf extracts.

The protection afforded by plants has been attributed to various phenolic antioxidants which are increasingly becoming of interest in the food industry because they retard oxidative degradation of lipids and thereby improve food quality (Kähkönen et al., 1999). The results of the study strongly suggest that polyphenolics are important components of S. mahagoni which could be attributed to play an important role in adsorbing and neutralizing free radicals, quenching singlet oxygen or decomposing peroxides.
5.2.2. DPPH’ radical scavenging activity

DPPH’, a relatively stable organic radical with a characteristic strong absorption band at 517 nm in visible spectroscopy (deep violet colour), was used to evaluate the free radical scavenging ability of the investigated samples (Table 9). DPPH radical scavenging activity is a measure of non-enzymatic antioxidant activity. When an antioxidant scavenges off the free radical by hydrogen donation, the purple colour of the DPPH solution becomes light yellow. The decrease in the absorbance was taken as a measure of the extent of radical scavenging activity. The concentration of an antioxidant needed to decrease the DPPH concentration by 50% (IC50) is a parameter widely used to estimate antioxidant activity (Tsimogiannis and Oreopoulou, 2004; Atoui et al., 2005; Kouri et al., 2007). A lower IC50 value corresponds with the highest antioxidant power. Higher levels of DPPH activity have been correlated with tolerance to different stress conditions. In the present study, the per cent DPPH radical scavenging activities of all the extracts were dose dependent. This antiradical activity of extracts would be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability (Brand-Williams et al., 1995). Among the extracts studied, the higher DPPH radical scavenging activity was shown by methanol extract of the stem bark with an IC50 of 24.76 ± 0.09μg/ml followed by the water extract (26.65 ± 0.07μg/ml) of leaf powder. The higher levels of total phenolics, and tannins recorded in the methanol extract (Table 8) might have acted as reductones in conferring the radical scavenging activities. In similar lines Siddhuraju et al., (2002) reported that high concentration of tannins (proanthocyanidins) extracted from stem bark of Cassia fistula possessed elevated DPPH radical quenching capacity. Amarowicz et al., (2000) also reported that the tannins extracted from canola and rapeseed hulls exhibited a high scavenging efficiency toward DPPH radicals. The results revealed that the plants contain powerful free radical inhibitor compounds, which may act as primary antioxidants. However, the antioxidants like BHA and gallic acid were found to be more potent in their hydrogen donating/electron transfer ability than all other extract samples.
5.2.3. Hydroxyl radical scavenging activity

Hydroxyl radical is the most dangerous radical in the body. It can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions, such as copper or iron. Hydroxyl radicals have the highest 1-electron reduction potential (2310 mV) and can react with everything in living organisms at the second-order rate constants of 109–1010 mol/s. It reacts with lipids, polypeptides, proteins, and DNA molecules, especially thiamine and guanosine. When a hydroxyl radical reacts with aromatic compounds, it can add across a double bond, resulting in hydroxycyclohexadienyl radical. The resulting radical can undergo further reactions, such as reaction with oxygen, to give peroxy radical, or decompose to phenoxy-type radicals by water elimination (Lee et al., 2004). The hydroxyl radical (OH•) in the cells can easily cross cell membranes at specific sites, react with most biomolecules and furthermore cause tissue damage and cell death. Thus, removing OH• is very important for the protection of living systems. Therefore, it is considered important to assess the protective ability of the extract against hydroxyl radicals. Hydroxyl radical scavenging activity is estimated by generating the hydroxyl radicals using ascorbic acid iron-EDTA. The hydroxyl radical is formed by the oxidation reaction with dimethyl sulfoxide (DMSO) to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent (Singh, et al., 2002). In the present investigation, all the samples exhibited between 3.54 ± 0.70% and 75.60 ± 0.60% of hydroxyl radical scavenging activity at five different concentrations in the reaction mixture (Table 10). The methanol extract of the stem bark rendered the maximum hydroxyl radical scavenging activity with an IC₅₀ of 18.87 ± 0.05μg/ml. The higher levels of total phenolics, and tannins recorded in the methanol extract (Table 8) might have acted as reductones in conferring the hydroxyl radical scavenging activity. The potential scavenging abilities of phenolic substances might be due to the active hydrogen donor ability of hydroxyl substitution (Siddhuraju, 2007). Hagerman et al., (1998) have also suggested that high molecular weight, and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical scavenging by tannins than their specific functional groups. From the above findings, it can be
concluded that the polyphenolic richness (Table 8) of S. mahagoni has made them an imminent free radical quencher.

5.2.4. Super oxide radical scavenging activity

Superoxide anion plays an important role in the formation of various reactive oxygen species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA (Halliwell, 1991). Superoxide anion, a highly toxic species, is a reduced form of molecular oxygen which is generated by numerous biological and photochemical reactions (Govindarajan et al., 2003). It has been implicated in several pathophysiological processes due to its transformation into more reactive species. Superoxide radicals are more detrimental due to their role as second messengers in fibroblast proliferation in inflammation and mediators of tissue destruction (Windrow et al., 1993). In the present study, the superoxide radical scavenging activity of different solvent extracts of S. mahagoni was determined using riboflavin-NBT-light system in vitro. The extracts are found to be efficient scavengers of superoxide radicals. Photochemical reduction of flavins generates O2•- which reduces NBT, resulting in the formation of blue formazan (Beauchamp and Fridovich, 1971). The scavenging ability of the sample extracts increased with increasing concentration. The percentage activity of all the tested samples ranged between 4.74 ± 0.08% and 75.26 ± 0.13%. The acetone extract of stem bark (22.09 ± 0.04µg/ml) followed by the methanol extract of the same (32.70 ± 0.03µg/ml) showed maximum scavenging ability compared to the leaf and fruit extracts. The superoxide radical scavenging activity of the acetone extract of stem bark was comparable with that of the standard antioxidant BHA. The activity of different solvent extracts of S. mahagoni leaf, stem bark and fruit against superoxide radical is of significance because superoxide can decrease the activity of other antioxidant defence enzymes such as catalase and glutathione peroxidase as well as being cytotoxic by generating more reactive species like peroxy nitrite (Halliwell and Gutteridge, 1989).

5.2.5. Nitric oxide radical scavenging activity

Virtually all cellular components including lipids, proteins, nucleic acids, carbohydrates are susceptible to oxidative damage (Pacifici and Davies, 1991). ROS
like O2•− may react with NO and give rise to various other reactive nitrogen species (RNS) such as NO2, N2O4 and peroxynitrite. Both ROS and RNS together attack and damage various cellular molecules, resulting in several pathological conditions including cancer (Nathan, 1992). A number of polyphenolic phytochemicals such as resveratrol, quercetin (Kawada et al., 1998), α-tocopherol (Arroyo et al., 1992) and catechin (Pannala et al., 1998) have been found to inhibit the RNS effect. Therefore, utilization of these significant sources of natural antioxidants to prevent or improve ROS or RNS mediated injury becomes very important. In the present study, nitrite ions generated by sodium nitro prusside (SNP), in aqueous solution at physiological pH was extensively scavenged by S. mahagoni in a concentration dependent manner (Table 12). The percentage activity of all the tested samples ranged between 4.69 ± 0.23% and 75.53 ± 0.23%. The acetone and methanol extract of stem bark (26.47 ± 0.02μg/ml and 26.49 ± 0.03μg/ml respectively) exhibited higher nitric oxide scavenging activity thereby inhibiting the nitrite formation. It can be seen from the data presented in Table 12 and fig. 8, that all the extracts were capable of scavenging NO•. Further, the scavenging activity of these plant extracts might help to arrest the chain of reactions initiated by excess generation of NO• that are detrimental to the human health. Kumaran and Karunakaran, (2006) have made similar observation that the plant/plant products may have the property to counteract the effect of NO• formation and in turn may be of considerable interest in preventing the ill effects of excessive NO• generation in the human body.

5.2.6. Ferric reducing antioxidant power (FRAP)

Reducing power is a novel antioxidation defense mechanism; the two mechanisms available to affect this property are: electron transfer and hydrogen atom transfer (Dastmalchi et al., 2007). This is because the ferric-to-ferrous iron reduction occurs rapidly with all reductants with half reaction reduction potentials above that of Fe³⁺/Fe²⁺, the values in the Ferric reducing antioxidant property (FRAP) assay expresses the corresponding concentration of electron-donating antioxidants (Halvorsen et al., 2002). The reducing powers of the different solvent extracts of S. mahagoni were assessed based on their ability to reduce Fe³⁺ to Fe²⁺ and the results are presented in Table 13. The results revealed that all extracts exhibited reducing power at a dose of 20μg/ml. Since the antioxidant activity of
phenolics is mainly due to their redox activities, this allows them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice et al., 1996).

5.2.7. Metal chelating activity

Transition metal ions, particularly iron is an essential mineral for normal physiology, but excess can result in cellular injury. If they undergo the Fenton reaction, the reduced metals may form highly reactive hydroxyl radicals and thereby contribute to oxidative stress (Hippeli and Elstner, 1999). The resulting oxyradicals cause damage to cellular lipids, nucleic acids, proteins, and carbohydrates and lead to cellular impairment. Since ferrous ions are the most effective pro-oxidants in food systems, their removal from circulation could be one of the promising approach to prevent oxidative stress-induced diseases. Although metal chelating agents are not antioxidants, they play a vital role in the stabilization of fatty acids against rancidity (Yen and Duh, 1994). Since Fe$^{2+}$ has been shown to cause the production of oxyradicals and lipid peroxidation, minimizing Fe$^{2+}$ concentration in Fenton reactions affords protection against oxidative damage. Therefore, it was considered to be important to screen the iron (II) chelating ability of the extracts. Hence, metal chelation is considered as one of the important properties of an antioxidant. Several plant extracts/ constituents have been reported to exert antioxidant activity by chelating the catalytic metals (Niu et al., 2000; Dillon et al., 2003). In the present investigation, it was observed that all the sample extracts of S. mahagoni offered pronounced antioxidant activities as they were able to chelate ferrous metal ion more efficiently with their values ranging between 65.66 ± 7.04 to 681.27 ± 8.77 mg EDTA/ g extract (Table 14). The extracts may be able to play a protective role against oxidative damage by sequestering iron (II) ions that may otherwise catalyze Fenton-type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions (Dorman et al., 2003). The iron (II) chelating properties of the additives may be attributed to their endogenous chelating agents, mainly phenolics. It has been reported that certain phenolic compounds have properly oriented functional groups, which can chelate metal ions (Thompson et al., 1976). Gua et al., (1996) reported that polyphenols with dihydroxy groups can conjugate metals, preventing metal catalyzed free radical formation. Chelating agents may serve as secondary
antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions.

5.3. In vivo studies

5.3.1. Acute toxicity

Toxicity can be induced by chemical compounds, organic drugs, a stable metabolite or a reactive metabolite in a dose dependent manner. Adverse or toxic effects in a biological system are not produced by a chemical agent unless the agent or its metabolites or conversion products reach appropriate receptors in the system at a concentration and for a length of time sufficient to initiate the toxic manifestation. Toxic effects may be produced by acute and/or chronic exposure to chemical agents. Mostly animal models, in particular Swiss albino mice which are the most sensitive, are used to determine the acute toxicity dose of the new chemicals or drugs. In acute toxicity study, substances with LD$_{50}$ between 5000 and 15,000 mg/kg b.w. are regarded practically non-toxic (Loomis and Hayes, 1996). In the present study, systematic administration of graded doses of the ethanolic extract of S. mahagoni, leaves at different doses failed to show any mortality or changes in the behavioural pattern up to 4000 mg/kg b.w. (Table 15). Therefore, the oral administration of S. mahagoni leaves extracts can be considered to have fairly high margin of safety.

5.3.2. Antinociceptive activity of S. mahagoni leaves

Plant extracts have been used for centuries, as popular remedies against several health disorders. The study of plants that have been traditionally used as pain killers should still be seen as a fruitful and logical research strategy in the search for new analgesic drugs and study of pain mechanisms (Calixto et al., 2000). Inflammation is usually associated with pain as a secondary process resulting from the release of algesic mediators (Osadebe and Okoye, 2003). For treatment of inflammation related diseases including arthritis, asthma and cardiovascular diseases, the most clinically important medicines are steroidal or non-steroidal anti-inflammatory chemical therapeutics. Though these have potent activity, long-term administration is required for the treatment of chronic disease. Furthermore, these drugs have various and severe adverse effects. Therefore, there is a need to
identify naturally occurring agents with reduced side effects to substitute the chemical therapeutics (Conforti et al., 2009).

In the present study, peripheral analgesic activity was assessed by acetic acid-induced writhing test. The ethanol extract of S. mahagoni showed significant (P<0.01) suppression of writhes in the experimental rats (Table 16). Pretreatment with extract at doses of 200 and 400 mg/kg reduced the number of writhes in a dose dependant manner. Similarly, paracetamol (300 mg/kg, i.p.) profoundly reduced the number of writhes elicited by acetic acid by 80.19%. Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response (Bartolini et al., 1987). The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and thereby brought a reduction in the number of writhes in the animals. Satyanarayana et al., 2004 has shown that acetic acid produced writhing or nociception by stimulating the production of prostaglandin. Paracetamol, a standard analgesic drug (Rang et al., 2011), has been shown to inhibit prostaglandin synthesis in the brain (Flower et al., 1972). It is, therefore, not surprising that paracetamol significantly attenuated acetic acid-induced nociception in this study. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting antinociceptives. The results indicate that the analgesic effect of ethanol extract of S. mahagoni might be mediated by its peripheral effects by inhibiting the synthesis or action of prostaglandins. According to Koster et al., (1959), Williamson et al., (1996) and Eddy and Leimback, (1953), acetic acid writhing is used to evaluate peripherally and centrally acting analgesic drugs respectively. The results obtained in the present study are in accordance with the earlier reports of (Gosh et al., 2012). Much evidence has shown that the production of free radicals at the site of inflammation contributes to tissue damage and stimulation of pain. Painful stimulation increases the production of free radicals and it increases lipoperoxidation. The application of antioxidants, in the present case ethanol extract of S. mahagoni, increases the antioxidative capacity and thus enhances the protection against the consequences of pain. Antioxidants are known to protect CNS against free radicals and also decrease the sensation of pain (Kim et al., 2001a; Rokyta et al., 2003).
5.3.3. Anti-inflammatory activity

Inflammation is a complex reaction in the vascularized connective tissue which can be defined fundamentally a protective response against injury. It has been implicated in pathophysiology of various clinical disorders including cancer. Many different mediators such as prostaglandins, leukotrienes and platelet aggregating factor (PAF) are involved in inflammatory response. By modulating the release of these mediators one can effectively control this process (Cuzzocrea et al., 2001). A number of anti-inflammatory agents, both steroidal and non-steroidal are available. Most of these agents, though are effective, have a number of severe side effects, the most important being the gastrointestinal irritation (Ajith and Janardhanan, 2001). Therefore there is a need to identify naturally occurring agents with reduced side effects to substitute the chemical therapeutics.

The most effective and widely used model for the evaluation of anti-inflammatory activity is carrageenan induced paw edema. Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and is derived from Irish Sea grass, Chondrus crispus. The development of carrageenan-induced edema is biphasic; the first phase is attributed to the release of 5-HT and kinins while second phase is related to the release of prostroglandins (Brooks and Day, 1991). Carrageenan induces a protein rich exudate containing large number of neutrophils (Lo et al., 1982). In the present investigation, ethanolic extract of leaf of S. mahagoni significantly inhibited edema formation in rats (P<0.001) in a dose dependant manner (Table 18). The inhibition of edema formation by ethanol extract at the dose of 400 mg/kg was comparable to that of the standard drug indomethacin. The ethanolic extract of S. mahagoni leaves exhibited a weak inhibitory effect at early phase but was able to effectively inhibit the increase of paw volume during the late phase (4 h after carrageenan injection) of inflammation. Based on this observation and the biphasic nature of carrageenan induced paw edema, it is possible to propose that the significant activity observed in the last phase of inflammation may be due to the ability of the extract to inhibit the release and/or activity of the late mediators involved in carrageenan induced paw edema. Thus, it is confirmed that action of extract is more potent against the release of prostaglandins.
Biochemical and physiological methods of diagnosis constitute a promising approach to the problem of detecting the effects of toxic chemicals at the earliest possible stage. Since drugs may induce certain biochemical change in animals before the drastic cellular systematic dysfunction manifests, appropriate biochemical parameters could be used effectively as sensitive indicators. Considerable evidence suggests that oxidative stress and reactive oxygen species (ROS) play significant roles in several aspects of acute and chronic inflammation. The enzymic antioxidant defense system namely super oxide dismutase (SOD), catalase(CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) protect cells against O2 toxicity and lipid peroxidation (Ramprasad et al., 2005). The results of the present study showed that the activities of SOD, CAT, GST and GPx were decreased (Table 19) in carrageenan induced animals which may be due to enormous production of free radicals. After treatment with the ethanolic extract of S. mahagoni leaves, the alternations produced in the carrageenan induced animals with respect to lipid peroxidation and antioxidant enzyme activities were modulated to near normal levels.

Superoxide dismutase (SOD) has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It is reported that, SOD plays an important role in protecting cells against ROS (Yamaguchy, 1991). It catalytically scavenges the superoxide radical and thus provides the first line of defense against free radical damage. Decrease in the serum activity of SOD is the most sensitive enzymatic index of inflammatory damage. A reduced activity of SOD was observed in the disease induced group compared to sample and drug treated groups (Table 19). The higher the activity of SOD leads to increased scavenging of free radicals thereby improving the activity of antioxidant enzyme.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and its high activity indicates its radical scavenging activity. The catalytic decomposition of H2O2 into water and oxygen is mediated by catalase (Gaetani et al., 1996). One molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second. In the present study, CAT activity was found to decrease in carrageenan induced group but reverted back to
near - normal levels after the standard drug / plant extract administration, revealing the elevation of antioxidant enzyme as defense system (Table 19).

Glutathione is intra-cellular thiol rich tripeptide which plays a major role in the protection of cells and tissue structures (Meister, 1983). Glutathione peroxidase (GPx) is localized in the cytoplasm and mitochondria, which catalyses the degradation of various peroxides by oxidizing glutathione with the formation of its conjugates. GPx has more affinity for H₂O₂ than catalase. It catalyses the reduction of hydrogen peroxide in the presence of glutathione to form water and oxidized glutathione. It provides second line of defense against hydrogen peroxide before they can damage membranes and other cellular components (Chie et al., 1982). Decreased activity of glutathione peroxidase has been implicated in inflammation. In the present study, glutathione content was found to be low in the disease induced group while the same has been elevated in extract sample and standard drug treated groups (Table 19). Hence, an elevated level of GPx indicates protective role of ethanolic extract in inflammation.

Glutathione-S-transferase (GST) is a soluble protein located in cytosol. It plays an important role in detoxification and excretion of xenobiotics by conjugating them with glutathione (Boyer et al., 1984). The level of GST recorded a significant (P<0.001) decline in carrageenan induced rats when compared with normal control (Table 19). This significant reduction in the activity of the enzyme could be attributed to the increased oxidative stress that is evidenced in the carrageenan induced group whereas in S. mahagoni extract treated group of rats, the activity of GST attained normal level indicating that the pretreatment resulted in significant reversion to level comparable to that of the standard drug indomethacin treated group (Table 19). GST has a well established role in protecting cells from mutagens and carcinogens, as a free radical scavenger along with glutathione (Cleasseaud, 1979).

The activated macrophages and lymphocytes by adjuvant inoculation or their product monokines may be involved in abnormal lipid and protein metabolism (Eden et al., 1985). Lipid peroxides are formed by auto-oxidation of polyunsaturated fatty acids found primarily in cell membranes. An increase in level of lipid
peroxides in tissues, therefore, reflects membrane damage (Kawamura et al., 1992). In many diseases, such as inflammations and rheumatoid arthritis, membrane damage often occurs in some organ or tissue, which provokes lipid peroxidation in the membrane and accelerates the disorder in structure and function of these membranes (Yagi, 1987). The lack of an antioxidant defense leads to an increase in lipid peroxidation and subsequent deleterious effects (Scott et al., 1989). Increased lipid peroxidation in body tissue has been observed in carrageenan induced inflammation (Bardhan and Sharma, 1983). In the present investigation, the level of lipid peroxides increased in inflammation condition (Table 19). The increased level of lipid peroxides under inflammation condition might be due to poor antioxidant defense as well as inactivation of antioxidant systems. However, on administration of the S. mahagoni extract, the level of lipid peroxides was found to decrease (Table 19). Hence, the results of this present investigation elucidate that the studied plant extract inhibits both inflammation as well as accumulation of lipid peroxides in tissues, which lie on line with previous reports (Ramprasad et al., 2005).

The results obtained in the present study are in accordance with the earlier reports (Gosh et al., 2012) of S. mahagoni methanolic seed extracts on carrageenan induced paw edema which reveals the anti-inflammatory activity of leaves of S. mahagoni.

5.3.4. Hepatoprotective activity of S. mahagoni leaves

Liver is the most important organ concerned with the biochemical activities in the human body. It is also the organ highly affected primarily by the toxic agents and it has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore, damage to the liver inflicted by hepatotoxic agents is of grave consequence. There is an ever increasing need of an agent which could protect the liver from such damages. In view of the severe, undesirable side effects of synthetic agents, there is growing focus to evaluate the scientific basis for the traditional medicines which are claimed to possess hepatoprotective activity.

The present study brings about the hepatoprotective potential of leaves of S. mahagoni and gives insight into its mechanism of action. Paracetamol was used to induce hepatic damages in rats while studying the hepatoprotective potential of
S. mahagoni. Paracetamol induced hepatic injuries are commonly used in experimental models for the screening of hepatoprotective drugs (Davis et al., 1974). When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. The leakage of cellular enzymes into the plasma is an obvious sign of hepatic injury (Schmidt et al., 1975). Their estimation in the serum is a useful quantitative marker for the extent and type of hepatocellular damage.

The paracetamol induced hepatotoxicity is already evident in literature, as indicated by increased levels of alanine-leucine-transaminase, 24 h after a single injection to rats of 400-500 mg paracetamol per kg body weight (3.3-4.0 mmolkg\(^{-1}\)) (Tarloff et al., 1996). Its toxicity is associated with the depletion of hepatic GSH, followed by covalent binding of NAPQI, produced by cytochrome P\(_{450}\), to tissue proteins (Ray et al., 1993). When GSH levels are low, NAPQI fails to be detoxified by conjugation; it accumulates and causes liver injury (Mc Murtry et al., 1978). In the present study, when rats were exposed to paracetamol (750 mg/kg), the activity of the enzymes like SGOT, SGPT and ALP were significantly increased when compared to the control group (Table 21). Transaminases which play a vital role in protein metabolism (Bergmeyer, 1963) are located in the cytoplasm and in the mitochondria (Nemcsok et al., 1981). Aspartate and alanine transaminase functions as link enzymes between the protein and carbohydrate metabolism and also serve as an indicator of altered physiological or stress condition. The damage and lysis of the cells results in getting these enzymes in blood at relatively high concentrations causing a rapid increase of enzyme activity called “blood transaminases”. Elevated levels of serum transaminase enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lowhorn, 1978). Co-administration of S. mahagoni extract seems to preserve the structural integrity of the hepatocellular membrane. It may be capable of conditioning the hepatocytes and causes accelerated regeneration of parenchyma cells (Thabrew et al., 1987), thus protecting against membrane fragility and decreasing the leakage of marker enzymes into the circulation. This is in agreement with the commonly accepted view that serum levels of transaminases return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew et al., 1987).
Alkaline phosphatases are a group of enzymes found primarily in the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). The name implies, this enzyme works best at an alkaline pH (pH 10) and thus the enzyme itself is inactive in the blood. The primary importance of measuring alkaline phosphatase is to check the possibility of liver disease or bone disease. When the liver, bile ducts or gall bladder are not functioning properly or are blocked, this enzyme is not excreted through the bile and is released through the blood stream. Thus the serum alkaline phosphatase is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine. Ansari et al., 1991 demonstrated that the type of membrane involving a change in compartmentalization concerns the lysosome. Injury to lysosome will lead to the release of ACP, ALP and 5’Nucleotidase into the blood stream. Thus an increased ALP activity in the present study indicates the lysosomal damage and its release into the blood stream. It is evident from the results that ALP activity in serum was decreased significantly (P< 0.001) in ethanolic extract of S. mahagoni treated groups (Group IV, V) as compared with the paracetamol treated group (Group II). This might be due to protection of lysosomes by drug (S. mahagoni) against paracetamol induced damage. The results of the present study are in parallel with the finding of Emmanuel et al., 2001 who reported that treatment with leaves of Wedelia calendulacea reduced the elevated levels of ALP against paracetamol induced hepatotoxicity.

A significant increase (P<0.001) in the serum levels of bilirubin was recorded in paracetamol intoxicated rats against normal control rats. An increase in serum bilirubin in paracetamol treated rats is an indication of hepatic damage (Singh et al., 2001a) and might be due to the destruction of erythrocytes by toxic metabolites leading to overproduction or failure to excrete bilirubin (Germano et al., 1999). Co-administration of ethanol extract of S. mahagoni offered hepatoprotection which is evidenced by the inhibition of the rise in bilirubin level. The observation of the present study were supported by the studies of Singh and Handa (1995) where Apium graveolens and Hygrophylla auriculata were found to revert the increased bilirubin level in paracetamol and thioacetamide intoxicated rats.
Recently reported data suggest that paracetamol hepatotoxicity is mediated by an initial metabolic oxidation, covalent binding and subsequent activation of macrophages to form reactive oxygen and nitrogen species (Michael et al., 1999). Lipid peroxidation, an autocatalytic process resulting from oxidative stress contributes to the initiation and progression of liver damage (Kyle et al., 1987). Cells have a number of mechanisms to protect themselves from the toxic effects of ROS such as SOD, CAT and GPx. SOD removes superoxide by converting it into \( \text{H}_2\text{O}_2 \), which can be rapidly converted to water by CAT and GPx (Halliwell and Gutteridge, 1992). In order to elucidate the protective mechanism of S. mahagoni the lipid peroxidation levels as well as enzymatic antioxidant activities were examined.

Lipid peroxidation is initiated by free radicals leading to the formation of lipid peroxides, lipid alcohol and aldehydic byproducts like malondialdehyde (MDA) which has been used as a model for secondary products of lipid peroxidation. The lipid peroxidation is a highly destructive process and induces alteration in structure and function of cellular membranes (Kale and Sitasawad, 1990). The lipid peroxidative degradation of biomembrane is one of the principle causes of hepatotoxicity of paracetamol. In the present study, the increase in the thiobarbituric acid reactive substances (TBARS) of liver in paracetamol intoxicated rats indicates enhanced lipid peroxidation leading to tissue injury and failure of antioxidant defense mechanisms to prevent the formation of excess free radicals (Saraswathy et al., 1998). Co-administration with plant extract reversed these changes (Table 22). These results were supported by Shenoy et al., (2001) who reported an increased lipid peroxidation against paracetamol administration which was reverted to normal levels by Ginkgo biloba. Hence, the possible mechanism of hepatoprotection offered by the ethanolic extract of S. mahagoni may be due to its antioxidant effect.

Superoxide dismutase (SOD) has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It is suggested to a play a role in defense mechanism against endogenously generated superoxide anion (Prabhu, 2002). It scavenges the superoxide anion to form hydrogen peroxide, thus diminishing the Toxic effect caused by this radical. Decrease in the serum activity of SOD is the most sensitive enzymatic index of hepatocellular damage and liver injury.
(Curtis et al., 1972). In the present study also, the hepatic level of SOD was significantly reduced in the paracetamol treated rats when compared to the control group (Table 22), indicating liver injury. Co-administration of ethanolic extract of Swietenia mahagoni, however, reversed the hepatic SOD activity. The ethanol extract of Swietenia mahagoni exhibiting higher levels of antioxidant activities (Table 22) may interfere with the formation of NAPQI from paracetamol through an oxidative step, thereby preventing the formation of the inactive protein-NAPQI adducts. Parallel to this activity, the plant extract can also reduce free radicals that might result in decrease oxidative damage to liver tissues and improve the activities of this hepatic antioxidant enzyme.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and its highest activity is found in the red cells and in liver (Chance et al., 1992). It catalyzes the decomposition of hydrogen peroxide generated by the activity of SOD into less reactive gaseous oxygen and water molecule (Gaetani et al., 1996). One molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second. Therefore, the reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide. In the present study, CAT activity in liver tissue decreased in paracetamol intoxicated rats. The reduced activity of CAT observed in paracetamol treated rats confirms the hepatic damage to the rats (Kalpowitz et al., 1986). Decreased CAT activity is linked up to exhaustion of the enzyme as a result of oxidative stress caused by paracetamol. The CAT activity was brought to near normal in S. mahagoni ethanol extract treated rats. This evidently shows the antioxidant property of the ethanol extract of S. mahagoni against oxygen free radicals.

Glutathione (GSH) is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in the liver. Its functions are concerned with the removal of free radical species such as hydrogen peroxide, superoxide radicals and alkoxy radicals, maintenance of membrane protein thiols and serving as a substrate for glutathione peroxidase (GPx) (Prakash et al., 2001). GPx provides second line of defense against hydrogen peroxide before they can damage membranes and other cellular components (Chie et al., 1982). Blum and Fridorich (1985) reported that
O$_2^-$ reacts with selenium at the active site of GPx and thereby inactivating the enzyme. Prabhu (2002), clearly demonstrated that selenium dependant GPx diminish in paracetamol treated rats, which may be related to the increased cellular level of O$_2^-$ thus conforming the above postulate. In the present study, GPx activity was significantly (p<0.001) reduced in paracetamol treated group when compared with control group (Table 22). Reduced GPx activities confirm the damage to the hepatic cells (Singh et al., 1999). Oral administration of ethanolic extract of S. mahagoni restored the activities of GPx. The reversal of GPx activity by the plant extract may be attributed to its antioxidant activity (Table 22) by scavenging / detoxifying the endogenous metabolic peroxides generated after paracetamol injury in the liver tissue.

Glutathione-S-transferase (GST) is a soluble protein located in cytosol. It plays an important role in detoxification and excretion of xenobiotics by conjugating them with glutathione (Boyer et al., 1984). The level of GST recorded a significant (P<0.001) decline in paracetamol administrated rats when compared with normal control. The decreased activity of GST can be explained on the basis that superoxide radicals when produced in excess may inactivate the H$_2$O$_2$ scavengers (Attri et al., 2001). In the test drug treated groups of rats the activity of GST attained a near normal level. The detoxification of paracetamol can be mediated by GST catalyzed conjugation with GSH in the liver (Threkeld, 1997). The increased hepatic GST activity induced by ethanol extract can, therefore, reduce the acute paracetamol hepatotoxicity. In this regard, GST has been shown to play a critical role in preventing the binding of paracetamol to DNA in species resistant to paracetamol toxicity (Park, 1995).

The level of hepatoprotectivity provided by the ethanolic extract of S. mahagoni against paracetamol intoxication (Table 20-22) was comparable with that of the standard, silymarin treated group. Preliminary phytochemical analysis of S. mahagoni leaves indicated the presence of flavonoids and tannins and these types of polyphenols are well known natural antioxidants due to their electron donating property which either scavenge the (Sugihara et al., 1999) principal propagating free radicals or halt the radical chain. Thus S. mahagoni, because of the presence of natural antioxidants, must have exerted protective action against paracetamol.