5.1. PHOTOCHEMICAL ANALYSIS

The phytoconstituents are known to play an important role in bioactivity of medicinal plants. To explore the importance of any medicinal plant the initial step is to screen for its phytochemicals, as it gives a broad idea regarding the nature of compounds present in it. In the present study, the whole plant of *Combretum albidum* and root of *Salacia fruticosa* were preliminarily screened for the phytochemicals. The presence of carbohydrates, alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids, phenolic compounds, protein and lack of gum, in the extract of CA and SF were stated in Table-3. The presence of many biologically active phytochemicals in various plant extracts may be responsible for their respective pharmacological properties (Agarwal and Rangari *et al.*, 2003; Singh *et al.*, 2002). In recent years, Gas Chromatography-Mass Spectrometry (GC-MS) has been applied unambiguously to identify the structures of different phytoconstituents in plant extracts and biological samples with great success [Prasain *et al.*, 2004]. GC-MS is a reliable technique to identify the constituents of volatile matter, long-chain branched hydrocarbons, alcohols acids and esters [Anjali *et al.*, 2009]

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the ethanolic extract of *Combretum albidum* and *Salacia fruticosa*. These compounds were identified through mass spectrometry attached with GC. The results of the present study were tabulated in Table-4 and 5. The gas chromatogram shows (Fig7and 8) the relative concentrations
of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. In addition to this, the results of the GC-MS/MS profile can be used as pharmacognostical tool for the identification of the compounds in plant with different chemical structures. The compound identified by mass spectrum with chemical structure are shown in fig.-9, 10 and 11.

The compounds identified in ethanolic extract of CA with higher percentage of peak area 26.85% are 5-Hydroxymethyl furfural an aldehyde reported to have Preservative and Anti-inflammatory activity. The phytochemicals present in ethanolic extract of SF was identified with highest peak area 38.43%, are Myo-Inositol, 4-C-methyl, molecular formula C\textsubscript{7}H\textsubscript{14}O\textsubscript{6}, molecular weight 194, is a Phyto active compound have antidiabetic effect [Duke’s, 2013]. The identified Phytosterols namely Campesterol, Stigmasterol, have been reported for the treatment of diabetic mellitus by lowering fasting blood glucose levels by cortisol inhibition [Devaraj and Jialal, 2006]. Palmitic acid, linoleic acid, linolenic acid are essential fatty acids found in animals and plants which are primarily used to produce hormone like substances that regulate a wide range of functions, including blood pressure, blood clotting, blood lipid levels, the immune response, and the inflammation response to injury infection [Altieri et al. 2009]. Squalene is mostly used in cosmetic industry [McAnuff et al., 2005].
It is hypothesized that the presence of phenolic compounds [Gonzalez de Mejia et al., 2004], saponins, glycosides and flavonoids [Yoshikawa et al., 2001] present in plant extracts are capable of inhibiting hepatocellular damage induced by hepatotoxins. The nephroprotective medicinal plants were reported to inhibit xenobiotic induced nephrotoxicity in experimental animal models due to their potent antioxidant or free radical scavenging effects [Devipriya and Shyamaladevim, 1999; Annie et al., 2005]. In addition, alkaloids were found to strongly inhibit lipid peroxidation induced in isolated tissues via antioxidant activity [Kumaran and Karunnakaran, 2007]. The protection offered by the extract might be due to the presence of flavonoids and alkaloids [Donsky et al., 2007; Lucia et al., 2007].

The results of qualitative phytochemical analysis and GC-MS/MS studies on CA and SF of plants extracts reveals that the presence of various bioactive compounds confirms the application of *Combretum albidum* and *Salacia fruticosa* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

5.2. ACUTE TOXICITY STUDIES

Toxicology is a science to study adverse-effects of chemicals or physical agents on biological system and preclinical toxicology is a science to evaluate safety of drug (mostly) in animals to decide if the drug is safe for human use or not. Plants, vegetables and herbs used as food and in the folk treatment have been accepted currently as one of the main source of drug discovery and development, but only a few of them have been scientifically investigated, especially regarding their toxic aspects (Pereira et al., 2010).

Acute toxicity studies in animals are usually necessary for any pharmaceutical intended for human use. The information obtained from these studies
is useful in choosing doses for repeat dose studies, providing preliminary identification of target organs of toxicity and occasionally, revealing delayed toxicity. Acute toxicity studies may also aid in the selection of starting doses for Phase 1 human studies, and provide information relevant to acute overdosing in humans. It could also be used to estimate the therapeutic index (LD50/ED50) of drugs (Rang et al., 2001; Maikai et al., 2008).

In the present study, acute toxicity test was done to establish if any adverse effects of the administration of the ethanolic extract of Combretum albidum and *Salacia fruticosa* on some observable parameters. The results indicate no abnormal symptoms and no death of the rats.

Acute toxicity denotes the effects of a single dose or multiple doses of a substance during a 24-hour period (Sathya et al., 2012). It is one of the initial steps in the assessment of the toxic properties of compounds (Bhardwaj and Gupta, 2012; Akhila et al., 2007). This toxicity studies is carried out for short periods (usually 24 hours) to establish the toxicity, safety and efficacy profile of a new product. After a single administered dose, samples are usually observed for a few days or 2 – weeks (Pacific BioLabs, 2009). LD50 is the index for studying acute toxicity (Abdullah, 2011). No death was recorded when the ethanolic extract of CA and SF were administered to rats, hence, the median lethal dose (LD50) was estimated to be above 2000 mg/kg body weight. Clarke *et al.* (1975) reported that any substance with LD50 beyond 1000 mg/kg should be regarded nontoxic or have low toxicity when orally administered.

5.3. INVIVO HEPATOPROTECTIVE STUDIES

Prophylactic action in liver damage induced by carbon tetrachloride has widely been used as an indicator of the liver protective activity of drugs in general
[Clauson et al 1989]. Since the changes associated with CCl₄-induced liver damage are similar to that of acute viral hepatitis [Rubinstein et al., 1962]. Investigation of chronic administration of CCl₄-induced liver damage in animals was chosen as an experimental model.

It is well documented that carbon tetrachloride is bio-transformed under the action of cytochrome P-450 system in the microsomal compartment of liver to trichloromethyl or peroxy trichloromethyl free radical. These free radicals bind covalently to the macromolecules and induce peroxidative degradation of the membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides followed by pathological changes such as triacylglycerol accumulation, polyribosomal disaggregating, and depression of protein synthesis, cell membrane breakdown and even death [Recknagel et al., 1966; Noguchiet et al., 1982].

In general, the extent of liver damage is assessed by histopathological evaluation and serum levels of ALT, AST, ALP, TB and TP release in circulation [Plaa et al., 1994; Portmann et al., 1975]. When the liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage [Mitra et al., 1998].

In the present study, it was observed that administration of CCl₄ elevates the levels of serum marker enzymes ALT, AST, ALP and total serum bilirubin as well as decreases total serum protein level significantly. Ethanolic extract of Combretum albium, Salacia fruticosa and reference drug silymarin treated groups exhibited lower serum levels of ALT, AST, ALP and total bilirubin whereas increases total protein as compared to CCl₄ treated groups. The stabilization of serum ALT, AST, ALP, and
total bilirubin and the restoration of total protein levels by Eathnaolic extract of
*Combretum albidum* and *Salacia fruticosa* is a clear indication of the improvement of
functional status of the liver cells.

Hepatoprotective activity correlated with antioxidant activity, since it
is free radical mediated damage [Murthy *et al.*, 2002]. Elevated level of
malondialdehyde (MDA) reflects an enhanced lipid peroxidation leading to tissue
damage and failure of antioxidant defence mechanisms to prevent formation of
excessive free radicals [Souza *et al.* 1997]. Treatment with Eathnaolic extract of
*Combretum albidum* significantly reversed these changes. Hence it may be possible
that the mechanism of hepatoprotection by Eathnaolic extract of *Combretum albidum*
and *Salacia fruticosa* is due to its antioxidant effect.

The enzymatic antioxidant defence systems are the natural protector
against lipid peroxidation. SOD, CAT and GPx enzymes are important scavengers
of superoxide ion and hydrogen peroxide. These enzymes prevent generation of
hydroxyl radical and protect the cellular constituents from oxidative damage
[Scott *et al.*, 1991]. In the present study, it was observed that the Eathnaolic extract of
*Combretum albidum* and *Salacia fruticosa* significantly increased the hepatic SOD
activity in CCL₄ induced liver damage in rats. This shows that the Eathnaolic
extract of *CA and SF* can reduce reactive free radicals that might lessen oxidative
damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

Earlier studies regarding mechanism of CCL₄ induced hepatotoxicity
have shown that GSH plays a key role in detoxifying the reactive toxic metabolites
of CCL₄ and that liver necrosis begins when the GSH stores are marked in a
Eathnaolic extract of *CA and SF* increased the content of GSH significantly as compared to CCl₄ treated groups.

Catalase (CAT) is an enzymatic antioxidant widely distributed in all animal tissues and the highest activity, is found in the red cells and in the liver. CAT decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals [Chance et al 1952]. 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. Administration of the Eathnaolic extract of *CA and SF* increased the activities of CAT on CCl₄ induced liver damage in rats to prevent the accumulation of excessive free radicals and protected the liver from CCl₄ intoxication.

These findings can be further in corroborated by histopathological studies. The histopathological examination clearly reveals that the hepatic cells, central vein, and portal triad are almost normal in the liver section of rats treated with Eathnaolic extract of *CA and SF* in contrast to the liver section of rats which received CCl₄ only. Thus *Combretum albidum* and *Salacia fruticosa* can be considered to be an effective hepatoprotective as it ameliorate almost to normalcy the damage caused by CCl₄ to hepatic function.

It is well established that the phytoconstituents such as Flavonoids, triterpenoids and tannins are well known for their hepatoprotective activities [Manjunatha et al., 2008 Manjunatha et al., 2009]. The literature review revealed that preliminary phytochemical analysis of heart wood of *Combretum albdium* showed the presence of the higher percentage of tannins, flavonoids, triterpenes, saponins, and glycosides, and five triterpenoids namely betulin, betulinic acid, oleanolic acid, arjunolic acid, ellagic acid and other constituent beta sitosterol, gallic
acid were isolated and reported [Kumar et al., 2015; Sreedhar et al., 2013]. From the aerial parts (leaves and stem) of *Salacia fruticosa*, the presence of alkaloids, carbohydrates, phytosterols, saponins, phenolic compounds, proteins, free amino acids, flavonoids, and terpenoids [Saravanan et al., 2015] have been reported and Srinivasan et al 2009, reported the isolation of Mangiferin (2-Î²D glucopyranosyl-1,3,6,7-tetrahydroxyxanthen-9-one) from the roots of *S. fruticosa*. The hepatoprotective activity of *Combretum albidum* and *Salacia fruticosa* may be attributed due to the presence of these constituents. This study supports the traditional claims of the plant CA and SF could be added in traditional preparations for the various liver diseases.

**5.4. INVIVO NEPHROPROTECTIVE STUDIES**

Various environmental toxicants and clinically useful drugs, like paracetamol and gentamicin, can cause severe organ toxicities through the metabolic activation to highly reactive free radicals including the superoxide and oxygen reactive species [Abraham et al., 1999]. The selective renal accumulation of non-steroidal anti-inflammatory nephrotoxins including paracetamol in animal and human is thought to result in a chain of biochemical reactions which culminate in acute or chronic nephropathies [Schnellman, 2001]. In addition, paracetamol has been reported to promote hepatocyte and renal apoptosis [Ray et al., 2000; Boulares et al., 2002]. Paracetamol toxic overdose is often manifested by too many metabolic and uric acid derangements. Serum urea and Creatinine are considered the major nephrotoxicity markers [Adelman et al., 1981], although serum urea concentration is often considered a more reliable renal function predictor than serum creatinine [Palani et al., 2009]. Blood urea nitrogen is found in the liver protein that is derived from diet or tissue sources and is normally excreted in the urine. Creatinine, on the
other hand, is mostly derived from endogenous sources by tissue creatinine breakdown [Mayne, 1994]

In the present investigation, administration of a nephrotoxic dose of Acetaminophen to rats resulted in a significant \( (p < 0.01) \) elevation of serum levels of urea, uric acid and creatinine in APAP treated group within 48 hours of exposure to it when compared with the normal control group. These results are in agreement with those observed in Isik B et. al.( 2006), who noticed an elevation in serum urea and creatinine in rats after 1 g/kg body weight of paracetamol administration. This elevation in the levels of urea and creatinine was explained by the presence of strong correlation between nephrotoxicity and oxidative stress [Karadeniz et al., 2008; Ajami et al., 2010]. However, daily pretreatment with ethanol extract of Combretum albidum and Salacia fruticosa for 7 days to the Acetaminophen renal injured rats significantly \( (p < 0.001) \) decreased the concentration levels of urea, uric acid, and creatinine to normal status in a dose dependent manner and 500mg/kg dose offered maximum protection. This proved the potency of renal cell regeneration capacity of Salacia fruticosa.

The important function that blood cells perform, together with the susceptibility of this highly proliferative tissue to intoxication by xenobiotic, makes the hematopoietic system unique as a target organ [Adeneye et al., 2008]. The various blood cells (erythrocytes, leucocytes, and platelets) are produced at a turnover rate of about 1 to 3 million per second in a healthy human adult and this value could be altered in certain physiological or pathological states including hemolytic anemia and inflammation [Guyton, 1991]. Certain drugs including alkylating cytotoxic agents could also affect blood formation rate and the normal range of hematological parameters [Adeneye et al., 2008]. In the present study, treatment with APAP oral
dose significantly decreased the level of Hb, PCV, PLC, MCH & MCHC, whereas the level of MCV were increased significantly. After the pretreatment with ethanol extract of *Combretum albidum* and *Salacia fruticosa* these levels are retrieved normally, as compared to the normal control group. Results of this study shows that the *Salacia fruticosa* extract may contain an active molecule which recover the hematotoxic effect of acetaminophen and assured the improvement of hematopoietic system.

Several researches have shown that APAP- induced nephrotoxicity is associated with lipid peroxidation. This is attributed to a free radical-mediated chain reaction that damages cell membranes [Zhar *et al.*, 1998; Abraham *et al.*, 2005] and MDA is a good indicator of the degree of lipid peroxidation. In this study, we observed a significant increase in MDA levels in the renal tissue of rats treated with APAP alone compared with normal control. Administration of the plant extract inhibited the increase in lipid peroxidation level in renal tissue. It is likely that the action of ethanol extract of Combretum albidum and *Salacia fruticosa* in reducing the membrane damage is mainly related to its ability to scavenge lipid peroxidation initiating agents.

Depletion of renal GSH is one of the primary factors that permit lipid peroxidation, it is suggested to be closely related to APAP tissue damage. It has been reported that renal glutathione content, and glutathione peroxidase and reductase activity of kidney tissue, which are critical constituents of the GSH-redox cycle, were significantly reduced by treatment with adriamycin, and the authors proposed that impairment of the kidney antioxidant defense mechanisms could permit enhanced free radical- induced kidney damage in adriamycin nephrotoxicity [Simic, 1996]. Similarly, in the current study, administration of ethanol extract of *Salacia*
fruticosa to APAP treated rats also increased the GSH level and the GSH-Px, CAT, and SOD activity of renal tissue. The increase in both non-enzymatic and enzymatic antioxidants might play a significant role in the mechanism of the Nephroprotective effect of Salacia fruticosa.

These findings can be further incorporated with histopathological studies. APAP induced renal damage is consistent with acute tubular necrosis. In the present study, the results of histopathological examination showed a clear evidence of nephrotoxicity following the administration of APAP in an overdose. Acute tubular necrosis was the most relevant histopathological change. These results are in agreement with those of the previous investigation describing the renal histological alterations following the administration of APAP in an overdose [Ghaisas et al., 2010].

Treatment with ethanol extract of Combretum albidum and Salacia fruticosa ameliorated the APAP induced histopathological renal changes.

It is well established that medicinal plants with nephroprotective properties to mediate their protection via antioxidant and/or free radical scavenging activities due to the high concentration of flavonoids and alkaloids they contain [Miller et al., 1997; Adeneye et al., 2008]. The identification of these biologically active compounds by GC-MS/MS analysis of EECA & EESF, may be contribute to the hepatoprotective, nephroprotective and anti-oxidant potential of the plants.