# RESULTS AND DISCUSSION

# 4.0 RESULTS AND DISCUSSION

The plant kingdom is a treasure house of potential drugs and in recent years there has been an increasing awareness about the importance of medicinal plants. The chemical constituents of plant medicines are a part of the physiological activities of living plants and hence they are believed to have a better compatibility with the human body. There is a growing tendency all over the world to shift from synthetic to natural hared products including medicinal plants (Cathrine and Nagarajan, 2010).The present study was carried out in four phases to evaluate the Antioxidant, Hepatoprotective and Thrombolytic effect of ***Alternanthera sessilis*(L).R.Br.ex DC.**

- In the first Phase of the study, the leaf extract of *Alternanthera sessilis* was screened for phytochemical constituents and antioxidant potential.
- In Phase II, hepatoprotective effect of the plant was evaluated using experimental rats. The extent of hepatic damage was assessed by the level of selected biochemical parameters namely Total protein, Albumin, Bilirubin, Total cholesterol, High Density Lipoprotein(HDL),Low Density Lipoprotein(LDL), Triglycerides, Aspartate transaminase(AST), Alanine transaminase(ALT) and Thiobarbituric Acid Reactive substancse(TBARS).
- Cytotoxic effect of the plant sample was detected by Brine Shrimp Lethality Bioassay in Phase III
- Phase IV involves the determination of thrombolytic activity of the plant using human blood.

## 3.3.1 PHASE I

### PHYTOCHEMICAL CONSTITUENTS

Phytochemicals are known to have protective and disease preventive properties. The phytochemical screening of plant materials to determine the presence of bioactive constituents is, thus, vital in the knowledge of the therapeutic properties of plants. Such bioactive constituents analyzed in this study are indicated in table I.

TABLE I

PHYTOCHEMICAL CONSTITUENTS OF *Alternanthera sessilis*

| S. No | Phytoconstituents | Solvents | | |
|---|---|---|---|---|
| | | Water | Ethanol | Petroleum ether |
| 1 | **Alkaloids** | + | + | + |
| 2 | **Flavonoids** | + | + | + |
| 3 | **Saponins** | + | + | - |
| 4 | **Phenols** | + | + | - |
| 5 | **Glycosides** | + | + | + |
| 6 | **Tannins** | + | + | - |
| 7 | **Carbohydrates** | + | + | + |
| 8 | **Steroids** | + | + | - |
| 9 | **Terpenoids** | + | + | + |
| 10 | **Anthraquinones** | + | + | + |

**+ Presence of phytoconstituents**

**- Absence of phytoconstituents**

Investigated phytochemicals such as alkaloids, flavonoids, glycosides, carbohydrates, terpenoids and anthraquinones were found to be present in both aqueous and organic (ethanolic and petroleum ether) extract of *Alternanthera sessilis* whereas saponins, phenols, tannins and steroids were detected only in aqueous and ethanolic extract and not in petroleum ether extract.

Phytochemical screening of aqueous extract of *Samanea saman* revealed the presence of phytochemicals namely tannins, flavonoids, steroids, saponins, cardiac glycosides and terpenoids Prasad *et al.*, (2008). Boxi *et al.*, (2010) have reported the presence of flavonoids, saponins and phenol compounds in the aqueous extract of *Commicarpus chinensis.* Ayoola *et al.*, (2008) have shown the presence of flavonoids, saponins, tannins, alkaloids and cardiac glycosides in the ethanolic extract of the leaves

of *Carica papaya* Linn. Rose and Cathrine, (2011) have indicated the presence of phytochemicals namely flavonoids, triterpenoids, carbohydrates and anthraquinones in both ethanolic and petroleum ether extract of *Vitex negundo*.

## TOTAL ANTIOXIDANT POTENTIAL

The total antioxidant potential of the leaves of *Alternanthera sessilis* was analyzed and the results are shown in the table II and figure III.

**TABLE II**

**TOTAL ANTIOXIDANT CAPACITY OF *Alternanthera sessilis***

| S. No | Concentration of the extract (µg/ml) | Total Antioxidant Capacity (µg/g) |
|---|---|---|
| 1 | 200 | 10.13 ± 0.02 |
| 2 | 400 | 11.05 ± 0.03 |
| 3 | 600 | 11.22 ± 0.05 |
| 4 | 800 | 11.28 ± 0.04 |
| 5 | 1000 | 12.14 ± 0.01 |
| | CD(P<0.05) | 0.083 |

Values are mean± S.D of triplicates

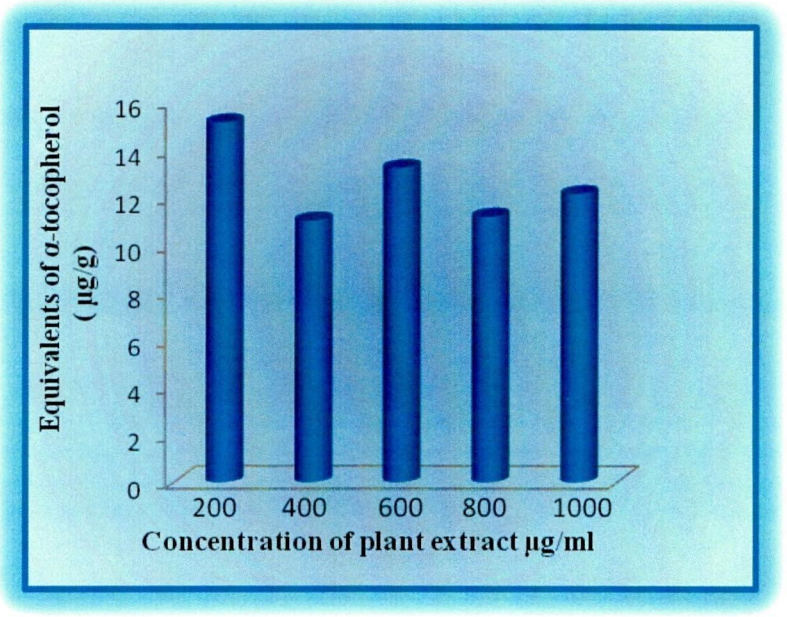
The extract with highest concentration (1000µg/ml) of leaves recorded the maximum total antioxidant capacity whereas the least value was observed in the extract with the lowest concentration (200µg/ml) of leaves. These results reveal that the total antioxidant capacity increased with increase in concentration of the plant extract.

This finding is in agreement with the report of Alam *et al.*, (2011) who have indicated that the antioxidant activity of the ethanolic extract of *Brassia nigra* is in the increasing trend with the increasing concentration of the plant extract. Kowti *et al.*, (2010) have observed significant dose dependent antioxidant activity in the leaves of *Spathodea campanulata*. Thus the obtained results suggest that the leaves of *Alternanthera sessilis* were found to be a good source of antioxidants. (Ramya and

Lakshmidevi, 2010) have also indicated that the ethanolic extract of *Tinospora cordifolia* leaves showed the highest total antioxidant activity when compared to other solvent extracts.

**FIGURE III**

**TOTAL ANTIOXIDANT CAPACITY OF *Alternanthera sessilis***




**TOTAL PHENOLS AND FLAVONOIDS**

The antioxidant activity of the plant products is associated with their bioactive compounds, mainly phenolics and flavonoids, because of their ability to scavenge free radicals. In the present study the content of total phenols and flavonoids was determined and given in table III.

TABLE III

CONTENT OF TOTAL PHENOLS AND FLAVONOIDS IN *Alternanthera sessilis*

| Total Phenols (mg/g) | Total Flavonoids (mg/g) |
|---|---|
| 1.41 ± 0.05 | 0.42 ± 0.08 |

Values are mean± S.D of triplicates

It is evident from the content of Total Phenols and Flavonoids in the leaves of *Alternanthera sessils* was found to be 1.41± 0.05 (mg/g) and 0.42±0.08 (mg/g) respectively. Phenolics compounds have attracted more and more attention as potential agents for preventing and treating many oxidative stress-related diseases. Several studies showed that phenolic compounds were the main antioxidant ingredients in several medicinal plants Li *et al.*, (2008). Flavonoids exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic and anti-ulcer actions. They are potent antioxidants and have free radical scavenging abilities (Kalpana *et al.*, 2011). Methanolic extract of *Flemingia strobilifera* showed higher amount of flavonols and phenolics and it was also considered as potential source of natural antioxidants (Madan *et al.*, 2010). Gan *et al.*, (2010) have indicated that the phenolic compounds could be the main contributor of the antioxidant activity of medicinal plants. The findings of the present study is in agreement with the above reports suggesting that total antioxidant capacity of *Alternanthera sessilis* might be due to the presence of phenols and flavonoids.

### 3.3.2 PHASE II

### DETERMINATION OF HEPATOPROTECTIVE EFFECT OF *Alternanthera sessilis*

Ethanolic extract of *Alternanthera sessilis* was evaluated for the hepatoprotective effect using experimental rats. After the experimental period, animals were anaesthetized, blood was collected and serum was separated. Liver was surgically removed and weighed. Liver homogenate was prepared using phosphate buffered saline. Serum and Liver samples were analyzed for various biochemical parameters.

## WEIGHT OF THE LIVER SAMPLES OF EXPERIMENTAL RATS

The net weight of the liver from different groups of experimental animals was recorded and given in the table IV.

### TABLE IV

### WEIGHT OF THE LIVER SAMPLES OF EXPERIMENTAL RATS

| Group No | Treatment | Weight of liver samples (g) |
|---|---|---|
| I | **Control** | 2.66 ± 0.025 |
| II | **$CCl_4$** | 3.24 ± 0.036 |
| III | **$CCl_4$+Silymarin** | 3.13 ± 0.059 |
| IV | **$CCl_4$+Plant extract(200mg/kg)** | 3.07 ± 0.038 |
| V | **$CCl_4$+Plant extract(400mg/kg)** | 3.02 ± 0.065 |
| | **CD(P<0.05)** | **0.047** |

Values are mean± S.D of six samples in each group

From the tabulated values, it is clear that $CCl_4$ induced a significant increase in liver weight. There was a significant reduction in the liver weight in group III, IV, V when compared to that of group II $CCl_4$ intoxicated rats. Comparison among groups III, IV and V indicates that the plant extracts reduced the weight of the liver to a greater extent than the drug silymarin. Plant extract at high concentration (400mg/kg) caused greater weight reduction than the extract at lower concentration (200mg/kg).

Qureshi *et al.,* (2009) have observed that silymarin and the ethanolic extract of *Cordia macleodi* leaves prevented the increase of liver weight in rats. Samudram *et al.*, (2008) stated that the significant reduction in the liver weight was seen in animals treated biherbal ethanolic extract of *Eclipta alba* and *Piper longum* when compared to $CCl_4$ intoxicated rats. Verma, (2010) has indicated that *Anisochilus carnosus* (L) Wall leaf extracts possessed a protective effect against Rifampicin induced hepatotoxicity in rats.

## TOTAL PROTEIN, ALBUMIN AND BILIRUBIN CONTENT IN EXPERIMEN TAL RATS

Table V, Figure IV indicate the content of Serum total protein, albumin and table VI, figure V reveal the bilirubin content in the experimental rats.

### TABLE V

### TOTAL PROTEIN AND ALBUMIN CONTENT IN EXPERIMENTAL RATS

| Group No | Treatment | Total protein(mg/dl) | Albumin(mg/dl) |
|---|---|---|---|
| I | **Control** | 8.01 ± 0.639 | 5.58 ± 0.182 |
| II | **$CCl_4$** | 4.83 ± 1.140 | 1.71 ± 0.148 |
| III | **$CCl_4$+Silymarin** | 7.73 ± 0.930 | 4.60 ± 0.139 |
| IV | **$CCl_4$+Plant extract (200mg/kg)** | 5.55 ± 0.403 | 3.34 ± 0.215 |
| V | **$CCl_4$+Plant extract (400mg/kg)** | 5.73 ± 0.638 | 4.35 ± 0.126 |
| | **CD(P<0.05)** | **0.94** | **0.22** |

Values are mean± S.D of six samples in each group

The content of total protein and albumin in serum of rats in the control group was found to be 8.01 ± 0.639 (mg/dl) and 5.58± 0.182(mg/dl) respectively. Administration of $CCl_4$ led to significant decrease in the level of total protein and albumin concentration. In groups (III-V) treated with silymarin and different doses of plant extracts, the level of total protein and albumin was found to be improved when compared to $CCl_4$ intoxicated group.

Animals treated with hydro alcoholic extract of the aerial part of *Cajanus cajan* at the dose of 400mg/kg b.wt showed significant increase in the total protein when compared to the $CCl_4$ (Singh *et al.*, 2011). Mujeeb *et al.*,(2011) have shown that treatment with ethanolic extract of *Ficus carica* Linn leaves at a dose of 50mg/kg, 100mg/kg and 200mg/kg significantly increased the level of total protein and albumin when compared to the $CCl_4$ intoxicated rats. Kumar *et al.*, (2010) opined that the

animals treated with $CCl_4$ significantly decreased the level of total protein and albumin. Treatment with silymarin and different doses of *Sesamun indicum* (400mg/kg and 700mg/kg) reversed the parameters, as compared to $CCl_4$ treated group.

**TABLE VI**

**BILIRUBIN CONTENT IN EXPERIMENTAL RATS**

| Group No | Treatment | Serum Bilirubin (mg/dl) |
|---|---|---|
| I | **Control** | 1.28 ± 0.152 |
| II | **$CCl_4$** | 3.25 ± 0.210 |
| III | **$CCl_4$+Silymarin** | 1.54 ± 0.117 |
| IV | **$CCl_4$+ Plant extract (200mg/kg)** | 1.29 ± 0.159 |
| V | **$CCl_4$+ Plant extract (400mg/kg)** | 1.39 ± 0.176 |
| | **CD(P<0.05)** | **0.215** |

Values are mean± S.D of six samples in each group

From table VI and figure V it is evident that the level of bilirubin in the serum was found to be increased in $CCl_4$ treated rats. Treatment with ethanolic extract of *Alternanthera sessilis* (200mg/kg and 400mg/kg) caused significant hepatoprotective effect and it was almost comparable to that of silymarin, the known hepatoprotective agent.

Kumar and Kumar, (2010) have shown that $CCl_4$ caused significant elevation of serum bilirubin and treatment with methanolic extract of *Mimosa pudica* (200mg/kg and 400mg/kg) brought down the raised bilirubin level. Ethanolic extract of *Aerva lanta* Linn significantly reversed the elevated level of bilirubin indicating the effectiveness of the extract in normal functional status of the liver (Manokaran *et al.*, 2008). The substantially elevated level of serum bilirubin was restored normalization significantly by the ethanolic extract of *Gmelina asiatica* aerial parts (Merlin and Parthasarathy, 2011).

**FIGURE IV**

**TOTAL PROTEIN AND ALBUMIN CONTENT IN EXPERIMENTAL RATS**

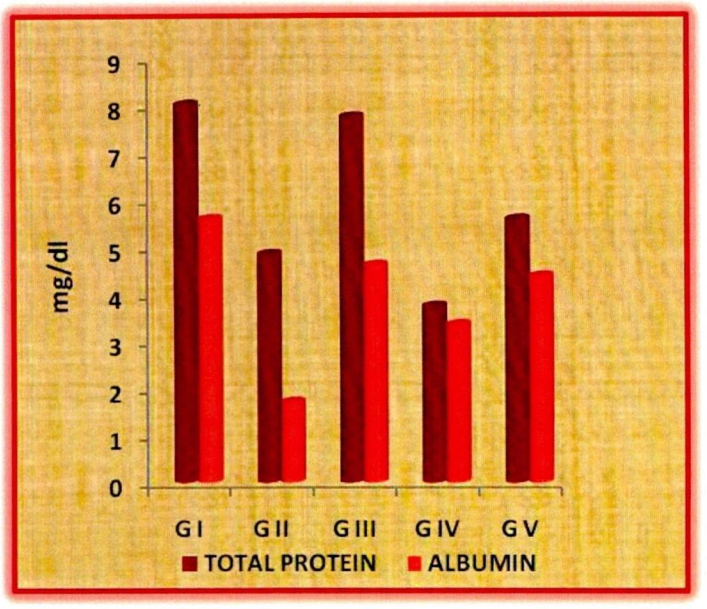
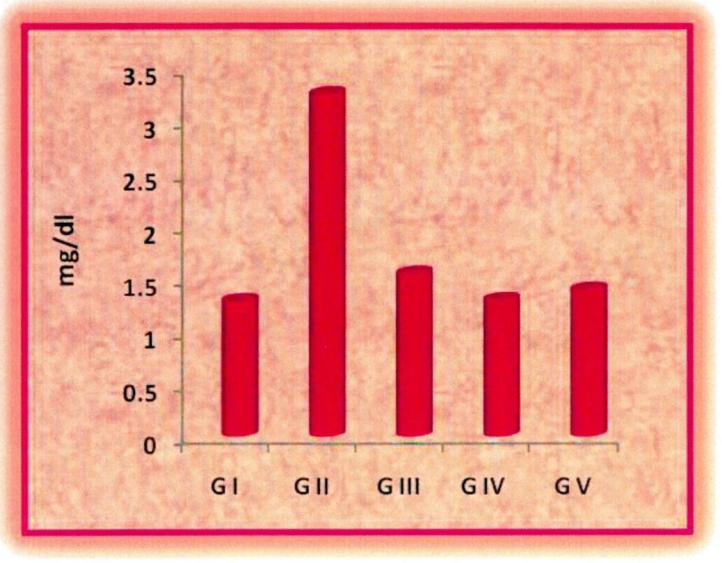



**FIGURE V**

**BILIRUBIN CONTENT IN EXPERIMENTAL RATS**



## LIPID PROFILE OF SERUM AND LIVER SAMPLES OF EXPERIMENTAL RATS

Total cholesterol, HDL, LDL and Triglycerides were assessed in serum and liver samples of different groups of rats and depicted in table VII, VIII and figure VI and VII.

### TABLE VII

### LIPID PROFILE OF SERUM SAMPLES IN EXPERIMENTAL RATS

| G. No | Treatment | Total Cholesterol (mg/dl) | High Density Lipoprotein (mg/dl) | Low Density Lipoprotein (mg/dl) | Triglycerides (mg/dl) |
|---|---|---|---|---|---|
| I | Control | 130.1 ± 0.952 | 48.2± 1.390 | 172.3 ± 0.899 | 160.6 ± 1.660 |
| II | $CCl_4$ | 420.0 ± 0.550 | 30.9 ± 1.630 | 290.0± 1.260 | 430.7 ± 2.060 |
| III | $CCl_4$+Silymarin | 209.3 ± 1.668 | 55.0 ± 1.860 | 123.2 ± 1.240 | 130.5± 1.383 |
| IV | $CCl_4$+Plant extract (200mg/kg) | 103.1 ± 1.435 | 33.5 ± 1.304 | 97.3 ± 0.803 | 113.9 ± 1.861 |
| V | $CCl_4$+Plant extract (400mg/kg) | 83.8 ± 1.249 | 36.2 ± 1.789 | 86.7 ± 1.229 | 104.2 ± 1.287 |
| | **CD(P<0.05)** | **2.39** | **2.25** | **1.89** | **3.14** |

Values are mean± S.D of six samples in each group

TABLE VIII

LIPID PROFILE OF LIVER SAMPLES IN EXPERIMENTAL RATS

| G. No | Treatment | Total Cholesterol (mg/dl) | HDL(mg/dl) | LDL (mg/dl) | Triglycerides (mg/dl) |
|---|---|---|---|---|---|
| I | Control | 155.7 ± 1.593 | 44.8± 1.649 | 168.1 ± 0.828 | 148.6 ± 1.441 |
| II | $CCl_4$ | 437.9± 1.786 | 37.4 ± 1.630 | 433.8± 1.341 | 455.8± 1.588 |
| III | $CCl_4$+Silymarin | 126.1 ± 1.289 | 47.6± 1.303 | 133.5 ± 1.869 | 104.2± 1.137 |
| IV | $CCl_4$+Plant extract (200mg/kg) | 112.5± 1.277 | 44.2± 1.855 | 104.4.± 1.308 | 123.1± 1.456 |
| V | $CCl_4$+Plant extract (400mg/kg) | 103.2 ± 1.222 | 47.4± 1.125 | 96.5± 0.940 | 116.6 ± 1.251 |
| | **CD (P<0.05)** | **2.48** | **1.89** | **1.70** | **1.81** |

Values are mean± S.D of six samples in each group

The liver injured non treated group showed a significant increase in serum cholesterol, LDL and triglycerides and a significant decrease in HDL when compared to control group. The groups treated with silymarin and *Alternanthera sessilis* leaf extracts showed a significant decrease in serum cholesterol, LDL and triglycerides and significant increase in HDL. A similar trend was observed in the lipid profile of liver samples.

This observation coincides with the report of Venukumar and Latha, (2002) who have indicated an increase in the levels of total cholesterol, LDL and triglycerides in serum and liver samples of experimental animals with $CCl_4$ treatment and its recovery towards near normal value in methanolic extract of *Curculigo orchioides* administered rats. Sundari *et al.*, (2011) observed that administration of ethanolic extract of *Sphaeranthus indicus* (L.) significantly decreased the serum lipid profile in paracetamol toxicity induced rats because of its hypolipidemic effects.

**FIGURE VI**

**LIPID PROFILE OF SERUM SAMPLES IN EXPERIMENTAL RATS**

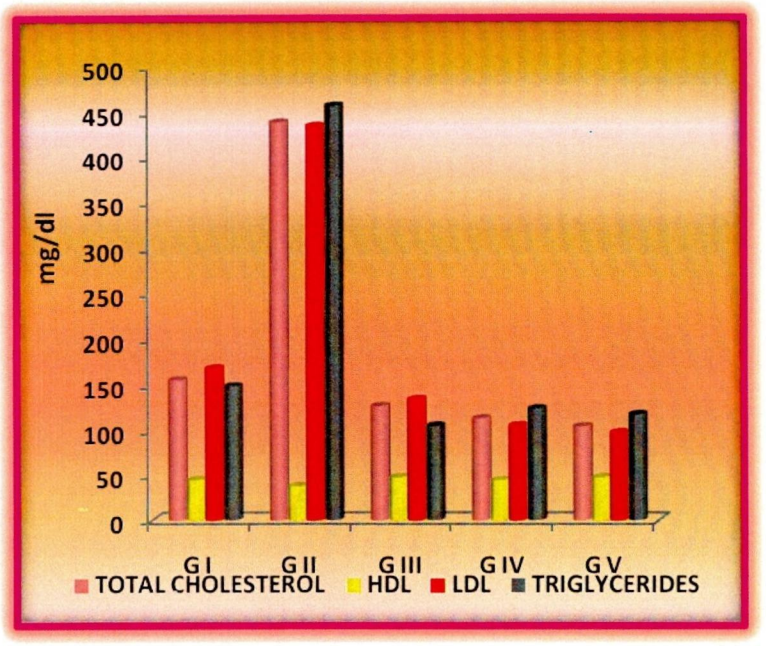
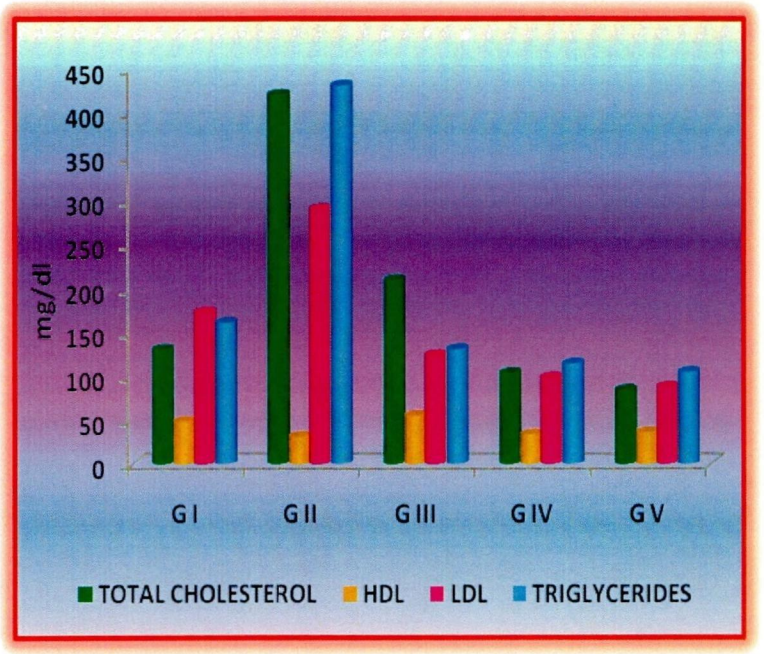



**FIGURE VII**

**LIPID PROFILE OF LIVER SAMPLES IN EXPERIMENTAL RATS**



## SERUM AND LIVER MARKER ENZYMES

The activity of hepatic marker enzymes namely AST, ALT was assessed in serum and liver samples of different groups of rats and depicted in table IX, X and figure VIII, IX.

### TABLE IX

### HEPATIC MARKER ENZYMES IN SERUM SAMPLES OF EXPERIMENTAL RATS

| Group No | Treatment | AST(IU/l) | ALT(IU/l) |
|---|---|---|---|
| I | **Control** | 136.4 ± 1.482 | 113.6 ± 1.589 |
| II | **$CCl_4$** | 385.2 ± 1.423 | 456.1 ± 1.245 |
| III | **$CCl_4$+Silymarin** | 116.2 ± 1.024 | 95.8 ± 1.633 |
| IV | **$CCl_4$+Plant extract (200mg/kg)** | 345.5 ± 1.696 | 115.6 ± 1.504 |
| V | **$CCl_4$+ Plant extract (400mg/kg)** | 242.6 ± 1.230 | 103.5 ± 1.295 |
| | **CD(P<0.05)** | **1.92** | **1.90** |

Values are Mean ± S.D of six samples in each group

### TABLE X

### HEPATIC MARKER ENZYMES IN LIVER SAMPLES EXPERIMENTAL RATS

| Group No | Treatment | AST(IU/l) | ALT(IU/l) |
|---|---|---|---|
| I | **Control** | 150.2 ± 0.968 | 109.6 ± 0.071 |
| II | **$CCl_4$** | 399.2± 0.560 | 456.6 ± 0.568 |
| III | **$CCl_4$+ Silymarin** | 200.3± 1.460 | 230.3 ± 2.790 |
| IV | **$CCl_4$+ Plant extract (200mg/kg)** | 323.5 ± 1.476 | 104.5 ± 1.084 |
| V | **$CCl_4$+ Plant extract (400mg/kg)** | 224.6 ± 1.971 | 97.4 ± 1.188 |
| | **CD(P<0.05)** | **1.73** | **2.12** |

Values are mean± S.D of six samples in each group

IU – Concentration of enzyme that catalyzes the formation of 1µmole of product per minute

In $CCl_4$ group, significant acute hepatocellular damage was indicated by the elevated level of AST and ALT. The rats which received silymarin and ethanolic extract of the plant extract showed a significant decrease in the elevated levels of AST and ALT. The ethanolic extract of the plant at higher concentration (400mg/kg) exhibited greater hepatoprotective effect than the plant extract at lower concentration (200mg/kg). The findings revealed that both the drug silymarin and ethanolic extracts of the plant restored the altered level of the enzymes.

In the study conducted by Lin *et al.*, (2006) with *Alternanthera sessils*(L.)DC, it has been shown that treatment with *Alternanthera sessilis* reduced the elevated levels of AST and ALT in mice detoxicated with $CCl_4$.The results obtained from the study of Verma and Khosa, (2010) indicated that the ethanolic extract of *Zanthoxylum armatum* exhibited hepatoprotective effect against $CCl_4$ induced liver damage by normalizing the elevated levels of the hepatic enzymes.

**FIGURE VIII**

**HEPATIC MARKER ENZYMES IN SERUM SAMPLES OF EXPERIMENTAL RATS**

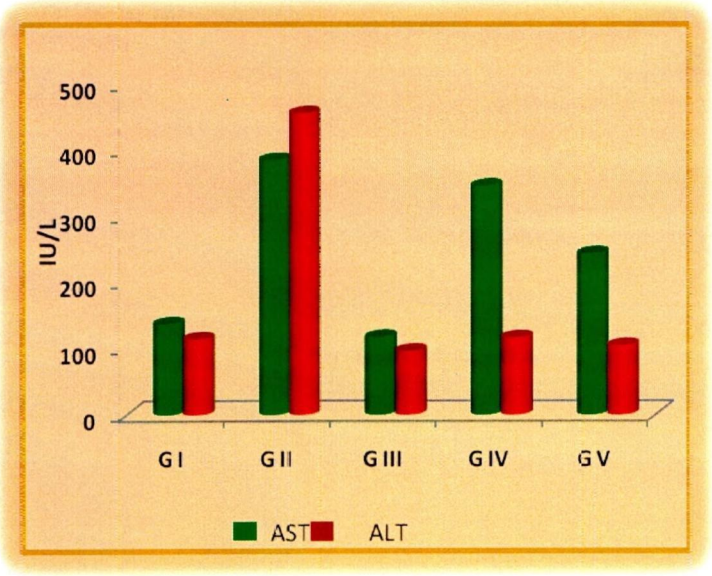
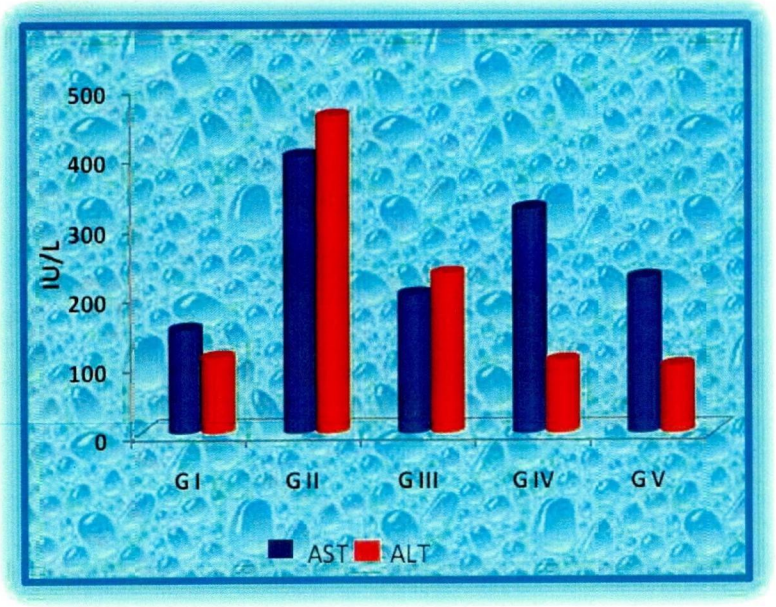



**FIGURE IX**

**HEPATIC MARKER ENZYMES IN LIVER SAMPLES OF EXPERIMENTAL RATS**



## TBARS IN LIVER AND SERUM SAMPLES OF EXPERIMENTAL RATS

Thiobarbituricacid reactive substances in serum and liver samples of different groups of rats are given in table XI and figure X, XI.

### TABLE XI

### LEVEL OF TBARS IN LIVER AND SERUM SAMPLES OF EXPERIMENTAL RATS

| Group No | Treatment | TBARS(mg/dl) | |
|---|---|---|---|
| | | Serum | Liver |
| I | **Control** | 134.3 ± 3.448 | 142.0 ± 3.453 |
| II | **$CCl_4$** | 217.8 ± 1.689 | 226.4± 4.017 |
| III | **$CCl_4$+Silymarin** | 130.2 ± 3.218 | 138.8 ± 1.649 |
| IV | **$CCl_4$+ Plant extract (200mg/kg)** | 190.8 ± 2.838 | 159.8 ± 1.871 |
| V | **$CCl_4$+ Plant extract (400mg/kg)** | 182.3 ± 2.225 | 153.8 ± 2.809 |
| | **CD(P<0.05)** | **1.86** | **1. 24** |

Values are mean± S.D of six samples in each group

The level of TBARS in serum and liver tissues of $CCl_4$ intoxicated rats was significantly elevated when compared to the level in control animals. The administration of silymarin (100mg/kg) and ethanolic extract of *Alternanthera sessilis* at a dose level of (200mg/kg and 400mg/kg) decreased the level of TBARS significantly in serum and liver samples but the decrease was found to be maximum in the group treated with silymarin.

TBARS is the most popular method of estimation of MDA level, which is an indication of lipid peroxidation and free radical activity (Bhaskar and Balakrishnan, 2009). Olaleye *et al.*, (2010) have also reported that oxidative stress is associated with increase in the formation of TBARS in the paracetamol intoxicated group. Swathi *et al.*, (2010) have indicated that supplementation with *Cassia auriculata* leaf extract to rats decreased the elevated level of TBARS content significantly in the serum.

**FIGURE X**

**LEVEL OF TBARS IN SERUM SAMPLES OF EXPERIMENTAL RATS**

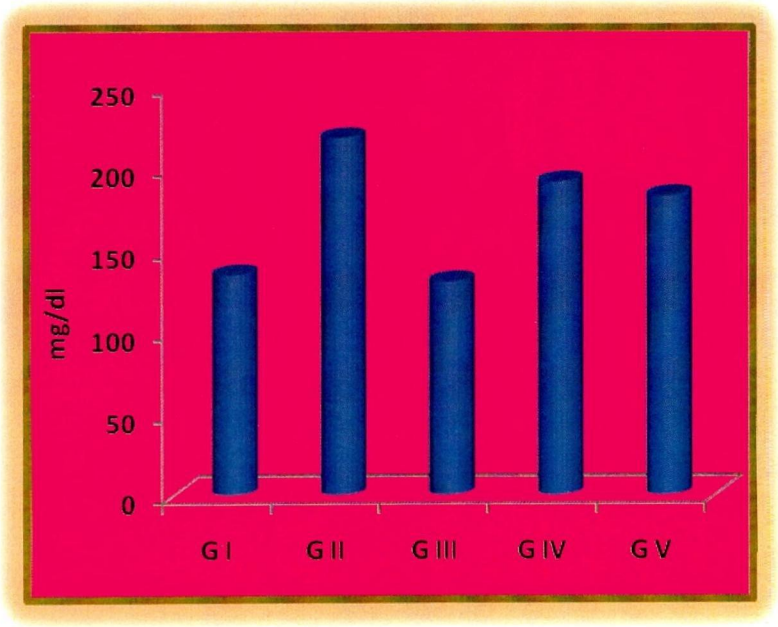
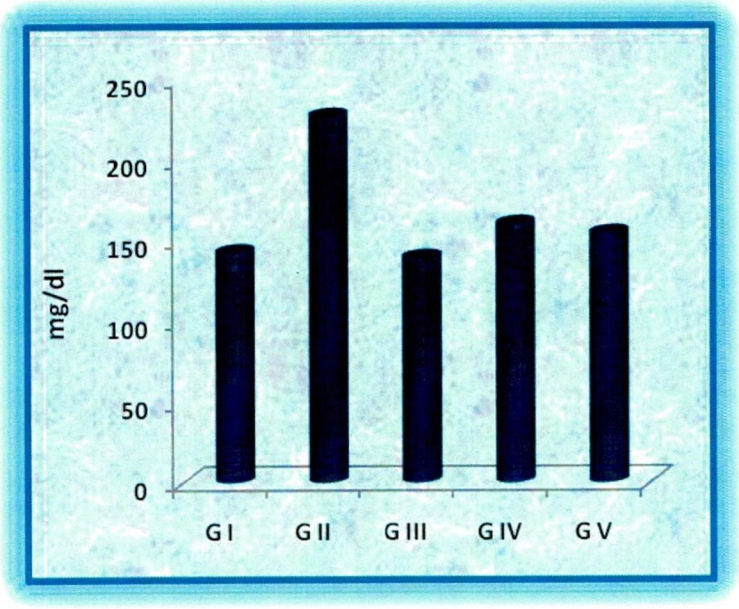



**FIGURE XI**

**LEVEL OF TBARS IN LIVER SAMPLES OF EXPERIMENTAL RATS**



## HISTOPATHOLOGICAL CHANGES OF EXPERIMENTAL RATS

Histopathological examination of sections of liver of control and experimental rats of the various groups was carried out to test hepatoprotective effect of ethanolic extract of *Alternanthera sessilis* leaves. The cellular changes in liver of control and experimental groups of rats are indicated in plate III and the findings of histopathological examination examination are discussed below:

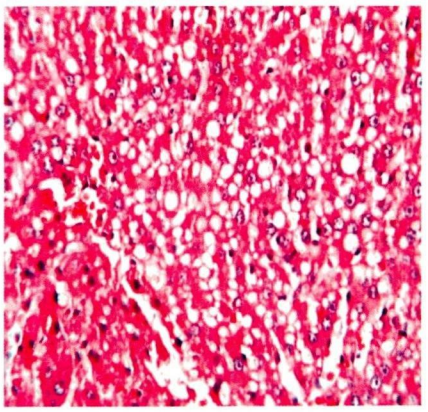
Histology of the liver sections of control animals showed intact hepatocytes arranged in cord like structure around the normally appearing portal triad. Liver sections of $CCl_4$ group animals revealed micro to macro fatty vesicular changes of hepatocytes in which eccentrically displaced nucleus was visible. Necrotic changes of nucleus were noticed in some of the hepatocytes. Hyperplasia of Kupffer cells was also evident along the sinusoidal space. In the case of liver histology of animals treated with silymarin, radiating pattern of cords with normal hepatocytes was observed indicating protective efficacy of the drug. Animals treated with low concentration (200mg/kg) of the plant extract showed hepatocytes with moderate foamy appearance of cytoplasm revealing the partial alleviating effect of the drug. Liver histology was found to be improved in animals treated with high concentration (400mg/kg) of plant extract and it was revealed by normal parenchyma with hepatocytes showing minimal vacuolar degeneration. These findings suggest the hepatoprotective effect of the selected plant *Alternanthera sessilis*.

Karthikeyan, (2011) reported that the sections of liver treated with ethanolic extract of *Spermacoce hispida* revealed better hepatoprotective activity. Dubey and Batra, (2008) have indicated that the ethanolic fraction of *Thuja occidentalis* Linn. prevents fatty degenerative changes. In the histopathological study carried out by Joshi *et al.*, (2010) it is indicated that the animals pretreated with ethanolic extract of *Stachytarpheta indica* L. (Vahl) hepatic damage was minimal with distinct preservation of architectural frame of the hepatic cells.

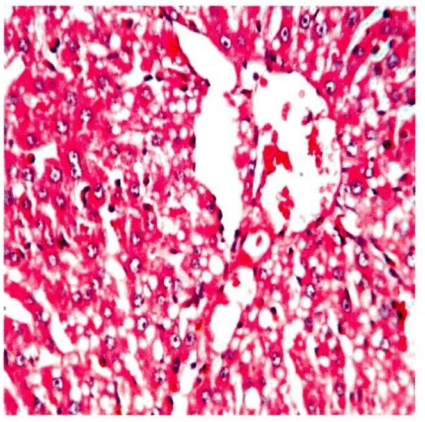
## PLATE III

## HISTOLOGICAL SECTIONS OF LIVER IN DIFFERENT TREATMENT GROUPS OF RATS

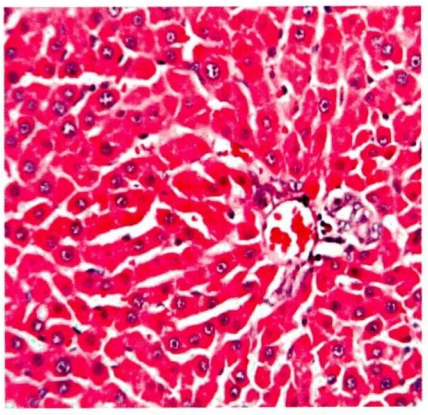
**Control**



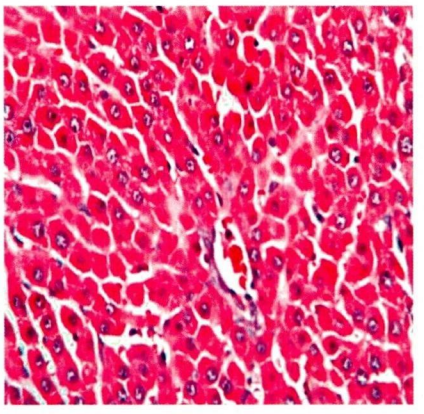
**$CCl_4$**



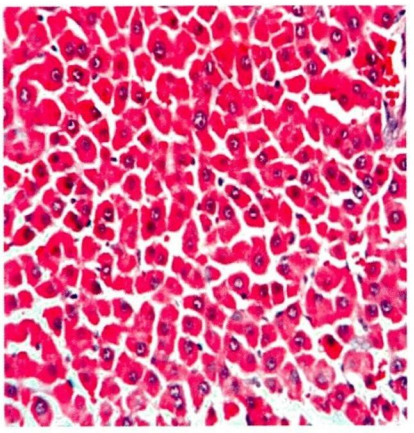
**Silymarin (100 mg/kg)**



**Plant extract (200mg/kg)**



**Plant extract (400mg/kg)**



## PHASE III

### 3.3.3 BRINE SHRIMP LETHALITY BIOASSAY

Brine Shrimp Lethality was carried out using brine shrimp larvae (*Artemia salina)* to test the cytotoxicity of the plant extract. The lethality of the ethanolic extract of *Alternanthera sessilis* to brine shrimp, was determined after 24 hours of exposure and the findings are represented in the table XII

**BRINE SHRIMP**

**TABLE XII**

**BRINESHRIMP LETHALITY BIOASSAY OF *Alternanthera sessilis***

| Concentration (mg/ml) | Lethality (%) | Potassium dichromate |
|---|---|---|
| **10** | 40 | 40 |
| **20** | 45 | 50 |
| **30** | 50 | 60 |
| **40** | 60 | 70 |
| **50** | 75 | 80 |
| **$LC_{50}$** | **228** | **200** |

Values are mean of duplicates

The results of Brine Shrimp Lethality Bioassay are expressed in percentage lethality. Maximum mortality was observed at a concentration of 50mg/ml and minimum mortality at a concentration of 10mg/ml. Thus there was a gradual increase in the percentage lethality with the increase in concentration of the ethanolic extract of *Alternanthera sessilis*. $LC_{50}$ Value for potassium dichromate was found to be 200 and for leaf extract, it was 228. The lowest value is found to be the most potent. The $LC_{50}$ of the leaf extract was greater than the value of potassium dichromate revealing lesser toxicity than potassium dichromate. Thus the finding suggests plant extract can be regarded as a promising candidate for a plant derived anti-tumor compound.

Ramachandran *et al.*, (2011) have shown aqueous and alcoholic extracts of *Agava cantula* leaves exhibited potent Brine Shrimp Lethality ($LC_{50}$ as 15 and 25mg respectively). Peteros and Uy, (2010) they have tested four Philippine medicinal plants for cytotoxicity and shown that the significant lethality of the crude plant extracts to brine shrimp is indicative of the presence of potent cytotoxic compounds.

## 3.3.4 PHASE IV

### ASSESSMENT OF THROMBOLYTIC ACTIVITY

The leaf extract of *Alternanthera sessilis* was screened for thrombolytic activity and the results are given in the following table XIII and figure XII.

**TABLE XIII**

**THROMBOLYTIC ACTIVITY OF *Alternanthera sessilis***

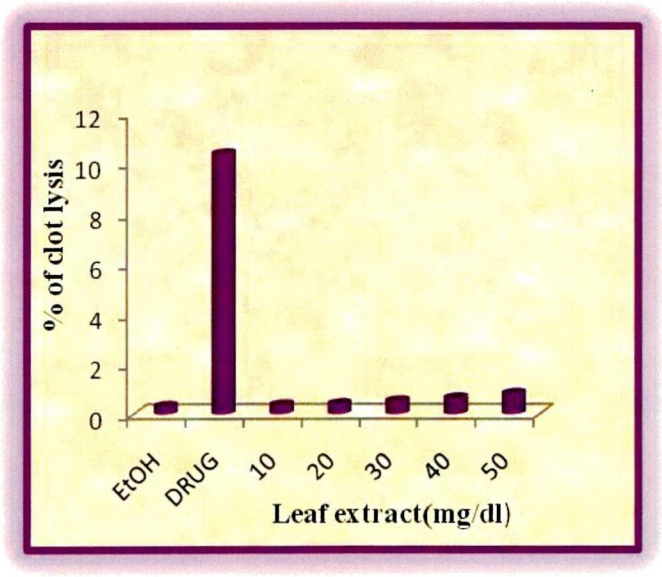
| Samples | % Clot lysis |
|---|---|
| **Ethanol** | 0.33±0.05 |
| **Streptokinase(IU/L)** | 10.40±1.10 |
| **Leaf extract(mg/ml)** | |
| **10** | 0.38±0.13 |
| **20** | 0.42±0.13 |
| **30** | 0.53±0.05 |
| **40** | 0.65±0.08 |
| **50** | 0.83±0.07 |
| **CD(P>0.05)** | **0.29** |

Values are expressed by mean ± SD

In the present study five different concentrations of leaf extract *Alternanthera sessilis* were tried for evaluation of possesses thrombolytic activity. Streptokinase the standard drug used for thrombolysis served as positive control and ethanol was used as negative control. Comparison of clot lysis by positive control and negative control clearly demonstrates that there was no lysis when the clot was treated with ethanol. Moderate lysis was noticed with the plant extract. A gradual increase in the thrombolytic activity was observed with increase in concentration of the plant extract.

**FIGURE XIII**

**THROMBOLYTIC ACTIVITY OF *Alternanthera sessilis***




Prakash and Manavalan, (2011) reported that multiple solvent extracts of *Andrographis paniculata* have shown significant clot lysis which is similar to the effect exhibited by the drug streptokinase. Chowdhury *et al.*, (2010) have indicated that the crude methanolic extract of *Aponogeton undulates* exhibited significant thrombolytic activity at a dose of 10mg/ml. Prasad *et al.*, (2007) have observed significant thrombolytic activity by *Fagonia arabica.*