4. RESULTS

The result of various parameters such as phytochemical constituents, haematological studies, enzymatic antioxidant activities, non-enzymatic antioxidant activities, hepatoprotective enzymes and histopathological studies were given in detail (Table 1-8).

4.1. PHYTOCHEMICAL CONSTITUENTS

4.1.1. Qualitative analysis

The results of the phytochemical analysis of leaves of both *A. marmelos* and *E. prostrata* are given in Table 1. The qualitative phytochemical analysis of aqueous extracts revealed the presence of carbohydrate, alkaloids, proteins, phytosterols, tannins and phenols in both the plants, absence of flavonoids in *E. prostrata* and saponin in *A. marmelos*.

4.1.2. Trace elements

The concentration of important trace elements were determined in both the plants by Atomic Absorption Spectroscopy (AAS). The analysis showed the presence of trace elements such as sodium, potassium, calcium, zinc, copper, manganese and iron. Trace element concentration of *E. prostrata* was higher than that of *A. marmelos* except in zinc and manganese (Table 2 and Fig.11).
4.1.3. Gas Chromatography-Mass Spectroscopy analysis

In GC-MS analysis, totally 33 compounds were identified from the methanolic fraction of *A. marmelos* (Table 3; Figure 3). The plant samples revealed the synthesis of 1,2,5,63-Piperidinol, 1-methyl-, Carbamic acid, phenyl ester, 2(1H)-Pyridinone, 6-hydroxy-, Proline, N-methyl-, butyl ester, 2-Octanone, 5-Hepten-2-one, 2H-Pyran, 2-ethoxy-3,4-dihydro-, 1-Butanol, 2-methyl-, acetate, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 2-Methyl-pyrrolidine-2-carboxylic acid, Benzofuran, 2,3-dihydro-Coumaran, 2-Methoxy-4-vinylphenol, 2-Methyl-6-methylene-octa-1,7-dien-3-ol, cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, 2-Propenoic acid, 3-phenyl-, methyl ester, Cinnamic acid, methyl ester, 2,4(1H,3H)-Pyrimidinedione, 1,3-dimethyl-, 2-Butenoic acid, 4,4-dimethoxy-, methyl ester, 2-Propenoic acid, 3-phenyl-, Cinnamic acid, methyl ester, Ethanone, 1-[4-(1-methylethyl)phenyl]-, 2-Methoxy-4-vinylphenol, 2-Methyl-6-methylene-octa-1,7-dien-3-ol, cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, 2-Propenoic acid, 3-phenyl-, methyl ester, Cinnamic acid, methyl ester, 2,4(1H,3H)-Pyrimidinedione, 1,3-dimethyl-, 2-Butenoic acid, 4,4-dimethoxy-, methyl ester, 2-Propenoic acid, 3-phenyl-, Cinnamic acid, Ethanone, 1-[4-(1-methylethyl)phenyl]-, â-Selinene, D-Allose, Octanoic acid, 2-butyl-, Cyclopropane, 1-(2-methylene-3-but enyl)-1-(1-methylenepropyl)-, 1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl-, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, 1,4-Naphthalenedione, 2,3,6-trimethyl-, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Tetradecanoic acid, 12-methyl-, methyl ester, n-Hexadecanoic acid, 9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-, Phytol, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-, Linolenic acid and 2-Methyl-6-methylene-octa-1,7-dien-3-ol. The compounds detected are of pharmacological importance as they possess the properties such as antioxidant, anti-diabetic and anti-microbial activities.
On the other hand by the GC-MS analysis, totally 16 compounds were identified from the methanolic fractions of the *E. prostrata* (Table 4; Fig. 4). The plant samples revealed the synthesis of 2-Butenoic acid, 2- methyl-(E), Propanamide, 2-amino-3-phenyl,1,6;3,4-Dianhydro-2-O-acetyl-a-d allopyranose, n-Decanoic acid, 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl), 1,6-Anhydro-a-D-glucopyranose(levoglucosan), Dodecanoic acid, 1-Hexadecanol,3,7,11,15-tetramethyl, Tetradecanoic acid, 3,7,11,15-Tetramethyl-2-hexadecan-1-ol, Cyclohexane, 1-methyl-4-(1-methylethenyl)-,cis-, E-Z-2-,15-Octadecadien-1-ol acetate, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, Phytol and 1,E-11,Z-13-Octadecatriene. All these compounds are of pharmacological importance as they possess the properties such as anti-diabetic, antibacterial, and antifungal activities.

4.2. HAEMATOLOGICAL PARAMETERS

The levels of RBC, WBC and haemoglobin (Hb) in the control and experimental groups of rats are given in table 5 and Fig.12-19. All haematological parameters including RBC, WBC, haemoglobin (Hb), Packed Cell Volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and platelet count (PLT) showed an increase in their levels of blood samples treated with extracts of both the medicinal plants over the alcohol intoxicated samples.

Among the medicinal plants the extracts of *A. marmelos* recorded significantly increased the levels of all the haematological parameters
such as RBC (5.29 ± 0.12), WBC (7.29 ± 0.01), haemoglobin (11.85 ± 0.41), packed cell volume (36.12 ± 1.34), mean corpuscular volume (48.90 ± 1.60), mean corpuscular haemoglobin (16.37 ± 2.15), mean corpuscular haemoglobin concentration (31.49 ± 2.32) and platelet count (584.0 ± 99.00) when compared to the extracts of *E. prostrata*, RBC (5.09 ± 0.15), WBC (6.00 ± 1.43), haemoglobin (10.21 ± 0.32), packed cell volume (34.16 ± 1.94), mean corpuscular volume (46.92 ± 2.57), mean corpuscular haemoglobin (14.26 ± 1.41), mean corpuscular haemoglobin concentration (31.24 ± 1.60) and platelet count (564.0 ± 100).

### 4.3. ANTIOXIDANT ACTIVITIES

#### 4.3.1. Enzymatic antioxidant activities

The levels of antioxidant enzymes such as Superoxide dismutase (SOD), Lipid peroxides (LPO), Catalase (CAT), Glutathione (GSH), Glutathione peroxidase (GPX) and Glutathione-S-Transferase in the normal and experimental animals were studied and given in Table 6 and Fig.20-25.

The antioxidant enzyme superoxide dismutase level showed a significant decrease (3.58 ± 0.26) in alcohol intoxicated rats while the rats treated with extracts of *A. marmelos* and *E. prostrata* exhibited a significant increase in the level of SOD over the alcohol intoxicated rats. Among the plant extracts, the extract of *A. marmelos* induced the SOD level significantly (7.3 ± 0.201) when compared to the extracts of *E. prostrata* (5.21 ± 0.33).
Ethanol intoxicated rats showed a high level (9.29 ± 0.00) of lipid peroxidase over control (4.31 ± 0.07). The extracts of medicinal plants, such as *A. marmelos* and *E. prostrata*, significantly reduced the level of LPO in alcohol intoxicated animals and the levels were 7.6 ± 0.201 and 7.04 ± 0.132 respectively. Among the treatments, the animals received the extract of *E. prostrata* showed a significant reduction in LPO level as compared to animals received the extract of *A. marmelos*.

Inhibition of catalase activity was observed in alcohol intoxicated rats when compared to control. The administration of drugs of *A. marmelos* and *E. prostrata*, in alcohol induced rats, elevated the catalase activity considerably in the liver tissues and the increase in activity was more in animals treated with *A. marmelos* (59.44 ± 1.81) than with *E. prostrata* (37.93 ± 1.78).

The enzymatic antioxidants such as Reduced Glutathione, Glutathione peroxidase and Glutathione-S-Transferase levels were declined in the ethanol induced rats when compared to normal rats. The treatment of alcohol intoxicated animals with drugs of *A. marmelos* and *E. prostrata* revealed an enhancement in their levels over alcohol induced ones. The levels of GSH, GPₓ and GST on 42nd day were 14.93 ± 0.05, 10.87 ± 0.67 and 72.08 ± 0.10 respectively with *A. marmelos* and *E. prostrata* were 12.42 ± 0.04, 8.26 ± 0.20 and 44.94 ± 0.08 respectively.
4.3.2 Non-enzymatic antioxidant activities

Vitamin E and C

The non-enzymatic antioxidants such as vitamins E and C levels were estimated in control, alcohol intoxicated and extract administrated in addition to standard drug treated animals. Their levels were significantly decreased in alcohol intoxicated animals as compared to control. On the other hand, plant extracts administrated animals showed higher levels of these non-enzymatic antioxidants over alcohol intoxicated animals. Interestingly, the extract of *A. marmelos* increased the level of both vitamins in alcohol intoxicated animals over the standard drug, silymarin (Table 7 and Fig.26-27).

4.4. HEPATOPROTECTIVE ENZYMES

Hepatoprotective effects in terms of activities of hepatic enzymes such as Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamic pyruvic transaminase (SGPT), Alkaline phosphatase (ALP) and Gamma glutamate transpeptidase (GGT) and levels of total bilirubin and total protein were investigated and showed an increase in alcohol induced rats when compared to control. The extracts of both the test plant exhibited significant (p < 0.05) hepatoprotective activity against the alcohol induced liver models by improving liver function which was indicated by reduction in the levels of SGOT, SGPT, ALP, GGT, total bilirubin and total protein (Table 8 and Fig.28-33).

The levels of SGOT, SGPT, ALP, GGT, total bilirubin and protein were $102.16 \pm 3.66, 72.32 \pm 0.58, 38.78 \pm 0.43, 16.61 \pm 0.23, 0.71 \pm 0.01$
and 5.28 ± 0.26 respectively with *A. marmelos* administrated rat models and with *E. prostrata* treated rats the levels were 112.3 ± 2.11, 85.33 ± 0.01, 67.03 ± 1.30, 12.61 ± 0.15, 1.08 ± 0.00 and 4.09 ± 0.82 respectively. Here also the treatment of *A. marmelos* increased the hepatoprotective activity as against *E. prostrata* treatment (Table 8).

### 4.5. HISTOPATHOLOGICAL STUDY

In histological studies, liver section of normal (control) rats showed normal hepatocytes with well preserved cytoplasm. There was no sign of inflammation, fatty change or necrosis in these animals (Fig.6). Severe inflammations and cell swelling were observed in endothelial liver cells of alcohol treated rats and they also showed vacuoles in the cytoplasm as well as ballooning and degeneration of hepatocytes (Fig.7).

The liver section of *A. marmelos* (100 mg/kg.b.wt) treated rats showed higher recovery of inflammatory cells around portal tract. There were few portal traid with periportal lymphocytic infiltration, central vein and rest of the hepatic parenchyma appeared unremarkable. No centrizonal necrosis was identified (Fig.8).

The liver section of *E. prostrata* (100 mg/kg .b.wt) treated rats showed the recovery of inflammation (Fig. 9). They showed greater reduction in periportal and centrizonal inflammation without any centrizonal necrosis. Focal areas also showed degenerative changes of periportal hepatocytes which was lower than in *A. marmelos* treated rats. Silymarin treated animal groups showed a normal liver lobule with no
sign of necrosis in the centrizonal area and portal triad, only focal periportal inflammation was observed (Fig.10). Among the two plants, *A. marmelos* was more effective in hepatoprotective activity.

Hence, from the results of phytochemical analysis, haematological parameters, antioxidant activities, hepatoprotective activities and histopathological studies, it is clear that among the two test plants, *A. marmelos* had more potentiality than *E. prostrata* in alcohol induced albino rats when compared to standard drug silymarin.