Albino Wistar rats. The levels of lipid peroxidative markers thiobarbutric acid reactive substances (TBARS) and lipid hydroperoxides (HP), the levels of carbohydrate, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates). Materials Used

Absolute ethanol [AR] was obtained from Hayman Private Limited, England. Sunflower oil marketed by Gold Winner was purchased from local market, Puducherry, India. Sunflower oil was subjected to heating at 180°C for 30 minutes, twice. The oil was analyzed by gas chromatography and found to contain altered fatty acid composition. All chemicals and solvents used were of the highest purity and analytical grade.

Preparation of Plant Extract

The plants were collected from the fields near Puducherry. Leaves were separated and dried at room temperature, powdered, sieved and stored prior to further use. The powder (100 g) was homogenized with water (800 ml) and the homogenate was kept in a shaker at 40°C for 24 h and then filtered using Whatman No. 1 paper. The filtrate was evaporated to dryness under reduced pressure. The yield of the aqueous extract was 56 g of leaf powder. The extract was reconstituted in a known volume of distilled water and used for different experiments.

Experimental Design

The animals were divided into 4 groups of 6 rats each. Group 1 rats served as the control. Group 2 rats were given 20% ethanol (7.9g/Kg body weight)6 orally, using an intragastric tube and 15% heated sunflower oil mixed with the diet. Group 3 rats were given 20% ethanol orally, using an intragastric tube, 15% heated sunflower oil mixed with the diet and aqueous extract (leaf) of S. grandiflora (100 mg/kg body weight) using an intragastric tube. Group 4 rats were given aqueous extract (leaf) of S. grandiflora (100 mg/kg body weight) orally, using an intragastric tube.

At the end of the experimental period (45 days), the rats were sacrificed after an overnight fast by cervical dislocation. Liver tissues were removed, cleared off blood and immediately transferred to ice-cold containers containing 0.9% NaCl for various estimations. A known amount of tissue was weighed and homogenized in appropriate buffer (10%) for the estimation of various biochemical parameters.

Preparation of Plasma

Blood was collected in heparinised tubes and plasma was separated by centrifugation at 1000g for 15 minutes for the estimation of GGT and ALP.
Biochemical Investigations

The activity of plasma Gamma-glutamyl transferase (GGT) was assayed by the method of Fiala et al. The activity of alkaline phosphatase (ALP) was assayed by the method of King and Armstrong using a reagent kit. The concentrations of Thiobarbituric acid reactive substances (TBARS) was estimated by the method of Niehaus and Samuelsson and Lipid hydroperoxides (LH) by the method of Jiang et al. Ascorbic acid was estimated by the method of Fiala and vitamin E by the method of Baker and Sinha. Reduced glutathione (GSH) content in the tissue was determined by the method of Ellman. The activity of glutathione peroxidase (GPx) was assayed by the method of Rotruck et al, superoxide dismutase (SOD) by the method of Kakkar, and catalase (CAT) by the method of Sinha.

Statistical Analysis

Statistical analysis was done by analysis of variance (ANOVA) followed by Tukey's test. The values were considered statistically significant when p < 0.05.

RESULTS

Figure 1 and 2 show the changes in the activities of GGT and ALP. The activities of these liver marker enzymes were increased significantly in plasma of alcohol + DPUFA group when compared to normal. Co-administration of S.grandiflora decreased their activity significantly at P < 0.05. ANOVA followed by Tukey's test. Values sharing a common superscript do not differ significantly at P ≥ 0.05.

Figure 3 and 4 show the changes in the levels of TBARS and lipid hydroperoxides in liver. The levels of TBARS and HP were increased significantly in alcohol + ΔPUFA group when compared to normal. Treatment with S.grandiflora significantly decreased their levels. S.grandiflora control group did not show any significant change in the levels when compared to normal.

The changes in the levels of vitamin C, vitamin E and GSH in liver are given in Figure 5, 6 and 7 respectively. There was a significant decrease in the levels of vitamin C, vitamin E and GSH in alcohol + ΔPUFA group when compared to normal. The levels were significantly increased in S.grandiflora treated groups. S.grandiflora control group showed no significant change in their levels when compared to normal.

The activities SOD (Figure 8), CAT (Figure 9), GPx (Figure 10) showed a significant decrease in liver of alcohol + ΔPUFA group. Treatment with S.grandiflora significantly increased their activities. Administration of
Figure 6: Levels of Vitamin E in liver

(Values are mean ± S.D from 6 rats in each group)
ANOVA followed by Tukey's test. Values sharing a common superscript do not differ significantly at $P \leq 0.05$

Figure 7: Levels of Reduced Glutathione in liver

(Values are mean ± S.D from 6 rats in each group)
ANOVA followed by Tukey's test. Values sharing a common superscript do not differ significantly at $P \leq 0.05$

S. grandiflora alone did not alter the activities of these enzymic antioxidants.

In histopathological observations (Figure 11), the liver of alcohol+ heated PUFA treated rats showed fibrosis (Fig B), and *Sesbania grandiflora* treated rats showed only sinusoidal dilatation (Fig C). The histology of liver was not affected in both normal (Fig A) and *Sesbania grandiflora* control (Fig D) rats.

**DISCUSSION**
Alcoholic liver disease is progressive and major cause of morbidity and mortality. Ethanol stimulates free radical formation that causes lipid peroxidation.

Figure 11: Histopathology of liver

**Figure 8: Activities of Superoxide Dismutase in liver**

(Values are mean ± S.D from 6 rats in each group)
ANOVA followed by Tukey's test. Values sharing a common superscript do not differ significantly at $P \leq 0.05$

Units - enzyme reaction which gives 50% inhibition of NBT reduction / minute

**Figure 9: Activities of Catalase in liver**

(Values are mean ± S.D from 6 rats in each group)
ANOVA followed by Tukey's test. Values sharing a common superscript do not differ significantly at $P \leq 0.05$

Units - $\mu$M of $H_2O_2$ liberated/ minute.

**Figure 10: Activities of Glutathione Peroxidase in liver**

(Values are mean ± S.D from 6 rats in each group)
ANOVA followed by Tukey's test. Values sharing a common superscript do not differ significantly at $P \leq 0.05$

Units - mmoles of glutathione liberated / minute.
The resultant comparatively long lived carbon radical species may undergo reaction with molecular oxygen to form hydroperoxy fatty acids which can decompose to form aldehydic products such as malondialdehyde (MDA), hydroxyalkenals, 4-hydroxy nonenal (4HNE) and 4-hydroxyhexanal (4HHE) and other aldehydes. These products can react with TBA and hence the levels of TBARS and the secondary products of LPO, hydroperoxides are increased during ethanol and PUFA ingestion[21].

Chronic alcohol consumption can increase oxidative stress through several mechanisms. In normal metabolism there is a balance between the generation of free radicals and antioxidant defense mechanism. This balance is disturbed by the chronic consumption of alcohol. Studies have shown that ethanol consumption results in increased formation of lipid peroxides and free radicals leading to depletion of antioxidants present in the biosystems[22].

PUFA can also cause deterioration of the antioxidant status due to their liability to become oxidized. A high intake of PUFA has been demonstrated to increase the formation of lipid radicals and to deplete endogenous antioxidants[24]. In our study, the levels of enzymatic and non - enzymatic antioxidants were decreased in alcohol and PUFA group. The inactivation and depletion is due to the excess amount of reactive oxygen species produced. Sesbania garandiflora is considered a wonder plant with a variety of potential. It is known to contain phenolic and anthocyanins[5]. These compounds have hydrogen donating ability, forming arylxoyl radicals. It has been proposed that hydroxyl and hydroperoxy radicals initiate H abstraction from a free phenolic substrate to form phenoxy radical that can rearrange to quinonemethide radical intermediate, which is excreted via bile. These molecules are considered to scavenge free radicals and inhibit lipid peroxidation.

In our study, the administration of crude extract of Sesbania grandiflora has reduced the lipid peroxidation and restored the antioxidant status. This may be because of its effective antioxidant property. Our histopathological observations were also in correlation with our biochemical parameters. Studies have shown that the leaf juice of Sesbania grandiflora scavenges nitric oxide and 2-diphenyl 1 - picryl hydrazyl free radicals[25].

The crude extract of Sesbania grandiflora effectively protected the liver and prevented the release of liver markers and decreased the extent of lipid peroxidation and reactivated the antioxidant machinery. Hence this wonder plant Sesbania grandiflora can be considered as a hepatoprotector against alcohol and PUFA induced hepatotoxicity.

REFERENCES
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