



Antioxidant and hepatoprotective effects of *Clitoria ternatea* leaf extracts by using *in vivo* model

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Abstract: Herbal medicines are in great demand because of their great efficacy and little or no side effects. Many plant extracts show promising antioxidant and free radical scavenging activity. The plant used in the present study is *Clitoria ternatea* (Sanskrit-Sankupushpam) belongs to the family *Fabaceae*. The *in vivo* studies were conducted using male Wistar rats. Liver cells participate in a variety of metabolic activities and contain a host of enzymes. CCl₄ is a known hepatotoxin whose action is mediated by the cytochrome P450 (CYP), the extent of liver damage caused by ethanol-CCl₄ treatment was studied by estimating the serum marker enzymes and the circulating lipid profile. The effect of leaf extracts was significantly higher in all the antioxidants suggests *Clitoria ternatea* a strong protection against oxidative damage in CCl₄ induced hepatotoxicity in rats. The observations made *in vivo* were also confirmed by histopathological studies.

Keywords: Antioxidants; *Clitoria ternatea*; free radical; *in vivo*.

Introduction

Antioxidants act as a defense mechanism that protects against oxidative damage, and include compounds and repair enzymes to remove or repair damaged molecules. However, the natural antioxidant compounds become important (Malpure *et al.*, 2006). of antioxidants is supposed to protect against diseases. Nitric oxide radical is a labile compound, which has a brief half life and is rapidly converted to the stable end products nitrite (NO₂⁻) and nitrate (NO₃⁻) (Celiktar *et al.*, 2007).

One of the plants that deserves attention is *Clitoria ternatea* (Sanskrit-Sankupushpam) belongs to the family *Fabaceae*, is widely used in traditional Indian system of medicine as a brain tonic. *Clitoria ternatea* is a perennial twinning herb bearing blue or white flowers (Gomez and Kalamani, 2003). In the present study is the antioxidant activity was analysed by

using male Wistar rats as an animal model and thus the *Clitoria ternatea* leaf extracts were analysed by using male Wistar rats as an animal model.

Materials and methods

Plant material

Clitoria ternatea habit is a twining shrub with alternate imparipinnate leaves. About 2-3 pairs of leaflets which are in opposite, ovate and obtuse at base and apex. Flowers are white or blue which are solitary or in pairs. The classification of *Clitoria ternatea* is given below

Family	Fabaceae
Subfamily	Faboideae
Genus	<i>Clitoria</i>
Species	<i>ternatea</i>
Botanical name	<i>Clitoria ternatea</i>
Common name	Sankupushpam

Experimental study

Male Wistar rats were treated with and without *Clitoria ternatea* leaf extracts (Both blue and white flowered leaf varieties) along with the administration of alcohol-CCl₄ oxidant and the standard antioxidant silymarin was compared with the antioxidant effect of the leaf extracts.

Adult male Wistar rats weighing 100-120g were obtained from Tetrex Animal Breeding Centre, Madurai. The animals were housed in spacious cages kept in a well-ventilated, hygienic atmosphere. The animals were maintained on a commercial rat feed manufactured by Hindustan Lever Ltd., Mumbai, India. Water and food were provided *ad libitum* to the animals. They were given a weeks' time to get acclimatized to the laboratory conditions. Before the experiment, the clearance of the ethical committee for experimentation on animals was obtained (623/02/b/CPCSEA).

Treatment Groups

The animals were randomly divided into nine groups of six animals each as follows:

Group I	Untreated control
Group II	Alcohol treated (0.1ml of 50% alcohol / 100g body weight)
Group III	Alcohol + CCl ₄
Group IV	Alcohol + CCl ₄ + white flowered leaf extract
Group V	Alcohol + CCl ₄ + blue flowered leaf extract
Group VI	White flowered leaf extract
Group VII	Blue flowered leaf extract
Group VIII	Alcohol + CCl ₄ + Silymarin
Group IX	Silymarin alone

The experimental period was for twenty-one days. CCl₄ administration was carried out as a single, subcutaneous injection at a dose of 2ml/kg body weight, diluted equally in paraffin oil. Alcohol was administered at a dose of 0.1ml of 50% alcohol/100g body weight throughout the experimental period for inducing the activity of cytochrome P450 2E1 (CYP2E1). In this study, alcohol was pre-administered to the animals prior to the CCl₄ assault, in order to

maximize the oxidative effects of CCl₄. CCl₄ was administered subcutaneously to rats, once on day 21 alone. The plant extract as administered by intragastric intubation at a dose of 500mg/kg body weight, once per day for 21 days. A suspension of the standard antioxidant silymarin in paraffin oil was administered at a dose of 25mg/kg body weight for 21 days, once a day by intragastric intubation. This was done to compare the antioxidant activity of the plant extract with the standard antioxidant silymarin. At the end of the experimental period (22nd day), the rats were sacrificed after an overnight fast. The liver tissue slices were blotted dry and immediately transferred to cryovials containing 0.1M Tris-HCl buffer (pH 7.4) and stored in the deep freezer (-80°C) till analysis. A small portion of the liver was cut and fixed in 10% formalin for histopathology study.

Estimation of Free fatty acids

Non-esterified free fatty acids were estimated (Falhot *et al.*, 1973). An accurate amount (0.01ml) of serum, 1.0ml of phosphate buffer, 6.0ml of extracting solvent and 2.5ml of copper reagent, 3.0 ml of the upper layer was transferred, 0.5ml of diphenyl carbazide and mixed. Standard palmitic acid was treated with copper reagent and diphenyl carbazide.

Assay of serum marker enzymes

The marker enzymes for hepatic damage namely aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and γ -glutamyl transpeptidase (γ -GT) were assayed in the serum. All the enzymes were assayed using kits available commercially (Dia Sys India Pvt Ltd., Chennai).

Assay of AST and ALT (Bergmeyer et al., 1978)

Serum 0.1ml, 1.0ml of the working reagent (from reagent kit) was added, mixed and incubated at 37°C for 60 minutes for AST and 30 minutes for ALT and the decrease in absorbance was read at 430nm. The enzyme activities were expressed as IU/L.

Assay of ALP (Schlebusch et al., 1974)

The working reagent was prepared from PNPP substrate with 5.0ml of buffer. To 20 μ l of serum, 1.0ml of working reagent, after one minute, the increase in absorbance was measured at 415nm. The ALP activity was expressed as IU/L.

Determination of serum lipids

The levels of cholesterol, triglycerides, free fatty acids and phospholipids were estimated in serum and analyzed using commercially available kits (Ark Diagnostic Pvt. Ltd, Mumbai).

Estimation of cholesterol (Allain et al., 1974)

Take 0.01ml of serum and standard and 1.0ml of the working reagent (using kit solution) was added, mixed and kept at 37°C for 5 minutes., read at 510nm. The serum cholesterol was expressed as mg/dL.

Estimation of triglycerides (Bucolo and David, 1973)

About 0.1ml of the triglyceride mono reagent, 0.01ml of standard and serum were added and incubated at 37°C for 10 min and measured at 550nm .The serum free fatty acids were expressed as mg/dL.

Estimation of phospholipids (Zilversmit et al., 1950)

An exact amount (0.01ml) of serum ,1ml of 5N sulphuric acid , 1.0ml of water heated in a boiling water bath for 5 minutes. 1.0ml of ammonium molybdate 0.1ml of ANSA added and measured at 680nm. The serum phospholipids were expressed as mg/dL.

*Estimation of enzymic antioxidants**Super oxide dismutase (SOD) (Kakkar et al., 1984)*

For Superoxide dismutase active take *Clitoria ternatea* (0.5g) of leaves ,1.2ml of sodium pyrophosphate buffer, 0.1ml of PMS,

0.3ml of NBT, 0.2ml of enzyme , 0.2ml of NADH , incubation at 30°C for 90 seconds, stopped by the addition of 1.0ml of glacial acetic acid measured at 560 nm.

Catalase

Catalase activity in the leaves of white and blue variety of *Clitoria ternatea* (Luck et al., 1974) leaves were homogenized in 2.5ml of phosphate buffer, 3.0ml of hydrogen peroxide in phosphate buffer was adjusted at 240nm in a spectrophotometer. The time interval required for a decrease in absorbance by 0.05 units were recorded at 240nm.

Glutathione S transferase (GST)

Glutathione S-transferase activity was assayed (Habig et al., 1974) *Clitoria ternatea* leaves were homogenized in 5.0ml of phosphate buffer. The enzyme activity was determined by 340 nm in a spectrophotometer. , 0.1ml of both the substrates (GSH and CDNB), 0.1ml of the enzyme extract and the values have been expressed as nmoles of CDNB conjugated per minute per gram of leaves.

Glutathione reductase (GR) (David et al., 1983)

Clitoria ternatea leaves (0.5g) were homogenized, 1.0ml of potassium phosphate buffer, 0.1ml of EDTA, 0.1ml of sodium azide, 0.1ml of oxidized glutathione, 0.1ml of the enzyme source and 0.1ml of NADPH was added. The absorbance at 340 nm . The enzyme activity was expressed as μ moles of NADPH oxidized/minute/gram of leaves.

Glutathione peroxidase (Rotruck et al., 1973)

The reaction mixture 0.4ml of sodium phosphate buffer, 0.1 ml of sodium azide, 0.2ml of reduced glutathione, 0.2ml of tissue homogenate and 0.1ml of H₂O₂ , 0.3ml of disodium hydrogen phosphate and 1.0ml of DTNB solution were added was read at 412nm in a spectrophotometer. One unit of GPx activity was expressed as μ g of glutathione consumed/min.

Total protein (Lowry et al., 1951)

The protein content of the enzyme extracts were taken about 0.2ml to 1.0ml working standard and 0.1ml of the enzymes sources, 5.0ml of alkaline copper sulphate reagent stand for 30 minutes. Then 0.5ml of Folin-Ciocalteu reagent was added incubated at 37°C for 3 minutes. The blue colour developed was read at 660nm. The activities of the enzymes were expressed as units/mg protein.

Ascorbic acid

Ascorbic acid, a scavenger of oxyradicals was estimated (Roe and Keuther, 1943). An accurate amount (1g) of leaves were homogenized in 4% TCA and made upto 10ml. Centrifuged and remove the charcoal residue and the supernatant obtained was used for the estimation. For estimation, Aliquots of 0.5 - 1.0ml of this supernatant and 0.2 to 1.0ml of standard ascorbate were made up to 2.0ml with 4% TCA. 0.5ml of DNPH reagent was added to all the tubes, followed by 2 drops of 10% thiourea solution. The contents were mixed and incubated at 37°C for 3 hours. The osazones formed were dissolved in 2.5ml of 85% sulphuric acid, in cold. After incubation for 30 minutes at room temperature, the absorbance was read spectrophotometrically at 540nm.

Tocopherol

The method (Rosenberg, 1992) was followed for the estimation of tocopherol. An exact amount (2.5g) of the homogenized plant tissue (with 5ml 0.1N sulphuric acid). Aliquots of the filtrate were used for the estimation into 3 stoppered centrifuge tubes, 1.5ml of plant tissue extract, 1.5ml of the standard and 1.5ml of water were pipetted out respectively. To all the tubes, 1.5ml of ethanol and 1.5ml of xylene were added, mixed well and centrifuged. 0.1ml of the xylene layer was transferred into another stoppered tube and 0.1ml of 2, 2'-dipyridyl reagent was added to each tube and mixed. Pipetted out 1.5ml of the mixture into a spectrophotometer cuvette and the extinction was read at 460nm. 0.33ml of ferric chloride solution was added and mixed well, and after exactly 15 minutes, the

absorbance of the red colour produced was read against a blank at 520nm.

Vitamin A

Vitamin A was estimated (Bayfield and Cole, 1980). To 1.0ml homogenate, 1ml of saponification mixture was added. The tubes were gently refluxed for 20 minutes at 60°C. The tubes were cooled at room temperature, 20ml of water was added and mixed well. The volume of the extract was noted and 1.0ml of it was evaporated to dryness at 60°C. Aliquots of the standard were pipetted and were made up to 1.0ml with chloroform, 2ml of TCA reagent was added, the absorbance was recorded 620nm in a spectrophotometer.

Reduced glutathione

Estimation of reduced glutathione was done according to the procedure (Moron *et al.*, 1979). An exact amount (0.5g) of the plant sample was homogenized with 2.5ml of 5% TCA. The precipitated protein was centrifuged at 1000 rpm for 10 minutes. 0.1 ml of the supernatant was taken for the estimation. For estimation, an accurate aliquot (0.1 ml) of the supernatant was made up to 1.0 ml with 0.2M sodium phosphate buffer. Freshly prepared DTNB solution (2.0 ml) was added and the intensity of the yellow colour formed was read at 412nm.

Histological Architecture of the liver tissue

In support of the biochemical parameters in the liver of the rats, the cellular and tissue architecture of the organs was followed. Small bits of the liver were fixed in 10% formalin immediately after autopsy. This procedure (Luna, 1968) was followed for this study. The tissues were placed in 10% formal saline (10% formalin in 0.9% NaCl) for one hour to rectify shrinkage due to higher concentration of formalin. The tissues were dehydrated in ascending grades of isopropanol for one hour. The sections were counter-stained in 1% aqueous solution of eosin for 1 minute and incubate at 60°C for 5 minutes, they were mounted in DPX mountant.

Statistical Analysis

All the parameters studied were subjected to statistical treatment using Sigmastat statistical package (version 3.1), one way ANOVA, followed by post-hoc analysis using Fisher's LSD.

Results

All the four serum marker enzymes (AST, ALT, ALP and γ -GT) showed was significantly increased in the serum of rats treated with ethanol, which was further augmented by CCl₄ administration shown in Figure 1 and 2. The activities of SOD increased significantly in the ethanol treated group and decreased further in ethanol-CCl₄ treated group. *Clitoria ternatea* leaf administration significantly increased the activi-

ty of SOD. The activities of catalase in the liver caused a significant decrease in the activity over the untreated controls. The activities of white flowered leaf extracts were more pronounced than the blue flowered leaves. The activities of glutathione peroxidase in the liver of rats, Administration of ethanol decreased the activities and the leaf extract administration reverted the glutathione peroxidase activity. The activity of GST decreased significantly upon ethanol assault as well as in ethanol-CCl₄ exposure. The administration of leaf extracts and silymarin increased the activity of GST. The activity of glutathione reductase in the presence and the absence of *Clitoria ternatea* leaf extracts or silymarin was significantly altered shown in Figure 3 and 4.

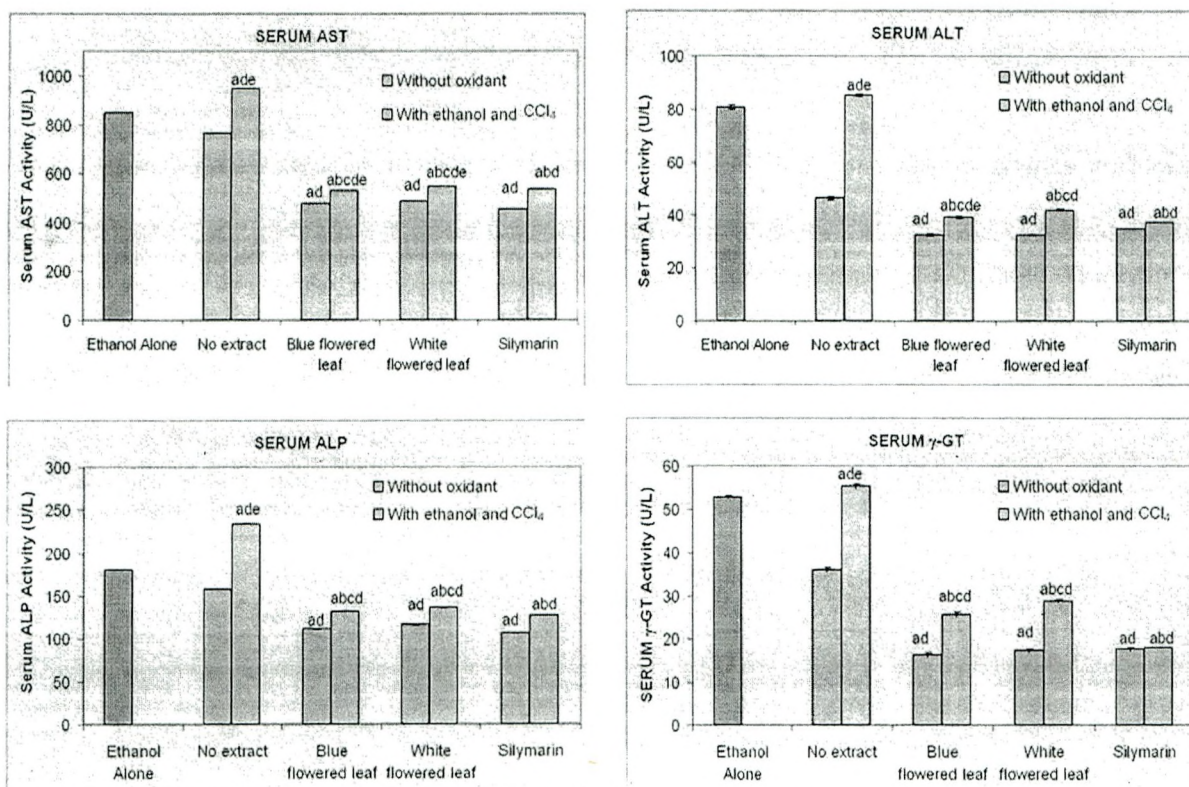


Figure 1: Effect of *Clitoria ternatea* leaves on serum AST, ALT, ALP, γ -GT activities in the rats exposed in vivo to alcohol CCl₄ induced oxidative stress.

The levels of vitamin C, E, A, GSH in the liver of rats exposed to alcohol-CCl₄ in the presence or absence of *Clitoria ternatea* leaf extracts or silymarin, Alcohol and alcohol-CCl₄ treated group alone caused a significant drop in

the levels The treatment of white flowered leaf extract caused a significant elevation in all vitamin levels in the liver compared to blue flowered leaves shown in Tables 1 to 4.

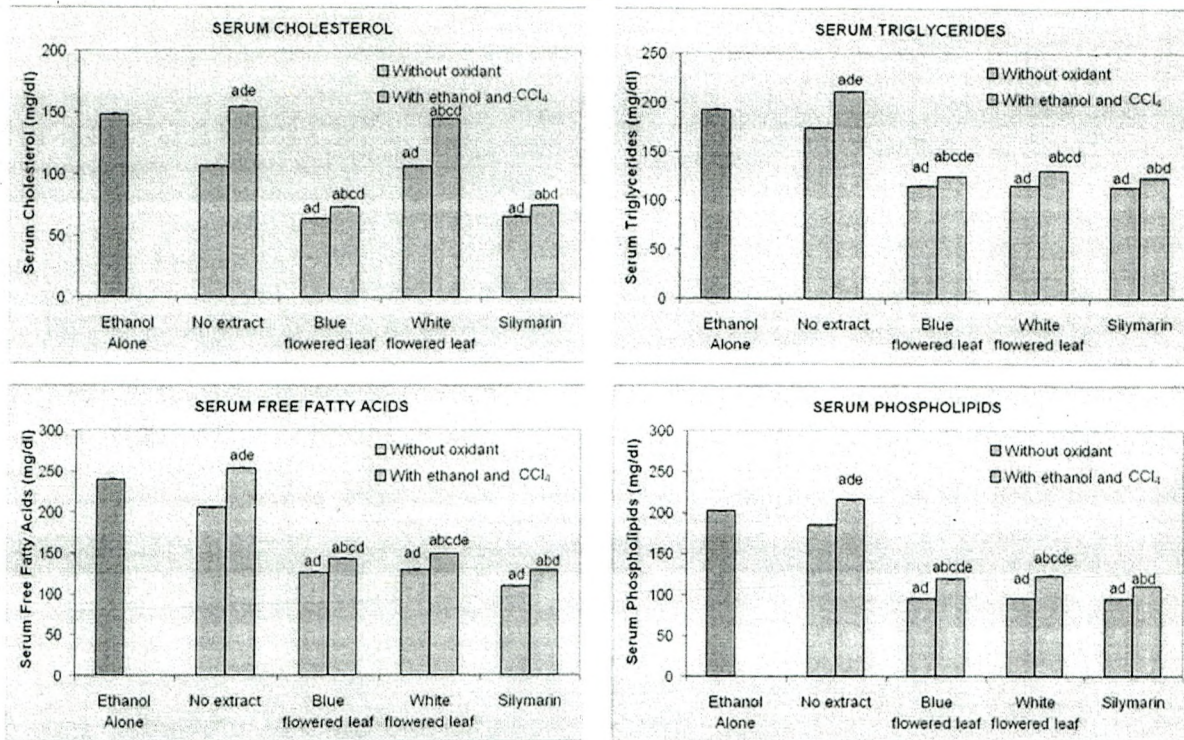


Figure 2: Effect of *Clitoria ternatea* leaves on serum lipid profile in the rats exposed *in vivo* TO alcohol CCl₄ induced oxidative stress

Table 1: Effect of *Clitoria ternatea* leaves on the levels of Vitamin C in the liver of rats exposed *in vivo* to alcohol-CCl₄ induced oxidative stress

Sample	Levels of vitamin C (mg/g tissue)	
	Without CCl ₄	With ethanol and CCl ₄
No extract	2.29 ± 0.29	1.59 ± 0.37 ^{a d e}
Blue flowered leaf	1.92 ± 0.17 ^{a d}	1.73 ± 0.39 ^{a b c d e}
White flowered leaf	2.40 ± 0.41 ^{a d}	3.00 ± 0.34 ^{a b c d e}
Silymarin	1.88 ± 0.19 ^{a d}	1.71 ± 0.27 ^{a b d}

LSD (5%) = 0.355, Values are mean ± SD (n=6)
Levels of vitamin C in ethanol treated group = 1.12 ± 0.17 mg/g tissue

- a - Statistically significant (P<0.05) compared to untreated control.
- b - Statistically significant (P<0.05) compared to ethanol + CCl₄ treated group.
- c - Statistically significant (P<0.05) compared to respective plant/silymarin control.
- d - Statistically significant (P<0.05) compared to ethanol alone treated group.
- e - Statistically significant (P<0.05) compared to ethanol + CCl₄ + silymarin treated group.

Table 2: Effect of *Clitoria ternatea* leaves on the levels of Vitamin E in the liver of rats exposed *in vivo* to alcohol-CCl₄ induced oxidative stress

Sample	Levels of Vitamin E (µg/g tissue)	
	Without CCl ₄	With ethanol and CCl ₄
No extract	33.41 ± 0.47	31.08 ± 0.17 ^{a d e}
Blue flowered leaf	38.55 ± 0.50 ^{a d}	33.53 ± 0.19 ^{a b c d e}
White flowered leaf	41.38 ± 0.32 ^{a d}	34.89 ± 0.22 ^{a b c d e}
Silymarin	40.15 ± 0.20 ^{a d}	35.12 ± 0.19 ^{a b d}

LSD (5%) = 0.39, Values are mean ± SD (n=6)
Levels of vitamin E in ethanol alone treated group = 30.88 ± 0.12 µg/g tissue

- a - Statistically significant (P<0.05) compared to untreated control.
- b - Statistically significant (P<0.05) compared to ethanol + CCl₄ treated group.
- c - Statistically significant (P<0.05) compared to respective plant/silymarin control.
- d - Statistically significant (P<0.05) compared to ethanol alone treated group.
- e - Statistically significant (P<0.05) compared to ethanol + CCl₄ + silymarin treated group.

Table 3: Effect of *Clitoria ternatea* leaves on the levels of Vitamin A in the liver of rats exposed *in vivo* to alcohol-CCl₄ induced oxidative stress

Sample	Levels of vitamin A (µg/g tissue)	
	Without CCl ₄	With ethanol and CCl ₄
No extract	48.25 ± 0.58	27.43 ± 0.52 ^{a d e}
Blue flowered leaf	44.27 ± 0.31 ^{a d}	35.24 ± 0.30 ^{a b c d e}
White flowered leaf	47.21 ± 0.26 ^{a d}	35.29 ± 0.34 ^{a b c d e}
Silymarin	45.24 ± 0.30 ^{a d}	37.35 ± 0.37 ^{a b c d}

LSD (5%) = 0.428, Values are mean ± SD (n=6)

Levels of vitamin A in ethanol alone treated group = g/g 30.94 ± 0.37 tissue

a - Statistically significant (P < 0.05) compared to untreated control.

b - Statistically significant (P < 0.05) compared to ethanol + CCl₄ treated group.

c - Statistically significant (P < 0.05) compared to respective plant/silymarin control.

d - Statistically significant (P < 0.05) compared to ethanol alone treated group.

e - Significant (P < 0.05) compared to ethanol + CCl₄ + silymarin treated group.

Table 4: Effect of *Clitoria ternatea* leaves on the levels of GSH in the liver of rats exposed *in vivo* to alcohol-CCl₄ induced oxidative stress

Sample	Levels of GSH (mg/g tissue)	
	Without CCl ₄	With ethanol and CCl ₄
No extract	47.22 ± 0.27	26.32 ± 0.33 ^{a d e}
Blue flowered leaf	44.32 ± 0.33 ^{a d}	40.15 ± 0.25 ^{a b c d e}
White flowered leaf	46.38 ± 0.38 ^{a d}	42.17 ± 0.24 ^{a b c d e}
Silymarin	42.43 ± 0.41 ^{a d}	34.92 ± 0.29 ^{a b d}

LSD (5%) = 0.370, Values are mean ± SD (n=6)

Levels of GSH in ethanol alone treated group = 35.04 ± 0.65 mg/g tissue

a - Statistically significant (P < 0.05) compared to untreated control.

b - Statistically significant (P < 0.05) compared to ethanol + CCl₄ treated group.

c - Statistically significant (P < 0.05) compared to respective plant/silymarin control.

d - Statistically significant (P < 0.05) compared to ethanol alone treated group.

e - Statistically significant (P < 0.05) compared to ethanol + CCl₄ + silymarin treated group.

Histopathological studies shown in Figure 5 demonstrated that the ethanol with CCl₄ induced centrilobular fatty change with individual cell necrosis in hepatic cells and the renal tubules revealed cloudy swelling of the lining cells with congestion in the interstitial vessels (Maheswari and Rao 2005; Yang 2006) PGM administration of CCl₄ to rats induced degeneration in hepatic cords and hepatocytes, and infiltration of lymphocytes.

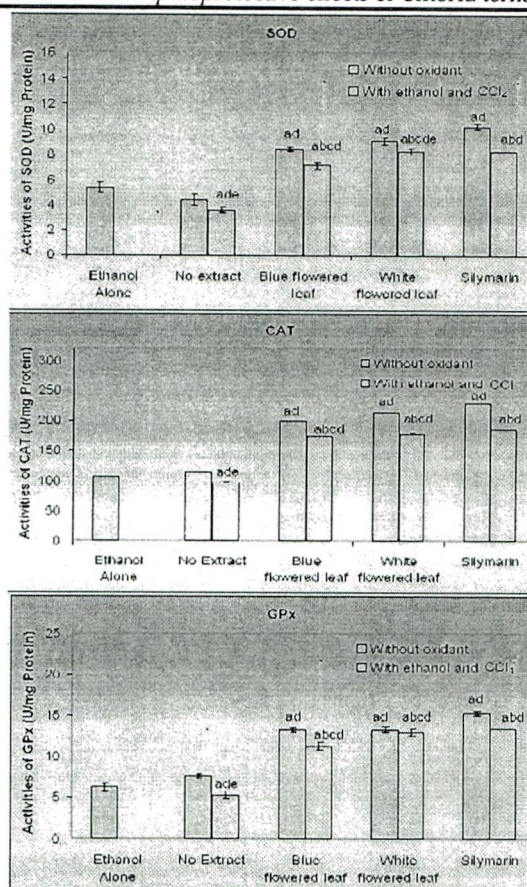


Figure 3: effect of *Clitoria ternatea* leaves on enzymic antioxidant activities in the liver of rats exposed *in vivo* to alcohol CCl₄ induced oxidative stress.

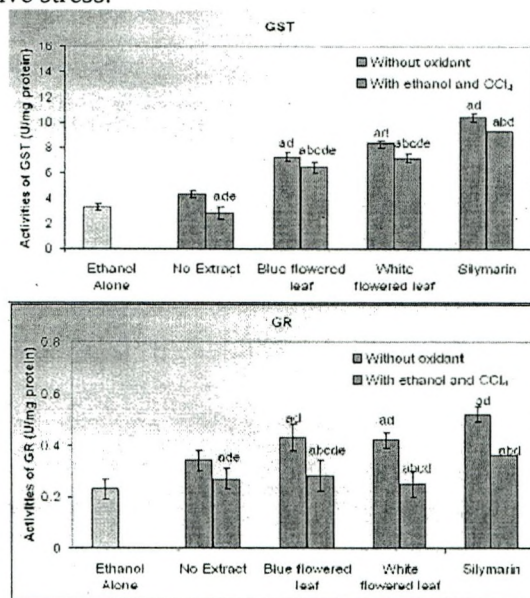


Figure 4: effect of *Clitoria ternatea* leaves on enzymic antioxidant activities in the liver of rats exposed *in vivo* to alcohol CCl₄ induced oxidative stress.

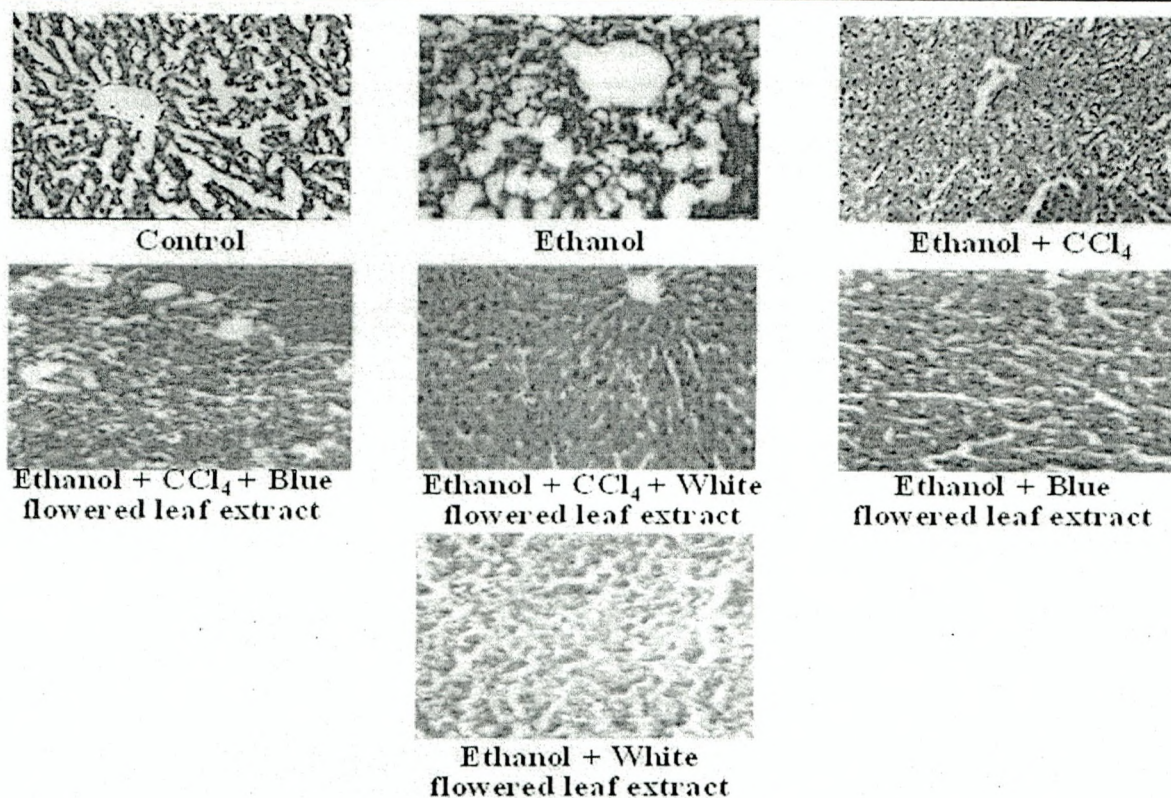


Figure 5: Histopathological architecture in the liver of control and experimental animals.

Discussion

The monitoring of marker enzymes in serum (AST, ALT, ALP and γ -GT) is a sensitive means of detecting the severity of liver damage. The marker enzymes analyzed were aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (γ -GT). Many medicinal plants have been reported for the hepatoprotective activity against CCl_4 induced liver damage in rats (Aniya *et al.*, 2005). The administration of ethanol increased the activity of liver marker enzymes such as AST, ALT, ALP and γ -GT, which was nullified by the administration of grape leaf extracts (Pari and Suresh, 2008). The levels of total cholesterol, triglycerides, phospholipids and free fatty acids were analyzed in the rats subjected to the different treatments. Administration of ethanol to rats caused changes in the metabolism of serum and tissue lipids (Remla *et al.*, 1991). On the administration of plant extracts, serum cholesterol level was normalized in these studies. *Dolichos biflorus* (Muthu *et al.*, 2005) and *Cassia auriculata* leaf extracts significantly decreased

the level of serum triglycerides, free fatty acids and phospholipids in rats with alcoholic liver injury.

Aqueous extracts of *Andrographis paniculata* exhibited its antioxidant effect by restoration in SOD enzyme (Tripathi and Kamat, 2007). Reduced SOD activity was observed in CCl_4 induced liver of rats, which was counteracted by the polyherbal formulation, Himoliv (Bhattacharyya *et al.*, 2003). Plant and herbal preparations have also been shown to exert a beneficial effect on GPx activity. The administration of allicin and amaranth leaves (Anilakumar *et al.*, 2007) significantly elevated the levels of enzymic antioxidants in different stress conditions in experimental animals. *Emblica officinalis* (Sultana *et al.*, 2005) caused a significant elevation of the decreased levels of SOD, CAT, GPx and GR in CCl_4 and in ethanol intoxication in rats. The protective effect of the juice of *Opuntia ficusindica* fruit against carbon tetrachloride induced hepatotoxicity may be related to the flavonoid fraction as well as vitamin C (Kamalakkannan *et al.*, 2005). That the same effect of CCl_4 treatment to rats in liver and

plasma. Diet derived antioxidants (vitamins C, E and carotenoids) are important as antioxidant defense and in maintaining healthcare. Re-treatment with green tea (*Camellia sinensis*) significantly improved the levels of vitamins E and A in the liver of ammonium metavanadate induced toxicity in rats (Dan *et al.*, 2005). That the chronic ethanol consumption depleted vitamin A stores in the liver of rats. Beta-carotene and other carotenoids have been thought to have anticancer activity, either because of antioxidant activity or because of their ability to be converted to vitamin A (Russel, 2004). Moderate accumulation of fatty lobules was observed in blue flowered leaf extracts treated rats. The silymarin treated group showed almost normalization of fatty accumulation and necrosis Shanmugasundaram and Venkataraman (2005). A similar effect was also observed by the administration of beta carotene in CCl₄ induced hepatic inflammation and fibrosis in rats and *Asteracantha longifolia* in CCl₄ induced hepatotoxicity in the liver of rats.

Conclusion

Our results showed that oxidative stress induced by ethanol-CCl₄ administration resulted in a significant depletion in the activities of all the enzymic antioxidants studied. The effect of white flowered leaf extract was significantly higher on all the enzymic antioxidant activities than the blue flowered leaf extracts. This enhanced effect and protective action on cellular antioxidant defense by *Clitoria ternatea* suggests a strong protection against oxidative damage in CCl₄ induced hepatotoxicity in rats. The observations made *in vivo* were also confirmed with histopathological studies. Thus, the outcome of the present study highlights the white flower bearing leaf extracts of *Clitoria ternatea* leaves are used in the preparation of medicinal aids to combat the wide spectrum of myriad diseases arising due to oxidative stress.

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