

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF
PRUNELLA VULGARIS BY CARBONTETRACHLORIDE INDUCED
HEPATOTOXICITY

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Summary

The hepatoprotective effect of ethanolic extract of leaves of *Prunella Vulgaris* against carbon tetrachloride (CCL₄) induced hepatic damage was investigated. The degree of protection was determined measuring levels of serum marker enzymes like Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP), Total and direct bilirubin and liver weight of rat. The histopathological studies were also carried out. Silymarin was used as standard drug for comparisons. Administration of ethanolic extract of *Prunella Vulgaris* (50,100 mg/kg p.o) markedly decrease CCL₄ induced elevation levels of Serum marker enzymes and liver weight in dose dependent manner. The effects of extract was compared with standard, Silymarin at 100 mg/kg dose. In ethanolic extract treated animals, the toxic effect of CCl₄ was controlled significantly by restoration of the levels of enzymes as compared to the normal and standard drug silymarin treated groups. Histology of the liver sections of the animals treated with extract showed the presence of liver cells, absence of necrosis and fatty infiltration, which further evidenced the hepato protective activity. It was concluded that Ethanolic extract of leaves of *Prunella vulgaris* possesses significant hepatoprotective activity.

Key words: *Prunella vulgaris*, Carbon tetrachloride, Hepato protective activity, Serum marker enzymes.

Introduction

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds. It is the most important first organ to encounter ingested nutrients, drugs and environmental toxicants that enter hepatic portal blood so regulate important metabolic function(1). There are several diseases can affect the liver are Wilson's disease, Hepatitis, Liver cancer and Cirrhosis, Whereas alcohol consumption alters the metabolism of the liver, Some medications have side effects that may harm liver are anticancer drugs((Methotrexate)(2). *Prunella vulgaris* (Family:Lamiaceae) also called as self heal or heart of the Earth grow along streams, around ponds and lakes, in road side ditches, wet prairies, as well as in drier habitats(3). It is a traditional Chinese drug and has hypotensive, anti bacterial, anti-viral, anti inflammatory, anti-tumor and hypoglycemic activities (4,5,6,7,8,9). Traditionally the leaves of the plant *Prunella vulgaris* was used for swelling of lymph glands, hepatic disorder, reduces excitement, nervousness and irritation. In spite of its reported use, no systematic clinical experimental studies have been carried out to possess the hepato protective activities of this species. Hence an effort has been made to evaluate the hepato protective effect of the ethanolic extract of leaves against CCL₄ induced liver damage in rats.

Methods

Preparation of the extract The leaves of *Prunella vulgaris* was collected from local area of Hyderabad and authenticated by Dr. Najma, Botanist, S.U.C.P college, Hyderabad. The leaves were shade dried and powdered and extracted with 70 % ethanol for 48 hrs in soxhlet apparatus. The extracts were filtered and concentrated in vacuum under reduced reduced pressure using rotary flash evaporator. The extract was subjected to qualitative phytochemical screening for the identification of phytoconstituents.

Animals Albino rats (160-180 g) of either sex were used and were maintained at standard housing conditions. The animals were fed with standard rodent diet and provided water *ad libitum* during the experiment. The study was permitted by the Institutional Animal Ethical Committee (IEAC) approved the use of animals for the present study, Ethical clearance number: IEAC/SUCP/03/2009.

Hepato protective activity: The animals were divided into 5 groups of 6 animals each **Group A** served as normal control and received subcutaneous administration of liquid paraffin (L.P) only 3ml/kg on alternate days for one week. All other groups **B, C, D** and **E** received Carbon tetrachloride (1ml/Kg) subcutaneously in the lower abdomen in a suspension of L.P in the ratio 1:2 v/v on alternate days for week. **Group B** animals were maintained as Carbon tetrachloride group. **Group C** animals were treated with Silymarin 100mg/kg (10) orally for 7 days. **Group D** animals were treated with Ethanolic extract of *Prunella vulgaris* 50 mg/kg orally for 7 days. **Group E** animals were treated with Ethanolic extract of *Prunella vulgaris* 100 mg/kg orally for 7 days. After drug treatment all the animals were sacrificed, blood was collected by puncturing the retro orbital plexus and was allowed to clot for 45 min at room temperature, serum was collected by centrifugation at 2500 rpm for 15 min, used for estimation of various bio-chemical parameters(11,12).

Assessment of Liver function: Bio-chemical parameters such as Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT)(13), Alkaline Phosphatase (ALP)(14) and Serum Bilirubin(15) were determined.

Statistical Analysis: Results are expressed as mean \pm SEM. Statistical analysis was performed with one-way ANOVA using Dunnett's t test. A value of $p < 0.01$ was considered statistically significant as compared with control.

Results

The results obtained from various parameters are summarized in the tables given below.

Table1: Effect of Ethanolic extract of *Prunella vulgaris* on Carbon tetrachloride induced Hepatotoxicity in Rats.

Group	SGPT (IU/L)	SGOT (IU/L)	ALKP (KA Units)	BILIRUBIN TOTAL (mg %)	BILIRUBIN DIRECT(mg %)
A-Control	36 \pm 0.73	49 \pm 0.73	30.64 \pm 0.47	0.98 \pm 0.005	0.185 \pm 0.009
B-Carbon tetrachloride	120 \pm 4.08 ^a	130 \pm 4.235 ^a	74.6 \pm 1.17 ^a	2.07 \pm 0.25 ^a	0.28 \pm 0.009 ^a
C-Carbon tetrachloride + Silymarin	70 \pm 0.96 ^c	68 \pm 0.96 ^c	48.35 \pm 0.84 ^c	1.34 \pm 0.05 ^c	0.18 \pm 0.01 ^c
D-Carbon tetrachloride + 50 mg/kg of Ethanolic Extract	108 \pm 1.54 ^b	88 \pm 0.96	68.3 \pm 0.87 ^b	1.69 \pm 0.03 ^b	0.23 \pm 0.007
E-Carbon tetrachloride + 100 mg/kg of Ethanolic Extract	94 \pm 1.18	78 \pm 0.96 ^a	51.4 \pm 0.62 ^a	1.30 \pm 0.07 ^b	0.21 \pm 0.009 ^a

Values are expressed as Mean \pm SEM. (n=6) ^a $p < 0.001$, ^b $p < 0.01$ as compared to control group, ^c $p < 0.01$ compared to CCL4 treated group

Table2: Effect of Ethanolic extract of *Prunella vulgaris*(PV) on Liver weight in Carbon tetrachloride induced Hepatotxicity in Rats.

Groups	Liver weight(gm)
A-Control	5.82 ±0.14
B-Carbon tetrachloride	9.46 ±0.02 ^b
C-Carbon tetrachloride + Silymarin	6.26 ±0.26 ^a
D-Carbon tetrachloride + 50 mg/kg of Ethanolic Extract	6.89 ±0.05
E-Carbon tetrachloride + 100 mg/kg of Ethanolic Extract	7.11 ±0.01 ^a

Values are expressed as Mean±SEM (n=6) ^ap<0.01, ^bp<0.001 compared to control group

Fig 1 Effect of *Prunella vulgaris* on SGPT levels in CCL4 induced hepatotoxicity in rats

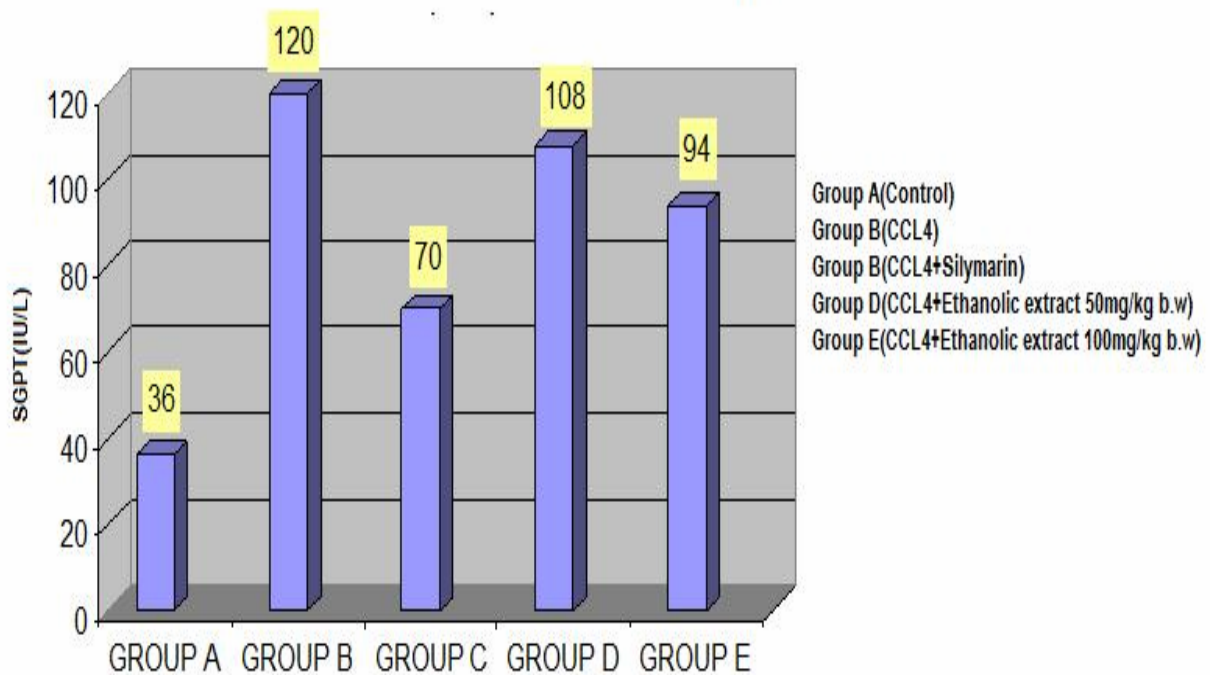


Fig 2 Effect of *Prunella vulgaris* on SGOT levels in CCL4 induced hepatotoxicity in rat

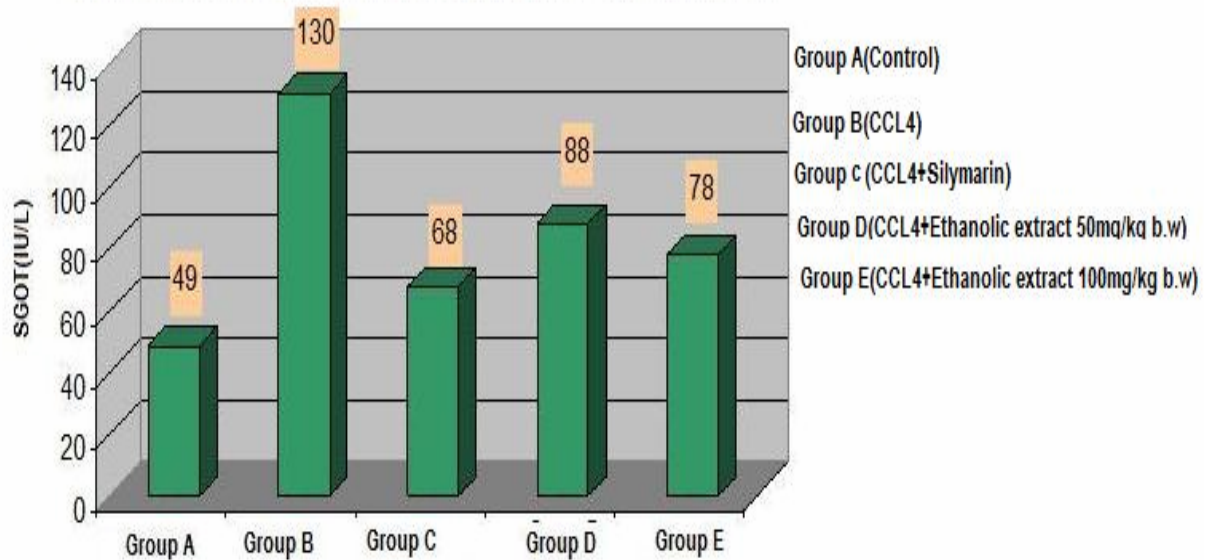
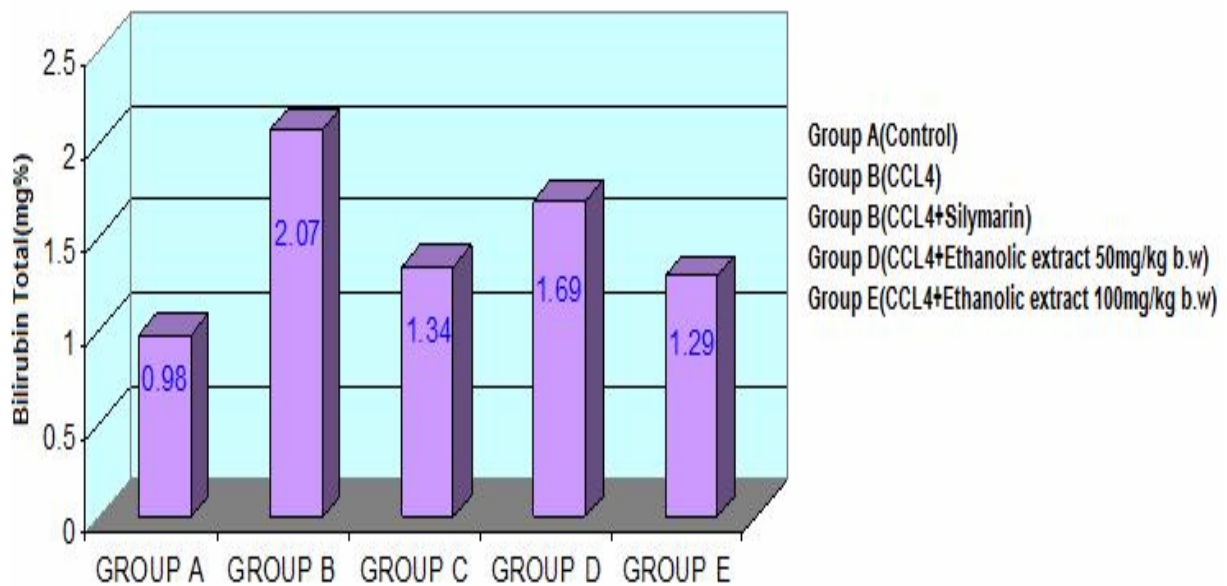


Fig 4 Effect of *Prunella vulgaris* on Total bilirubin levels in CCL4 induced hepatotoxicity in rats



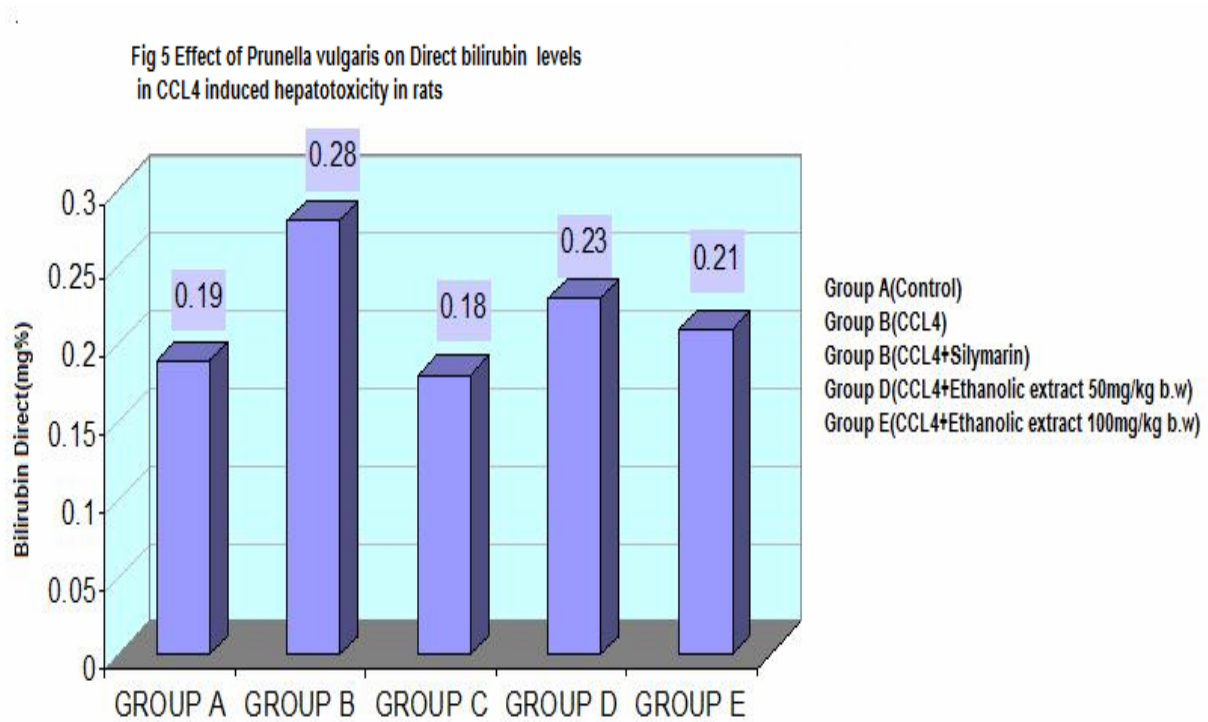


Fig 6 : Effect of ethanolic extract of *Prunella vulgaris* on CCl4 induced histopathological changes in rat liver

Fig 6a. Normal control rat: Liver section showing normal hepatic cells

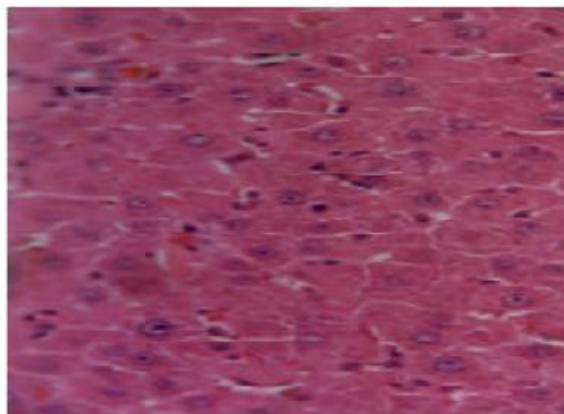


Fig 6b. CCl4 treated rat. liver section showing focal necrosis, fatty degeneration and vacuolization

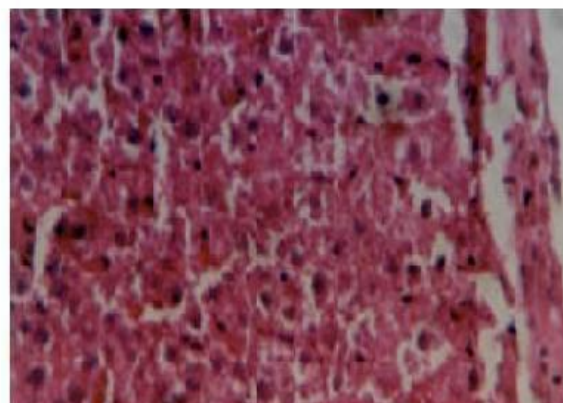


Fig 6c. Silymarin treated rat. Liver section showing normal hepatocytes and their lobular architecture was normal

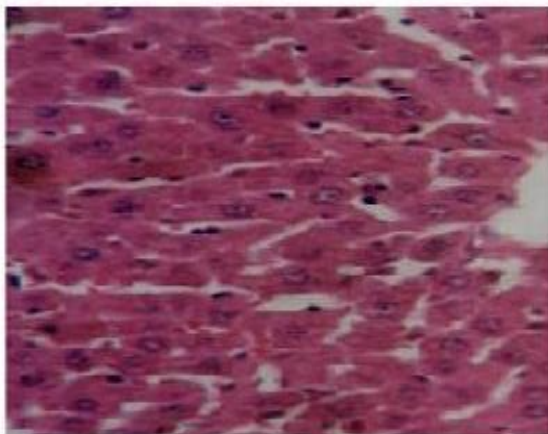
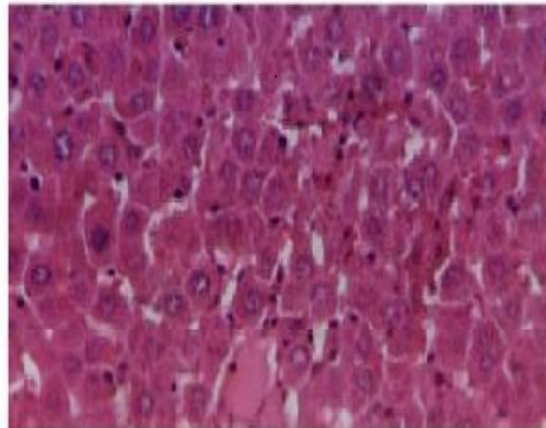


Fig 6d Ethanolic extract of *Prunella vulgaris*(100mg/kg), Liver section showing an almost normal architecture, barring a little deformity of hepatocytes with pyknosis and clearing of cytoplasm



Phytochemical screening The preliminary phytochemical tests indicate that the following chemical constituents alkaloids, sugars, steroids, tannins, phenols and flavanoids are found to be present in the ethanolic extract of leaves of *Prunella vulgaris*.

Effect of *Prunella vulgaris* extract on Biochemical parameters and liver weight Increased levels of SGPT, SGOT, ALP, Total and Direct bilirubin and liver weight were observed in CCL₄ treated group. The treatment with *Prunella vulgaris* decreases the elevated manner levels of biomarker enzymes of liver to the near levels in a dose dependent manner. The changes in biochemical markers are shown in **Table 1** compared to standard drug Silymarin 100 mg/kg.

Histopathological Observation Histopathological study of liver from **Group A** animals showed a normal hepatic architecture(**Fig 6a**). In CCL₄ treated group, severe hepatotoxicity was evidenced by profound central lobular fatty degeneration, necrosis, fibrosis and vacuolization an(**Fig 6d**). In **Group E** animals, the liver exhibited an almost normal architecture, barring a little deformity of hepatocytes with pyknosis and change of cytoplasm, shows moderate protection in CCL₄ induced liver damage. In **Group C** animals showed significant protection to considerable extent as evident from the formation of normal hepatic cells and absence of necrosis and vacuoles.

Discussion

The CCL₄ has been used as tool to induce hepatotoxicity in experimentals. This toxic chemical caused per oxidative degradation in the adipose tissue resulting in fatty infiltration of hepatocytes. The increase in the levels of serum bilirubin reflects the depth of jaundice and increase in transaminases and alkaline phosphatase was the clear indication of cellular leakage and loss of functional integrity of the cell membrane. The changes associated with CCL₄ induced liver damage are similar to that of acute viral hepatitis.

The CCL₄ is biotransformed by the cytochrome P₄₅₀ system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb Ca²⁺ homeostasis and finally result in cell death (16). Estimating the activities of serum marker enzymes, like SGOT, SGPT, ALKP, Direct and Total bilirubin can make assessment of liver function. When liver 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 liver damage. Hepatocellular necrosis leads to very high level of biomarker enzymes and bilirubin released from liver in the blood. Reduction in the level of SGOT, SGPT enzymes towards the respective normal values is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCL₄. Suppression of increased ALP activity with concurrent depletion of raised bilirubin level suggests the stability of the biliary dysfunction in rat liver (17).

The ethanolic extract of *Prunella vulgaris* decreases the CCL₄ induced elevated enzyme levels in dose dependent manner. It represents the ethanolic extract at 100mg/kg showed the protection of structural integrity of hepatocyte cell membrane and helps in the regeneration of damaged liver cells. The effectiveness of the normal functioning condition of the liver is indicated by the decreased levels of serum bilirubin. The results suggest that leaves of *Prunella vulgaris* prevent the formation of fatty liver comparable to Silymarin used as hepatoprotective agent. A complete histopathological study of liver from different groups further corroborated the hepatoprotective efficacy of *Prunella Vulgaris*. Further work is in progress to isolate and purify the active principle involved in hepatoprotective activity.

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