Animal models

Studies were carried out using Wistar strain albino rats (150-200 g) of either sex, which were procured from Central Animal House, National College of Pharmacy, Shivamogga. They werehoused under standard laboratory conditions (temp 23±2oC, relative humidity 55±10%) with dark light circle (14/10hr). Animals were allowed free access to standard pellet diet (Sai Durga Feeds, Bangalore) and water ad libitum. All experimental protocols were prepared and performed based on ethical guidelines of Institutional Animal Ethics Committee (No. 144/1999/CPCSEA/SMG).

Toxicity studies

Acute toxicity study was performed for all the extracts according to the staircase method of Ghosh [16]. Female albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extract was administered orally at doses of 100, 200 and 300mg/kg b.w. and observed for toxic symptoms up to 72 h. 200 mg/kg b.w was taken as the therapeutic oral dose for all the extracts.

Carbon tetrachloride induced hepatotoxicity

Rats were divided into twelve groups of six animals each. The rats of control group (I) received three doses of 5% gum acacia mucilage (1ml/kg b.w.) at 12 h intervals (0 h, 12 h and 24 h). The rats of CCl_4 group (II) received three doses of vehicle at 12 h intervals and a single dose of CCl_4 (1.25ml/kg b.w.) diluted in liquid paraffin (1:1) 30 minutes after the administration of 1st dose of vehicle. Group – III served as reference control, received silymarin (25mg/kg b.w.) once daily for 3 days. Group –IV to XII received, *T. cordifolia* extract (200 mg/kg b.w.) once daily for 3 days. Group III to XII received CCl_4 (3gm/kg b.w.) as single dose on day 3, thirty minutes after the administration of extracts and silymarin respectively. All the test drugs and CCl_4 were administered orally by suspending in 5% gum acacia mucilage. After 36 hour of administration of CCl_4, blood was collected by direct cardiac puncture under light ether anesthesia and serum was separated and used for determination of biochemical parameters such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin (TBL) [17].

Histopathological studies

The animals were sacrificed and the abdomen was cut open to remove the liver. The liver was fixed in Bouin's solution (mixture of 75 ml of saturated picric acid, 25 ml of 40% formaldehyde and 5ml of glacial acetic acid) for 12h, and then embedded in paraffin using conventional methods [18] and cut into 5µm thick sections and stained using haematoxylin-eosin dye and finally mounted in di-phenyl xylene. The sections were then observed under microscope for histopathological changes in liver architecture and their photomicrographs were taken.

Statistical analysis

Statistical analysis of the data was done by one-way ANOVA, followed by student t-test using software ezANOVA ver. 0.98. The level of significance was fixed at $P<0.05$.

RESULTS AND DISCUSSION

The preliminary phytochemical investigations show the presence of various secondary metabolites as tabulated in table 1. The same extracts have shown significant hepatoprotective activities at the concentration (200 mg/kg) tested, although its pure form remains to be studied. The results of biochemical parameters revealed the elevation of enzyme level in CCl_4 treated group, indicating that CCl_4 induces damage to the liver (Table 2). A significant reduction was observed in TBL, ALT, AST and ALP levels in the groups treated with silymarin and leaf extracts of *T. cordifolia*. But the groups which received other two extracts such as stem and root, as test drug at the dose of 200mg/kg showed a less significant decrease in the elevated levels of enzymes. The results obtained for biochemical parameters are comparable with silymarin, the standard hepatoprotective drug. Therefore, as like silymarin the leaf extracts have restored the altered level of enzymes significantly. The enzyme levels were almost restored to the normal. It was found that the extract decreased the CCl_4 induced elevated levels of the enzymes in group III to XII, indicating the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract. It was observed that the size of the liver was enlarged in CCl_4 intoxicated rats but it was normal in drug treated groups. A significant reduction in liver weight supports this finding.

Normally, AST and ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatic diseases is unusual. ALT is more selectively a paranchymal liver enzyme than AST [19]. Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and TBL which are enzymes originally present higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage [20]. Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. Decrease in serum

Table 1: Qualitative analysis of *T. cordifolia* plant extracts

Plant constituent	Leaf			Stem			Root		
	Pet. Ether ext.	Ethanol ext.	Aqueous ext.	Pet. Ether ext.	Ethanol ext.	Aqueous ext.	Pet. Ether ext.	Ethanol ext.	Aqueous ext.
Carbohydrates	–	+	+	–	+	+	–	+	+
Proteins	–	+	+	–	+	+	–	+	+
Tannins	–	+	+	–	+	+	–	+	+
Saponins	+	+	+	+	+	+	+	+	+
Triterpenoids	+	+	+	+	+	–	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Quinones	–	–	–	–	–	–	–	–	–
Sterols	+	–	–	+	–	–	+	–	–
Glycosides	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+

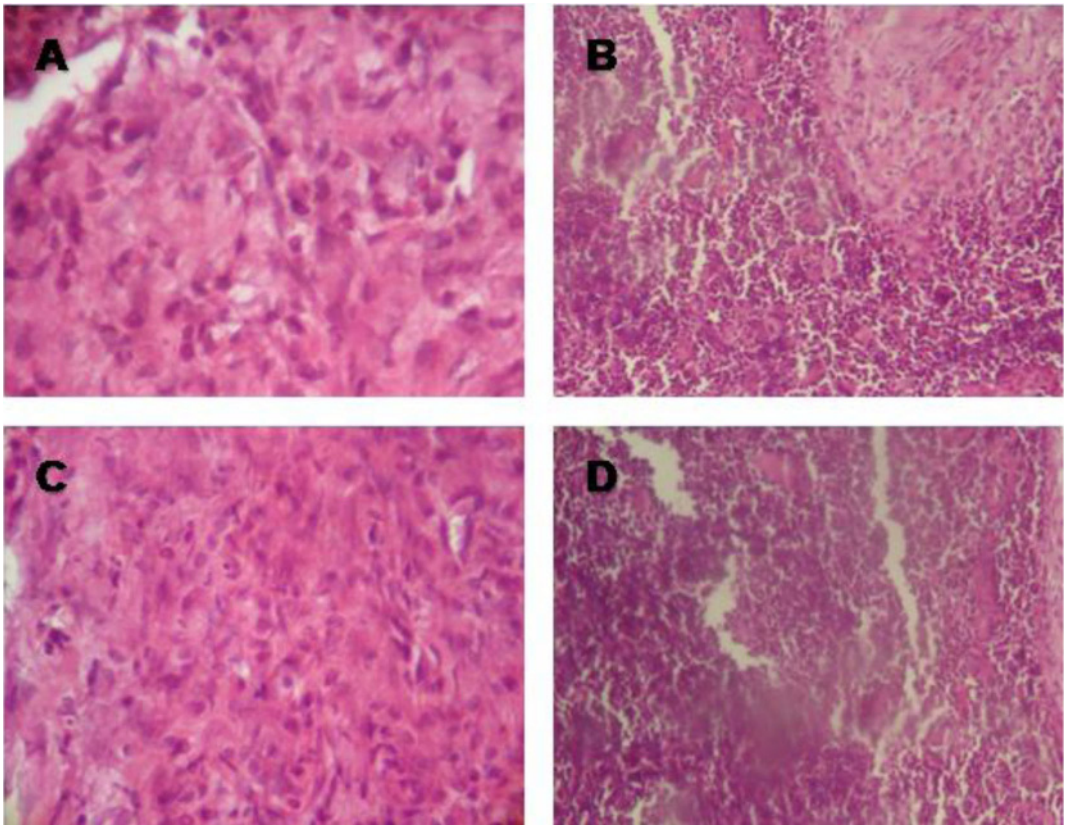
Phytochemical test: '+'- Present and '–' - Absent .

Table 2: Effect of leaf, stem and root extracts of *T. cordifolia* on CCl_4 induced hepatotoxicity in rats enzymes.

Group (n)	TBL (mg/dL) ± SE	ALT (IU/L) ± SE	AST (IU/L) ± SE	ALP (IU/L) ± SE
Control (I)	0.85 ± 0.02	202.50 ± 2.28	254.00 ± 2.50	300.83 ± 3.23
CCl_4 (II)	2.05 ± 0.03**	423.33 ± 3.17**	483.83 ± 2.39**	522.83 ± 2.23**
CCl_4 + S (III)	0.93 ± 0.03	194.83 ± 2.36	246.67 ± 2.16	291.83 ± 2.43
CCl_4 + PL (IV)	1.31 ± 0.03**	226.00 ± 1.79**	274.00 ± 2.28**	335.17 ± 2.26*
CCl_4 + EL (V)	0.95 ± 0.04**	204.33 ± 2.25**	253.83 ± 1.97**	303.67 ± 2.54**
CCl_4 + AL (VI)	1.11 ± 0.05**	213.67 ± 2.29**	265.33 ± 2.82**	329.67 ± 18.00**
CCl_4 + PS (VII)	1.46 ± 0.06	286.17 ± 2.10	334.67 ± 2.25	383.50 ± 2.47
CCl_4 + ES (VIII)	1.23 ± 0.05**	256.50 ± 2.22**	303.17 ± 2.48**	355.67 ± 2.06*
CCl_4 + AS (IX)	1.31 ± 0.02	275.17 ± 2.07	325.83 ± 2.75	381.33 ± 1.26
CCl_4 + PR (X)	1.47 ± 0.01	307.33 ± 1.91	355.17 ± 1.82	404.00 ± 2.38
CCl_4 + ER (XI)	1.28 ± 0.01**	285.83 ± 1.82**	334.50 ± 1.89*	384.00 ± 2.08**
CCl_4 + AR (XII)	1.32 ± 0.01	295.33 ± 1.93	344.83 ± 2.04	393.50 ± 2.19

PL-Pet ether ext. of leaf; EL-Ethanol ext. of leaf; AL-Aqueous ext. of leaf; PS- Pet ether ext. of stem; ES- Ethanol ext. of stem; AS- Aqueous ext. of stem; PR- Pet ether ext. of root; ER- Ethanol ext. of root; AR- Aqueous ext. of root. S- Silymarin, n = 6 animals in each group, The values are mean ± S.E. ** P<0.01 compared to standard.

Figure 1: Histopathological changes showing effect of Control (A); Positive control (B); Silymarin (C) and Drug treated (D).



bilirubin after treatment with the extract indicates the effectiveness of it in normal functional status of the liver.

Histopathological examination of the liver section of the rats treated with toxicant showed intense centrilobular necrosis and vacuolization. The rats treated with silymarin and extracts along with toxicant showed sign of protection against these toxicants to considerable extent as evident from formation of normal hepatic cards and absence of necrosis and vacuoles (fig. 1). The preliminary phytochemical studies have revealed the presence of flavonoids in the extracts of *T. cordifolia*, which supports the fact that usually flavonoids show hepatoprotective activity [21]. So in the present study, the hepatoprotective effect of *T. cordifolia* may be due to its flavonoid content. CCl_4 induced animals significantly lost their body weight and showed reduced food consumption as compared to control group. Animals of drug

treated group and standard group showed a significant increase in body weight and food consumption comparably. These findings suggested that extract administered has significantly neutralized the toxic effects of CCl_4 and helped in regeneration of hepatocytes [22]. In accordance with these results, it can be said that the leaf extracts of *T. cordifolia* exhibited a hepato protective effect against carbontetrachloride induced hepatotoxicity.

CONCLUSION

The plant is used in ayurvedic, "Rasayanas" to improve the immune system and the body resistance against infections. The Ayurveda literature reports that it can cause constipation, if taken regularly in high doses; it has no side effect and toxicity. Yet the safety and the potential indications in human beings have to be established using modern methods. These studies place this indigenous drug a novel candidate for bioprospection and drug development for the treatment of liver disorders where satisfactory cure managements are still not available. Efforts are in progress to isolate and characterize the active principle, which is responsible for the hepatoprotective efficacy of this valuable medicinal plant. Further studies are needed to explain the exact mechanism of action in neutralizing the toxic effects.

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