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RESEARCH ARTICLE

Hepatoprotective Activity of *Merremia tridentata* against Carbon Tetrachloride Induced Hepatotoxicity in Rats

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Abstract

Objectives: Liver performs a variety of function including storage and purification of blood, secretion of bile, synthesis of plasma proteins and drug detoxification. In an attempt to study the biochemical and connective tissue metabolism during the progression of hepatic fibrosis and to gain more insight into the mechanisms leading to the altered metabolic processes in human hepatic fibrosis, an experimental animal model was developed using administration of carbon tetrachloride (CCl_4). **Methods:** The present study evaluates the hepatoprotective activity of *Merremia tridentate* plant extract against CCl_4 induced liver damage in Wistar albino rats. CCl_4 caused a rise in liver and serum AST, ALT, ALP, ACP and total Bilirubin content. Pre-treatment and post treatment of rats with ethanolic and aqueous extracts (50,100 & 200 mg/kg b.wt) significantly reduced these serum parameters and antioxidant enzymes levels compared to CCl_4 induced rats. The post-treatment had better activity than the pre-treatment groups. **Findings:** Preliminary phytochemical analysis showed the presence of tannins, saponins, flavonoid, alkaloid, anthocyanin, quinones, glycosides, cardiac glycosides, terpinoids, phenol, coumarin and reducing sugar which may be responsible for the hepatoprotective activity. The liver stabilization activity is due to the presence of bioactive compounds in the *Merremia tridentate* extracts. **Conclusions:** Thus, *Merremia tridenta* extracts ameliorated the liver damage caused by the $CCl_4$and raised the liver and serum enzymes to normal levels.

Keywords: Liver; Detoxification; Carbon Tetrachloride; Merremia trident; Hepatotoxins

1 Introduction

The liver is the largest gland in the body. It performs a variety of function including storage and purification of blood, secretion of bile, synthesis of plasma proteins and drug detoxification. The interaction between the different cells that compose the liver is essential for maintaining the normal liver architecture and function. In an attempt to study the biochemical and connective tissue metabolism during the progression of hepatic fibrosis and to gain more insight into the mechanisms leading to the altered metabolic processes in human hepatic fibrosis, an experimental animal model was developed. Carbon tetrachlorideis specific hepatotoxin and has been shown to produce a reproducible model of hepatic fibrosis, as seen in human beings. This model differs from the other frequently used model of fibrosis induced by carbon tetrachloride in that there is no excessive accumulation of triglycerides in the hepatic tissue. [(1)] The animal models could replicate not only the histological picture of the disease condition but also the depicting feature of the condition in man. In addition, it can be used for studying the mechanisms leading to hepatic fibrosis and screening various therapeutic and anti-fibrotic agents against the disease. Since the pathogenic process of hepatic fibrosis leads to death and regeneration of liver tissue, studies on the alterations in the metabolism of connective tissue macromolecular components such as collagen, glycoprotein and glycosaminoglycan are of considerable clinical importance. [(2)] It was, therefore, that a through and systematic investigation on the biochemical, pathophysiological and connective tissue metabolism in this experimental model would be helpful to understand the various pathological events taking place in human hepatic fibrosis which may suggest a rational approach to therapy. [(3)]

The damage to the liver was assessed by variety of indices such as change in liver wet weight, DNA and RNA levels, total protein content and hydroxyproline content. The alteration of liver function was studied by assaying the activities of aspartate transaminase, acid and alkaline phophatase, lactate dehydrogenase and glutamyl transpeptidase. [(4)] The serum bilirubin, total protein and albumin levels were also studied. The changes in metabolic parameters were monitored in liver, serum and urine samples by estimating blood sugar, blood urea, ascorbic acid, lipid peroxides, total cholesterol phosphate and uric acid after induction of hepatic fibrosis. [(5)]

2 Materials and Methods

2.1 Plant material and preparation of the extract

As *Merremia tridentate* is locally available, the leaves of were procured in and around the Coimbatore district, Tamil Nadu, India. The plant sample was authenticated by Dr.C. Murugan, Tamil Nadu Agricultural University (TNAU Campus) Coimbatore, Tamilnadu. The collected leaves were washed thrice and air dried at room temperature. Finely powdered sample (500 g) has been defatted and then extracted with 1L of water by percolation technique. [(6)] The extract was concentrated under vacuum and used for the further analysis. Similarly procedure was carried out for the ethanolic extraction using 95% (V/V) ethanol.

2.2 Qualitative Phytochemical analysis of plant extract

Analysis of phytochemical constituents was carried out by qualitative tests. Several biochemical tests like test for carbohydrates, tannins, saponins, flavonoids, alkaloids, anthocyanin, betacyanin, glycosides, cardiac glycosides, terpenoids, reducing sugars, phytosterol, phenols, steroid, protein, Coumarin and Quinones. [(7)]

2.3 Animals

Wistar albino rats weighing 180-200 g were collected from Sree Venkateshwara Enterprises, Bangalore. Animals were kept in clean polypropylene cages and fed normal feed (Sai Feed P Ltd., Bangalore., India). The work was carried out in according to the ethical guidelines of the 466/01a /CPCSEA.

2.4 Acute toxicity studies

In order to determine the lethal dose (LD50), animals were kept fasting for overnight providing only water, during which the extracts were administered orally at various doses of 50,100,200 mg / kg and all rats were monitored for 30days for physical signs of toxicity. If the death was detected in 6 animals, the same dose was allocated as toxic dose one tenth of the toxic dose was used for the hepatoprotective analysis.

2.5 Experimental design on CCl_4 induced hepatotoxicity model in ethanolic extract

The animals were classified in to 10 groups consisting of 6 rats per group: [7]
Group I (Untreated group): The animals were maintained without any treatment and were given free access to food and drinking water.
Group II (Vehicle Control): The animals were treated with 100mg of plant extract dissolved in 2ml of 5% DMSO for 30 days.
Group III: Animals received hepatotoxic group (1ml/kg body wt) which received CCl_4 single dose daily for 30 days.
Group IV: Animals received silymarin (100mg/kg wt) simultaneously administered CCl_4 (1ml/kg body wt) single dose for 30 days.
Group V (Pre-treatment): Animals were pretreated with ethanolic plant extract (50 mg/kg wt) for 15 days. Hepatotoxicity was induced by CCl_4 (1ml/kg body wt) administered daily for next 15 days.
Group VI (Pre-treatment): Animals were pretreated with ethanolic plant extract (100 mg/kg wt) for 15 days. Hepatotoxicity was induced by CCl_4 (1ml/kg body wt) administered daily for next 15 days.
Group VII (Pre-treatment): Animals were pretreated with ethanolic plant extract (200 mg/kg wt) for 15 days. Hepatotoxicity was induced by CCl_4 (1ml/kg b. wt) administered daily for next 15 days.
Group VIII (Post-treatment: Animals were administered with CCl_4 (1 ml/kg b. wt) for 15 days consecutively. Then, the ethanolic plant extract (50 mg/kg) Single dose was given daily for next 15 days.|
Group IX (Post-treatment): Animals were administered with CCl_4 (1 ml/kg body wt) for 15 days consecutively. Then, the ethanolic plant extract (100 mg/kg) Single dose was given daily for next 15 days.
Group X (Post-treatment): Animals were treated with CCl_4 (1 ml/kg body wt) for 15 days consecutively. Then, the ethanolic plant extract (200 mg/kg) Single dose was given daily for next 15 days.

2.6 Experimental design on CCl_4 induced hepatotoxicity model in aqueous extract

The animals were classified into 10groups consisting 6 rats per group: [8]
Group I (Untreated Control): Animals were maintained without any treatment and were given free access to food and drinking water.
Group II (Vehicle Control): Animals were treated with 100mg of plant extract dissolved in 2ml of 5% DMSO for 30 days.
Group III: Animals received hepatotoxic group (1ml/kg b.wt) which received CCl_4 single dose daily for 30 days.
Group IV: Animals received silymarin (100mg/kg wt) simultaneously administered CCl_4 (1ml/kg b. wt) single dose for 30 days.

Group V (Pre-treatment): Animals were pretreated with aqueous plant extract (50 mg/kg wt) for 15 days. Hepatotoxicity was induced by CCl_4 (1ml/kg b. wt) administered daily for next 15 days.
Group VI (Pre-treatment): Animals were pretreated with aqueous plant extract (100 mg/kg wt) for 15 days. Hepatotoxicity was induced by CCl_4 (1ml/kg b. wt) administered daily for next 15 days.
Group VII (Pre-treatment): Animals were pretreated with aqueous plant extract (200 mg/kg wt) for 15 days. Hepatotoxicity was induced by CCl_4 (1ml/kg b. wt) administered daily for next 15 days.
Group VIII (Post treatment: Animals were administered with CCl_4 (1 ml/kg b. wt) for 15 days consecutively. Then, the aqueous plant extract (50 mg/kg) single dose was given daily for next 15 days.
Group IX (Post treatment): Animals were administered with CCl_4 (1 ml/kg b. wt) for 15 days consecutively. Then, the aqueous plant extract (100 mg/kg) single dose was given daily for next 15 days.
Group X (Post treatment): Animals were administered with CCl_4 (1 ml/kg b. wt) for 15 days consecutively. Then, the aqueous plant extract (200 mg/kg) single dose was given daily for next 15days.

After the 30 days of the treatment of all groups, the blood was extracted under mild ether anaesthesia through cardiac puncture. The serum was isolated using centrifuge at 2000 rpm for 15 minutes and tested for different biochemical parameters. The animals were then sacrificed by cervical decapitation method. The liver was easily dissected, cleaned with ice cold saline and blotted out free of blood, further the liver was subjected to biochemical analysis.

2.7 Biochemical analysis

Determination of Serum glutamic oxaloacetic transaminase (SGOT) and Serum glutamic pyruvic transaminase (SGPT) activities: Liver damage was evaluated by the estimation of serum activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) using test kit methods. The results were mentioned as units/ litre (IU/L).

2.8 Statistical analysis

The results are described as the mean ± S.D. The data were statistically analyzed using one –way Analysis of variance (ANOVA) followed by Tukeytest. The significance level was accepted with p=0.05.

3 Results and Discussion

3.1 Phytochemical analysis of plant extract

Qualitative Phytochemical analysis of *Merremia tridentate* plant extract was determined by various biochemical tests. The presence of several phytochemical compounds was presented in the Table 1. *Merremia tridentate* extracts showed the presence of Carbohydrate, Tannins, Saponins, Flavonoid, Alkaloid, Anthocyanin, Quinones, Glycosides, Cardiac glycosides, Terpinoids, Phenol, Coumarin, Protein and Reducing sugar.

Table 1. Phytochemical analysis of the *Merremia tridentate* plant extract

S.No	Phytochemical compound	Result
1	Carbohydrate	+
2	Tannins	+
3	Saponins	+
4	Flavonoid	+
5	Alkaloid	+
6	Anthocyanin	+
7	Betacyanin	-
8	Quinones	+
9	Glycosides	+
10	Cardiac glycosides	+
11	Terpinoids	+
12	Phenol	+
13	Coumarin	+
14	Protein	+
15	Reducing sugar	+
16	Steroids	-

3.2 Effect of ethanolic extracts of *Merremia tridentata* on serum enzymes in CCl_4 induced hepatotoxicity in albino wistar rats

Table 2. Effect of ethanolic extract on (50,100 and 200 mg/kg) and silymarin (100 mg/kg) (bio chemical parameters on serum enzymes) in CCl_4-induced hepatotoxicity in rats

Parameters	CCl_4 induced	CCl_4+Silymari (100 mg/kg)	Pre treatment		Post treatment	
			100mg/kg	200mg/kg	100mg/kg	200mg/kg
AST IU/L	145.81±19.28*	101.15±3.73*	123.02±9.8$^{*\#}$	106.34±8.5$^{*\#}$	113.96±32.96$^{*\#}$	104.09±3.4$^{*\#}$
ALT IU/L	133.50±1.08*	75.43±4.62*	85.09±1.18$^{*\#}$	80.7±3.05$^{*\#}$	76.98±11.98$^{*\#}$	68.91±4.33$^{*\#}$
ALP,KA unit	167.42±3.33*	101.41±2.68*	121.5±6.9$^{*\#}$	108.87±4.9$^{*\#}$	115.63±5.0$^{*\#}$	105.98±3.5$^{*\#}$
Bilirubin (mg/dl)	2.77±0.22*	1.05±0.06*	1.93±0.09$^{*\#}$	1.35±0.05$^{*\#}$	1.74±0.28$^{*\#}$	1.11±0.17$^{*\#}$

Values are denoted as mean ± standard deviation where n=6 rats in each group.* = significant increase at p<0.05 compared with control.*# = significant difference at p<0.05 compared withCCl_4 treated rats.

The effects of ethanolic extracts of *Merremia tridentata* on serum enzymes in CCl_4 induced hepatotoxicity in rats were determined for various doses of plant extracts (50mg/kg, 100mg/kg, 200mg/kg). The estimation of serum hepatic enzymes is an essential marker for evaluating the extent and type of liver damage. *Merremia tridentata* group

showed significant decreases in AST, ALT, ALP and bilirubin levels on compared to the CCl_4 sensitized group and CCl_4 + Silymarin treated group (Table 2). AST, ALT, ALP and bilirubin levels of CCl_4 induced group (Group III) were 145.81±19.28IU/L,133.50±1.08IU/L,167.42±3.33KA/unit, 2.77±0.22mg/dl. Pretreatment groups treated with 50mg/kg (Group V), 100mg/kg (Group VI) and 200mg/kg (Group VII) ethanolic extracts showed139.09±9.8IU/L, 123.02±9.8IU/L and 106.34±8.5IU/L of AST, 89.01±1.1IU/L, 85.09±1.18IU/L and 80.7±3.05IU/L of ALT, 128.71±4.5KA units,108.87±4.9KA units and 108.87±4.9KA units of ALP,2.51±0.07mg/dl, 1.93±0.09mg/dl and 104.09±3.4mg/dl of total bilirubin. Posttreatment groups treated with 50mg/kg (Group VIII), 100mg/kg (Group IX) and 200mg/kg (Group X) ethanolic extracts showed 126.9.8±34.66IU/L, 113.96±32.96IU/L and 107.09±1.0IU/L of AST, 84.78±14.0IU/L,76.98±11.98IU/L and 68.91±4.33IU/L of ALT, 123.89±2.3KA units,115.63±5.0KA and 105.98±3.5KA units of ALP, 2.21±0.03mg/dl, 1.74±0.28mg/dl and 1.11±0.17mg/dl of total bilirubin levels. 200 mg/kg ethanolic extracts treated group (Group VII and Group X) showed higher hepatoprotective activity than the other concentrations

3.3 Effect of ethanolic extracts of *Merremia tridentata* on liver enzymes in CCl_4 induced hepatotoxicity in albino wistar rats

Table 3. Effects of ethanolic extract on (50,100 and 200 mg/kg) and silymarin (100 mg/kg) (Biochemical parameters on liver enzymes) in CCl_4-induced hepatotoxicity in rats.

Parameters	CCl_4	CCl_4+Silymarin (100 mg/kg)	Pre treatment		Post treatment	
			100mg/kg	200mg/kg	100mg/kg	200mg/kg
AST IU/L	123.81±8.18*	100.15 ±6.70*	114.02±9.8*#	107.34±8.5*#	111.96±32.96*#	103.09±1.0*#
ALT IU/L	141.50±1.08*	69.73±1.62*	106.09±8.18*#	81.7±12.05*#	96.98±15.98*#	74.91±8.09*#
ALP,KA unit	158.8±11.64*	101.8±12.93*	129.5±6.9*#	116.87±8.9*#	119.63±15.0*#	107.98±9.5*#
Bilirubin mg/dl	2.42±0.14*	1.07±0.06*	1.46±0.09*#	1.25±0.07*#	1.51±0.8*#	1.12±0.06*#

Values are denoted as mean ± standard deviation where n=6 rats in each group.* = significant increase at p<0.05 compared with control.*# = significant difference at p<0.05 compared withCCl_4 treated rats.

The effects of ethanolic extracts of *Merremia tridentata* on liver enzymes in CCl_4 induced hepatotoxicity in rats was determined for various doses of plant extracts (50mg/kg, 100mg/kg, 200mg/kg). *Merremia tridentata* group showed significant decreases in AST, ALT, ALP and bilirubin levels on compared to the CCl_4 sensitized group and CCl_4 + Silymarin treated group (Table 3). AST, ALT, ALP and bilirubin levels of CCl_4 induced group (Group III) were 123.81±8.18IU/L, 141.50±1.08IU/L, 158.8±11.64KA/unit, 2.42±0.14mg/dl. Pretreatment groups treated with 50mg/kg (Group V), 100mg/kg (Group VI) and 200mg/kg (Group VII) ethanolic extracts showed 122.09±0.8IU/L, 114.02±9.8IU/L and 107.34±8.5IU/L of AST, 133.01±6.1IU/L, 106.09±8.18IU/L and 81.7±12.05IU/L of ALT, 145.6±10.5KA units, 129.5±6.9KA units and 116.87±8.9KA units of ALP,2.27±0.11mg/dl, 1.46±0.09mg/dl and 1.25±0.07mg/dl of total bilirubin levels. Post-treatment groups treated with 50mg/kg (Group VIII), 100mg/kg (Group IX) and 200mg/kg (Group X) ethanolic extracts showed 119.8±34.98IU/L,111.96±32.96IU/L and 103.09±1.0IU/L of AST, 125.78±9.0IU/L, 96.98±15.98IU/L and74.91±8.09 of ALT, 134.89±12.3KA units, 119.63±15.0KA units and107.98±9.5KA units of ALP. 2.26±0.16mg/dl, 1.51±0.8mg/dl and 1.12±0.06mg/dl of total bilirubin levels. 200 mg/kg ethanolic extracts treated group (Group VII and Group X) showed higher hepatoprotective activity than the other concentrations.

3.4 Effect of ethanolic extracts of *Merremiatridentata* on antioxidant enzymes in CCl_4 induced hepatotoxicity in rats

The effects of ethanolic extracts of *Merremia tridentata* on antioxidant enzymes in CCl_4 induced hepatotoxicity in rats was determined for various doses of plant extracts (50mg/kg, 100mg/kg, 200mg/kg). The estimation of antioxidant enzymes is a useful marker for determining extent and type of hepatocellular damage (Table 4). *Merremia tridentata* group showed significant decreases in Total protein, Gpx, GSH and LPO levels on compared to the CCl_4 sensitized group and CCl_4 + Silymarin treated group. CCl_4 induced group (Group III) showed 10.11±2.70mg/dl of total protein, 5.09±1.62moles of MDA liberate/min of GPx, 7.8±2.64μg/mg protein of GSH and3.2±0.68μmoles of MDA/min/mg protein of LPO. Pre-treatment groups treated with 50mg/kg (Group V), 100mg/kg (Group VI) and 200mg/kg (Group VII) ethanolic extracts showed 14.34±3.5,13.02±4.8 and 11.09±0.8 mg/dl of total protein,6.19±1.05,6.09±2.18 and 5.99±1.1moles of MDA liberate/min of GPx, 7.8±2.9, 6.5±1.7 and 6.37±1.5μg/mg protein of GSH,2.10±0.89, 2.0±0.62 and 1.88±0.31μmoles of MDA/min/mg protein of LPO.

Table 4. Effect of ethanolic extract on (50,100 and 200 mg/kg) and silymarin (100 mg/kg) on antioxidant enzymes in CCl_4-induced hepatotoxicity in rats

Parameters	CCl_4	CCl_4+Silymarin (100 mg/kg)	Pre treatment		Post treatment	
			100mg/kg	200mg/kg	100mg/kg	200mg /kg
Total protein (mg/dl)	13.66±1.28*	10.11±2.70*	13.02±4.8*#	11.09±5.8*#	13.96 ±2.96*#	10 .8±4.98*#
GPx (moles of MDA liberate / min)	7.4±1.08	5.09±1.62	6.09±2.18*#	5.99±1.1*#	5.9±1.18*#	5.15±1.1*#
GSH (μg/mg protein)	7.8±2.64	6.14±2.93	6.5±1.7*#	6.37±1.5*	6.09±2.65*#	6.21±4.3*#
LPO (μmoles of MDA/min/mg protein)	3.2±0.68	1.32±0.13	2.0±0.62*#	1.88±0.31*#	1.76±0.82*#	1.56±0.45*#

Values are denoted as mean ± standard deviation where n=6 rats in each group.* = significant increase at p<0.05 compared with control.*# = significant difference at p<0.05 compared withCCl_4 treated rats.

Post-treatment groups treated with 50mg/kg (Group VIII), 100mg/kg (Group IX) and (μmoles of MDA/min/mg protein 200mg/kg(Group X) ethanolic extracts showed 15.09±2.0, 13.96 ±2.96 and10.8±4.98mg/dl of total protein, 6.00±1.09, 5.9±1.18 and 5.15±1.1moles of MDA liberate/min of GPx,6.7±3.5,6.09±2.65 and 6.21±4.3μg/mg protein of GSH, 1.90±0.13, 1.76±0.82 and 1.56±0.45μmoles of MDA/min/mg protein of LPO. 200 mg/kg ethanolic extracts treated group (Group VII and Group X) showed higher hepatoprotective activity than the other concentrations.

3.5 Effect of aqueous extracts of *Merremia tridentata* on serum enzymes in CCl_4 induced hepatotoxicity in albino wistar rats

Table 5. Effects of aqueous extract on (50,100 and 200 mg/kg) and silymarin (100 mg/kg) (bio chemical parameters on serum enzymes) in CCl_4-induced hepatotoxicity in rats

Parameters	CCl_4	CCl_4+ Silymarin (100 mg/kg)	Pre treatment		Post treatment
			100mg/kg	200mg/kg	100mg/kg
200mg/kg					
AST IU/L 110.09±6.0*#	148.81±10.28*	98.11±6.70*	126.02±8.5*#	115.12±5.1*#	118.18±15.3*#
ALT IU/L 79.41±6.09*#	131.1±2.08*	77.73±2.62*	87.09±4.46*#	74.7±5.33*#	83.98±8.42*#
ALP,KA unit 107.35±7.21*#	160.68±5.64*	104.8±7.93*	127.26±8.47*#	115.66±8.14*#	119.29±2.15*#
Bilirubin mg/dl 1.53±0.22*#	2.72±0.15	1.01±0.08	2.08±0.6*#	1.56±0.07*#	1.97±0.16*#

Values are denoted as mean ± standard deviation where n=6 rats in each group.* = significant increase at p<0.05 compared with control.*# = significant difference at p<0.05 compared withCCl_4 treated rats.

The effects of ethanolic extracts of *Merremia tridentata* on serum enzymes in CCl_4 induced hepatotoxicity in rats was determined for various doses of plant extracts (50mg/kg, 100mg/kg, 200mg/kg). *Merremia tridentata* group showed significant decreases in AST, ALT, ALP and bilirubin levels on compared to the CCl_4 sensitized group and CCl_4 + Silymarin treated group (Table 5). AST, ALT, ALP and bilirubin levels of CCl_4 induced group (Group III) were 148.81±10.28IU/L, 131.1±2.08IU/L, 160.68±5.64KA/unit, 2.72±0.15mg/dl. Pre-treatment groups treated with 50mg/kg (Group V), 100mg/kg (Group VI)and 200mg/kg(Group VII) ethanolic extracts showed 134.09±0.8IU/L, 126.02±8.5U/L and 115.12±5.1IU/L of AST,93.01±7.32IU/L,87.09±4.46IU/L and 74.7±5.33IU/L of ALT, 134.6±2.22KA units, 127.26±8.47KA units and 115.66±8.14KA units of ALP,2.67±0.11mg/dl, 2.08±0.6mg/dl and 1.56±0.07mg/dl of total bilirubin levels. Post-treatment groups treated with 50mg/kg (Group VIII), 100mg/kg (Group IX) and 200mg/kg (Group X) ethanolic extracts showed 128.72±12.36IU/L,118.18±15.3IU/L and 110.09±6.0IU/L of AST, 90.78±9.68IU/L, 83.98±8.42IU/L and 79.41±6.09IU/L of ALT, 127.37±6.56KA units, 119.29±2.15KA units and 107.35±7.21KA units of ALP, 2.45±0.05mg/dl, 1.97±0.16mg/dl and 1.53±0.22mg/dl of total bilirubin levels. 200 mg/kg ethanolic extracts treated group (Group VII and Group X) showed higher hepatoprotective activity than the other concentrations.

3.6 Effect of aqueous extracts of *Merremia tridentata* on liver enzymes in CCl_4 induced hepatotoxicity in albino wistar rats

Table 6. Effects of aqueous extract on (50,100 and 200 mg/kg) and silymarin (100 mg/kg) (bio chemical parameters on liver enzymes) in CCl_4-induced hepatotoxicity in rats

Parameters	CCl_4	CCl_4+Silymarin (100 mg/kg)	Pre treatment		Post treatment	
			100mg/kg	200mg/kg	100mg/kg	200mg/kg
AST IU/L	125.52±10.57*	99.81±4.25*	120.15±5.32*#	112.66±4.32*#	113.72±32.41*#	108.36±4.3*#
ALT IU/L	149.3±3.66*	68.46±3.35*	115.45±8.66*#	105.34±1.63*#	110.98±10.53*#	88.91±14.22*#
ALP,KA unit	164.68±10.39*	104.35±9.42*	131.14±10.42*#	120.66±5.6*#	126.44±8.15*#	112.18±6.98*#
Bilirubin mg/dl	2.35±0.21*	1.09±0.04*	1.61 ±0.1*#	1.32±0.9*#	1.74±0.12*#	1.25±0.09*#

Values are denoted as mean ± standard deviation where n=6 rats in each group.* = significant increase at p<0.05 compared with control.*# = significant difference at p<0.05 compared withCCl_4 treated rats.

The effects of ethanolic extracts of *Merremia tridentata* on liver enzymes in CCl_4 induced hepatotoxicity in rats was determined for various doses of plant extracts (50mg/kg, 100mg/kg, 200mg/kg). *Merremia tridentata* group showed significant decreases in AST, ALT, ALP and bilirubin levels on compared to the CCl_4 sensitized group and CCl_4 + Silymarin treated group (Table 6). AST, ALT, ALP and bilirubin levels of CCl_4 induced group (Group III) were 125.52±10.57IU/L, 149.3±3.66IU/L, 164.68±10.39KA/unit, 2.35±0.21mg/dl. Pre-treatment groups treated with 50mg/kg (Group V), 100mg/kg (Group VI) and 200mg/kg (Group VII) ethanolic extracts showed 124.15±1.5IU/L,120.15±5.32IU/L and 112.66±4.32IU/L units of AST, 137.25±8.36IU/L, 115.45±8.66IU/L and 128.1±1.05 IU/L of ALT,144.0.6±1.5 KA units, 131.14±10.42KA units and 120.66±5.6KA units of ALP,2.27±0.14mg/dl,1.61 ±0.1mg/dl and 1.32±0.9mg/dl of total bilirubin levels. Post-treatment groups treated with 50mg/kg(Group VIII), 100mg/kg (Group IX) and 200mg/kg (Group X) ethanolic extracts showed 120.15±22.62IU/L, 113.72±32.41IU/L and 108.36±4.3IU/L units of AST, 131.44±12.23IU/L,110.98±10.53IU/L and 88.91±14.22IU/L units of ALT, 138.55±8.13KA units,126.44±8.15KA units and 112.18±6.98KA units of ALP. 2.14±0.12mg/dl,1.74±0.12mg/dl and 1.25±0.09mg/dl of total bilirubin levels. 200 mg/kg ethanolic extracts treated group (Group VII and Group X) showed higher hepatoprotective activity than the other concentrations.

3.7 Effect of ethanolic extracts of *Merremia tridentata* on antioxidant enzymes in CCl_4 induced hepatotoxicity in rats

Table 7. Effect of aqueous extract on (50,100 and 200 mg/kg) and silymarin (100 mg/kg) on antioxidant enzymes in CCl_4-induced hepatotoxicity in rats.

Parameters	CCl_4	CCl_4+Silymarin (100 mg/kg)	Pre treatment		Post treatment	
			100mg/kg	200mg/kg	100mg/kg	200mg/kg
Total protein(mg/dl)	14.6±2.75*	11.8±2.54*	13.1±3.33*#	12.9±3.35*#	13.6±4.81*#	12.1±3.4*#
GPx (moles of MDA liberate/min)	7.1±1.72	5.67±2.58	6.00±3.85*#	5.97±1.47*#	6.3±1.25*#	5.52±1.7*#
GSH(μg/mg protein)	8.2±1.64	6.8±2.66	7.0±1.61*#	6.9±1.22*	7.1±2.33*#	6.7±2.41*#
LPO(μmoles of MDA/min/mg protein)	3.4±0.22	1.09±0.4	2.08±0.89*#	1.99±0.33*#	2.10±0.28*#	1.77 ±0.59*#

Values are denoted as mean ± standard deviation where n=6 rats in each group.* = significantly increases at p<0.05 compared with control.*# = significant difference at p<0.05 compared withCCl_4 treated rats.

The effects of ethanolic extracts of *Merremia tridentata* on antioxidant enzymes in CCl_4 induced hepatotoxicity in rats was determined for various doses of plant extracts (50mg/kg, 100mg/kg, 200mg/kg). The estimation of antioxidant enzymes is a useful marker for determining extent and type of hepatocellular damage (Table 7). *Merremia tridentata* group showed significant decreases in Total protein, Gpx, GSH and LPO levels on compared to the CCl_4 sensitized group and CCl_4 + Silymarin

treated group. CCl_4 induced group (Group III) showed 14.6±2.75mg/dl total protein, 7.1±1.72moles of MDA liberate/min of GPx, 8.2±0.64 μg/mg protein of GSH and 3.4±0.22 μmoles of MDA/min/mg protein of LPO. Pretreatment groups treated with 50mg/kg (Group V), 100mg/kg (Group VI) and 200mg/kg (Group VII) ethanolic extracts showed 13.8±5.61,13.1±3.33 and 12.9±3.35mg/dl of total protein, 6.8±3.18, 6.00±3.85 and 5.97±1.47 moles of MDA liberate/min of GPx, 7.9±3.21, 7.0±1.61 and 6.9±1.22 μg/mg protein of GSH, 2.87±0.37, 2.08±0.89 and 1.99±0.33μmoles of MDA/min/mg protein of LPO. Post-treatment groups treated with 50mg/kg (Group VIII), 100mg/kg (Group IX) and 200mg/kg (Group X) ethanolic extracts showed 14.1±1.0, 13.6±4.81 and 12.1±3.4 mg/dl of total protein, 6.9±2.84,6.3±1.25 and 5.52±1.7 moles of MDA liberate/min of GPx,7.8±3.16, 7.1±2.33 and 6.7±2.41 μg/mg protein of GSH, 2.6±0.59, 2.1±0.28, 1.77±0.59 (μmoles of MDA/min/mg protein of LPO. 200 mg/kg ethanolic extracts treated group (Group VII and Group X) showed higher hepatoprotective activity than the other concentrations.

4 Discussion

Carbon tetrachloride (CCl_4) is the most widely used hepatotoxins in the researches on liver diseases.[8] Lack of scientific evidences on its safety and efficiency has raised issues regarding toxicity. Administration of ethanolic and aqueous extracts of whole plant of *Merremia tridentata* has demonstrated a substantial hepatoprotective efficacy, when compared with the standard commercial drug silymarin. The acute toxicity test conducted revealed that *Merremia tridentata* extracts is found to be non-toxic up to a dosage of 200mg/kg body weight. The toxicity test performed confirms that the extract is safe as observed from its high LD_{50} value.[9]

Various phytochemical compounds like saponins, flavonoids, phenol and alkaloids were identified from the phytochemical analysis of *Merremia tridentata* extracts. Particularly Flavonoids are a large group of polyphenolic compounds that play an important role in detoxification of free radicals and are found in fruits, vegetables and medicinal plants.[10] These phytochemical are used in therapeutic properties and also antimicrobial, antiviral, antifungi, antidiabetic, immunostimulatory, antidepressant, antitumour, lipemic, anti-inflammatory to antisickling.[11–13] Some of these secondary metabolites have been reported to be toxic in some experimental studies.[14] Reduction in body weight and internal organ weight are considered as sensitive indices of toxicity after exposure to toxic substances.[15,16]

The transaminases (alanine amino transferase and aspartate amino transferase), creatine kinase, LDH, and alkaline phosphatases are good indices of liver, heart, and kidney damage respectively.[16–18]Hence, the raised levels of these serum biochemical are indication of potential toxicity of the extract to the liver, heart, and the kidney. The chronic administration of the leaf extracts of *Merremia tridentata* (Linn) elicited responses in some serum electrolyte concentration.

The findings reported in Tables showed no significant changes in untreated and vehicle control groups (Group 1 &2). The hepatotoxicity induced by oral administration of CCl_4 resulted in significant ($p \leq 0.05$), dose dependent rise in the levels of liver and serum Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) and Bilirubin enzymes. Eventually, the average total protein content was found to be increased for all six groups. The oral administration of aqueous extract of *Merremia tridentata* (200mg/kg b.wt) had major effect in all parameters as compared to normal and sensitized groups.

Eventhough, oral administration of the *Merremia tridentata* along with CCl_4 for 30 days ($p \leq 0.05$) evidently increased the toxic effects of CCl_4 in liver and serum levels in a dose dependent manner. Various researches have discussed the significant increase in these enzyme activities by CCl_4. Liver injury is determined by the sensitive indicators like ALT, AST and ALP enzymes**.** These are of significant importance in assessment and monitoring of liver cytolysis, thus increased secretions in the serum may provide information about organ dysfunction. ALT activity is an marker enzyme to determine the degree of cell membrane damage, The reason that ALT has historically served as a major marker for liver damage is attributed to its abundant expression in liver and low levels present in other tissues in both rats and humans[19] where AST is an major parameter of mitochondrial damage.[10] ALP is used as important indicator for plasma membrane and endoplasmic reticulum, located predominantly in the microvilli of the bile canliculi. Hence, rise in levels of ALP activity indicates the pathological disturbances in biliary flow.[20] It is clearly known that CCl_4 activated in the liver, produce products which are metabolized by the mixed function oxidase. The toxicity of the reactive compounds and their metabolites may result due to the covalent interactions with the critical target molecules such as DNA, lipid, proteins or carbohydrates or from alteration of reduced or oxidized glutathione. Oral administration of CCl_4 along with Merremia tridentata extract resulted in significant healing in CCl_4 induced toxicity in the liver of rats. The decrease in the levels of ALT and AST by the extracts is an indication of stabilization of plasma membrane and repair of liver tissue damages caused by CCl_4. The treatment with *Merremia tridentata* extract for 30 days along CCl_4 significantly decreased ALP and ACP enzyme levels in the liver and serum. The post-treatment had better activity than the pre-treatment groups. The liver stabilization activity is due to the presence of bioactive compounds in the

Merremia tridentate extracts. This indicates that potentiality of *Merremia tridentata* extracts to stabilize biliary dysfunction and suggests a protective mechanism against rupture of lysosomes thereby restoring the cell integrity. [21–24] Oral administration of the *Merremia tridentata* extractsalso enhanced the protein synthesizing function of the liver and increased the total protein content.

5 Conclusion

The hepatoprotective activity of *Merremia tridentata* plant extract against CCl_4 induced liver damage in Wistar albino rats was studied. CCl_4 caused a rise in liver and serum AST, ALT, ALP, ACP and total Bilirubin content. Pretreatment and post treatment of rats with ethanolic and aqueous extracts (50,100 & 200 mg/kg b.wt) significantly reduced these serum parameters and antioxidant enzymes levels compared to CCl_4 induced rats. The post-treatment had better activity than the pre-treatment groups. Preliminary phytochemical analysis showed the presence of tannins, saponins, flavonoid, alkaloid, anthocyanin, quinones, glycosides, cardiac glycosides, terpinoids, phenol, coumarin and reducing sugar which may be responsible for the hepatoprotective activity. Thus, *Merremia tridenta* extracts ameliorated the liver damage caused by the CCl_4 and raised the liver and serum enzymes to normal levels.

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