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HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *CORDIA SEBESTENA* IN ANIMAL MODEL

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ABSTRACT

The present study was designed to evaluate the hepatoprotective activity *Cordia sebestina* against carbon tetrachloride induced hepatotoxicity. In the present study carbon tetrachloride induced elevated liver enzymes and declined the antioxidant principle like SOD, GSH and catalase. The extract of *Cordia sebestina* extract in two dose reverted the enzyme parameters in normal level and histopathology also shows well preserved hepatic cells. The present study concluded that the extract of *Cordia sebestina* shows good hepatoprotection via antioxidant mechanism.

Key words: Hepatotoxicity, Liver function test, *Cordia sebestina*, Antioxidants

INTRODUCTION

Liver is the main organ responsible for xenobiotics metabolism; hence, it is vulnerable to damage induced by different chemicals. Hepatic injury is a major clinical problem associated with different xenobiotics including drugs and industrial chemicals. Hence, many hepatoprotective agents are studied to protect liver from toxic insults.[1] recently; interest in the discovery of natural antioxidants has risen exponentially. Principal candidates in this discovery process are medical plants.[2] Hepatic cell injury caused by carbon tetrachloride (CCl_4)[3,4] and others, as well as chronic alcohol consumption,[5] is well-studied. CCl_4-induced hepatic injury is one of the most investigated animal models, and in the past decades, molecular mechanisms for hepatic necrosis and steatosis induced by CCl_4 were well-documented.[6] Herbal remedies on hepatic diseases have gained popularity in recent due to their safety.[7-9] Herbal extract is composed of various kinds of phytochemicals, so it would be difficult to identify major components having pharmaceutical effect.

Cordia sebestena (L.) (Boraginaceae Family) is commonly known as the Geiger tree. Hawaiians refer to the plant as Kou Haole though, which roughly translates to "foreign plant" (Abbott, 1992). Recent archaeological evidence indicates that the plant is actually indigenous to the islands (Burney et al., 2001).

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Regardless of its origin, the plant has a long history of use in Hawaiian culture. The plant's large dark green leaves have often been used to dye kappa, or wood cloth, that was used for both clothing and bedding. C. sebestena's dark orange flowers are typically used to make Leis. The plant is best known in the Hawaiian Island for its wood, which due to their lightweight, durable and easily workable nature, are used for many traditional items ranging from canoes to food vessels. The plant can grow up to 25 feet tall in tropical and sub-tropical areas where it is widely distributed due to its extensive use in landscaping. The hypoglycemic, antioxidant and hypolipidemic activity of *Cordia sebestena* has been reported.[10] The present study was designed to evaluate the heptoprotective activity of *Cordia sebestena* against carbon tetrachloride induced hepatic injury in rat model.

MATERIALS AND METHODS

Chemicals and drugs

Nitro blue tetrazolium (NBT), NADH, Sodium lauryl sulphate, Bradford reagent, Griess reagent, Potassium dihydrogen phosphate, Phenazinemethosulphate, Sodium pyrophosphate, 5, 5^1-dithio-bis-2-nitrobenzoic acid (DTNB), Trichloroacetic acid (TCA), Thiobarbituric acid (TBA), Sodium dihydrogen phosphate, Disodium hydrogen phosphate, 1,1-diphenyl-2-picryl hydrazyl (DPPH) were purchased from Sigma life sciences, Banglore. Sodium chloride (0.9%), ferric chloride, sodium nitroprusside, NaOH, $K_3Fe(CN)_6$ and other chemicals used were of analytical grade with high purity. Diazepam injection (Ranbaxy, India) was used as the standard drug.

Animals

Albino wistar rats of sex (150±10 g) were selected for the study. The animals were housed in clean

polypropylene cages under hygienic and standard environmental conditions at 22±2°C, 12:12 h light: dark cycle and 60±5 % RH with free access to standard laboratory food and water *ad libitum* (SaiDurga Feeds and Foods, Bangalore). Rats were habituated to laboratory conditions for one week before the test. All the experiments were carried out during the light period (08:00-16:00) and conducted in accordance with the guidelines given by the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi (India) and the Institutional Animal Ethics Committee (1220/a/08/CPCSEA) approved the experimental protocol.

Plant material and preparation of extract

The whole plant of *Cordia sebestena*was collected from the rock crevices in tirumala forests, Tirupati, A.P, India in the month of January 2013 and was authenticated by Dr. K. MadhavaChetty, Professor and Head, Department of Botony, S. V. University, Tirupati and voucher specimen number was lodged (ANCP-MP-COL 02/13) and preserved in the herbarium which was retained in our lab for future reference. Whole plant of *Cordia sebestena*was shade dried and coarsely powdered. The 400g of the powdered plant material was defatted with ethanol 70% (60-80°C) using a soxhlet extractor each for 72 hrs and the extracts obtained from the solvents were filtered and concentrated using rota evaporator. The yield of the extract was found to be 10.9% .

Preliminary phytochemical screening

The ethanolic extracts of *Cordia sebestena*was screened for the presence of carbohydrates, flavonoids, phytosterols, glycosids, tannins and phenolic compounds, the standard procedures of phytochemical tests andHPTLC

IN VITRO ANTIOXIDANT ACTIVITY

DPPH assay

The free radical scavenging activity of ethanolic extract of *cordiasebestena*was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. A 0.004% DPPH solution in methanol was prepared and 4 ml of this solution was added to 1 ml of sample extract solution in water at different concentrations (10-500 mg/ml). Left it for 30 minutes at room temperature for the reduction of the DPPH free radical and the absorbance was measured at 517 nm. The procedure was repeated for Vitamin C which was used as standard. The antioxidant activity of the extracts was expressed as IC_{50}, which is the inhibitory concentration required to scavenge 50% of DPPH free radicals (BuritsMand Bucar F, 2000; CuendetM et. al, 1997; Blois MS, 1958). The percentage inhibition was calculated from the following equation:

% Inhibition =A_{blank}- (A_{sample}/ A_{blank}) × 100

Acute oral toxicity study

The acute oral toxicity study was performed according to up and down procedure. The ethanolic exract of *cordiasebestena*upto a dose of 2000 mg/kg did not produce any signs of toxicity and morality (Lorke, 1983).

HEPATOPROTECTIVE ACTIVITY:

The animals were divided into five groups of six animals in each group.

Group I was given with vehicle (10 ml/kg).

Group II was given with CCL_4 50% v/v in olive oil at a dose of 0.1 ml/kg on the 13th day only starting from 1st day of treatment.

Group III was given with Silymarin (2 mg/100g) for 12 days starting from the first day of treatment, and on 13th day it was treated with CCl_4 50% v/v in olive oil at a dose of 0.1 ml/kg and it was treated as standard group

Group IV was given with Plant extract (200 mg/kg) for 12 days starting from the first day of treatment, and on 13th day it was treated with CCl_4 50% v/v in olive oil at a dose of 0.1 ml/kg.

Group V. was given with Plant extract (400mg/kg) for 12 days starting from the first day of treatment, and on 13th day it was treated with CCl_4 50% v/v in olive oil at a dose of 0.1 ml/kg.

On the 14th day all the rats from all the groups were sacrificed, blood was collected from each animal for serum analysis and their livers were stored under freezing conditions for the estimation of endogenous anti -oxidants and one sample from each group was stored in 10% formalin for histopathological studies.

Assessment of biochemical parameters- The blood samples were centrifuged (3000 rpm for 20 min) and the serum obtained was separated out and used for the estimation of SGOT, SGPT, ALP, TB, TP, and CHOLESTROL.

Assessment of anti-oxidant activity parameters- The isolated rats liver were rinsed with 0.9% ice-cold normal saline and processed to get 10% homogenate in cold phosphate buffer using glass Teflon homogenizer. The homogenates obtained were used for the estimation of thiobarbituric acids reactive substance(TBARS) (Ohkawa et al., 1979), reduced glutathione (GSH) (Ellmann, 1959), SOD (Kakkar et al., 1984), Catalase (Abei, 1974), protein (Bradford, 1976)

Statistical analysis

Results were expressed as mean±SD and analysed using Graph Pad Prism Version 5.1 using One Way Analysis Of Variance (ANOVA) followed by Dunnett'spost test. $P< 0.01$ was considered significant.

RESULTS & DISCUSSION

The priliminary phytochemical analysisrevealed the presence of carbohydrates, Phytosterol, glycoside, tannin and phenolic compounds and flavonoids. HPTLC revealed that the extracts especially ethanolic extract are rich in flavonoids Several concentrations ranging from 10 to 500 μg/ml of Ethanolic extract of *C. sebestena* were tested for their antioxidant activity in different invitro models. It was observed that the extracts scavenged the free radicals in a concentration-dependent manner in all the models. The IC_{50} values of Etanolic extract of *C. sebestena* and Ascorbic acid in DPPH scavenging activity were 30.5 and 80.5 μg/ml. Table no 2, figure no. 3

Table no.2 DPPH (2, 2-diphynal 1-picryl hydrazyl) Assay

S. NO	CONC	DPPH ACTIVITY	ASCARBIC ACID
1	10mg/ml	45.35	36.13
2	50 mg/ml	66.01	40.79
3	100 mg/ml	93.38	62.52
4	250 mg/ml	101.34	66.37
5	500 mg/ml	92.13	107.96
6	IC_{50}	30.5	80.5

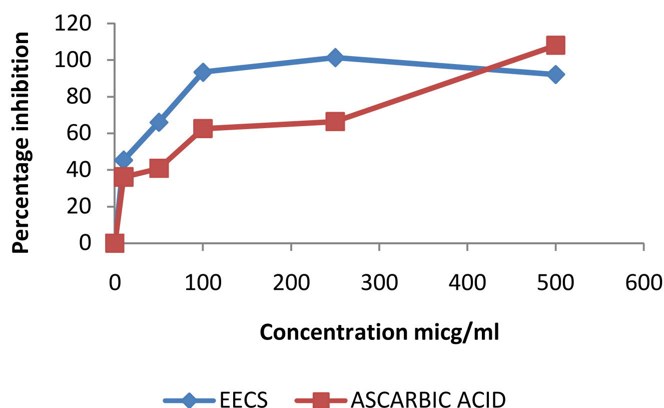


Figure3: DPPH inhibition activity of ethanolic extract of *C. sebestena*

In the present study the ethanolic extract of *C. sebestena* was subjected for the toxicity studies In the acute toxicity study, dose determination ethanolic extract was administered up to dose 4gm/kg body weight and extract did not any mortality. Thus $1/10^{th}$ (200mg) $1/20^{th}$ (400mg) of maximum dose tested were selected for the present study. LD50 of extract of *C. sebestena* were calculated and found to be 4000mg/kg . 30 Rats were divided in to 5 groups (n=6) and the duration of the experiment was 14 days. **G1** (Normal Control): Rats of this group received 0.5 ml of distilled water/100 g bw/rat/day for 14 days.**G2** (Toxic control): Rats of this group received 0.5 ml of distilled water/100 g bw/rat/day for 12 days and on day 13 received a single dose of CCl_4 injection intra peritoneally (0.1 ml/100 g bw, 50% v/v with olive oil). **G3:** Rats of this group received silymarin 2 mg/kg bw/rat/day for 12 days and on day 13 received a single dose of CCl_4 injection intra peritoneally**G4**: Rats of this group received *C. sebestena* 200 mg/kg bw/rat/day for 12 days and on day 13 received a single dose of CCl_4 injection intra peritoneally. **G5**: Rats of this group received *C. sebestena* 400 mg/kg bw/rat/day for 12 days and on day 13 received a single dose of CCl_4 injection intraperitoneally. All the rats of respective groups were treated under fasting condition. At the end of the treatment period, rats were deprived of food overnight and sacrificed on day 14 by light ether anaesthesia followed by decapitation after recording the final body weight. Blood was collected from each rat for biochemical estimation and liver was quickly isolated immersed in ice cold saline and weighed. Half of the liver was stored under freezer (-20 °C) for estimation of tissue antioxidant parameters and remaining part of the liver was preserved in buffered formalin (10%) for histopathological examination. SGPT, SGOT, ALP, total bilirubin and total protein levels were estimated in serum. The results were presented in Table no 3 and Graph no. 1,2. There is significant ($P<0.0001$) increase in the level of SGPT at group 2 when compared with Group 1. There is significant ($P<0.0001$) decrease in the level of SGPT at group 3, 4 and 5 when Compared with group 2. There is no significant increase in the level of SGOT at group 2 when compared with group 1. There is no significant increase in the level of SGOT at group 3 when compared to group 2. Group 4 showed no significant decrease in the level of SGOT compared to group 2. There is significant ($p<0.0001$) increase in the level of SGOT in group 5 compared to group 2.

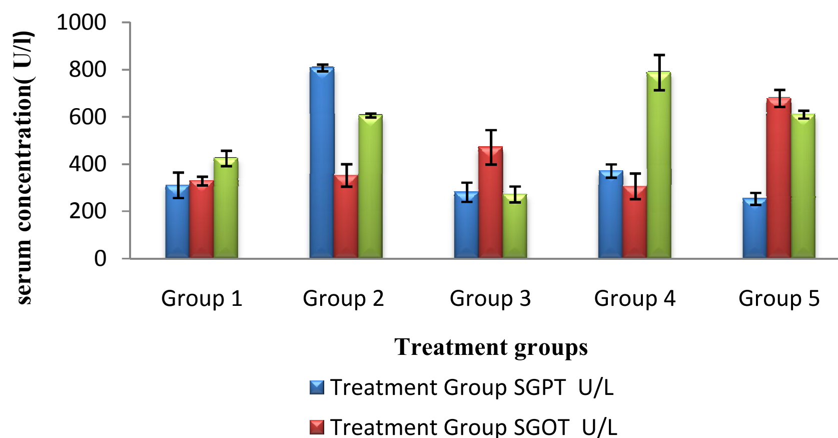
There is significance ($p<0.01$) increase in the level of ALP at group 2 when compared with group 1. There is significant ($p<0.0001$) decrease in the level of ALP at group 3 when compared to group 2. There is significance ($p<0.01$) increase in the level of ALP at group 4 when compared to group 2. There is no significance increase in the level of ALP at group 5 when compared to group 2. There is significance ($p<0.01$) increase in the level of TB at group 2 when compared with group 1. There is no significant decrease in the level of TB at group 3 when compared to group 2. There is significance ($p<0.0001$) increase in the level of TB at group 4, when compared to group 2. There is significance ($p<0.001$) increase in the level of TB at group 5 when compared to group 2. There is significance ($p<0.0001$) increase in the level of TP at group 2 when compared with group 1.

There is significant ($p<0.0001$) increase in the level of TP at group 3, 4 and 5 when compared with group 2. There is no significance increase in the level of cholesterol at group 2 when compared with group 1. There is significant ($p<0.0001$) increase in the level of cholesterol at group 3, 4 and 5 when compared with group 2.

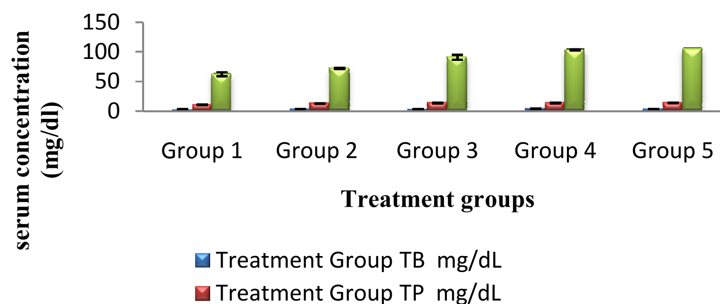
Table no: 3 Serum levels of SGOT, SGPT, ALP, TB, TP and Cholesterol

Treatment Group	Serum Parameters					
	SGPT U/L	SGOT U/L	ALP U/L	TB mg/dL	TP mg/dL	Cholesterol mg/dL
Group 1	309.9±53.7	328.1±18.7	423.8±32.8	2.3±0.6	10.3±0.3	61.9±3.4
Group 2	806.4±14.3[a]	351.2±47.4	605.3±7.9 [b]	2.7±0.1[b]	12.6±0.2[a]	71.7±0.9
Group 3	280.5±40.7 ***	470.4±73.1	271.4±33.5***	2.5±0.3	13.2±0.5***	90.6±4.2 ***
Group 4	370.3±28.1 ***	305.4±54.3	787.1±74.8*	3.7±0.1 ***	13.2±0.6***	103.1±0.8 ***
Group 5	252.4±25.4 ***	677.5±35.98***	609.0±16.6	2.7±0.4 **	13.6±0.2***	105±0 ***

All values expressed as mean±SEM. One Way Anova followed by Dennett's Multiple Comparison Test. a P<0.0001 vs Group 1; b – P<0.01 vs Group 1; *** - P<0.0001 vs Group 2; * - P<0.01 vs group 2;**-p<0.001vs group2
Group 1– normal control rats were treated with vehicle (distilled water 10 ml/kg bwp.o.)
Group 2– toxic control rats were treated with CCl_4 at a single dose 0.1 ml/100 g bwp.o.)
Group 3– rats were treated with Silymarin 2 mg/100 g body weight p.o
Group 4– rats were treated with *C. sebestena* 200 mg/kg body weight p.o.
Group 5– rats were treated with *C. sebestena* 400 mg/kg body weight p.o



Graph no. 1 Serum concentration of SGOT, SGPT, and ALP



Graph no 2 Serum Concentrations of TB, TP, and Cholesterol

Catalase, SOD, GSH, TBARS levels were estimated liver homogenization. The results were presented in Table 4 and Graph no. 3, 4, 5. There is significance ($p<0.0001$) decrease in the level of catalase at group 2 when compared to group 1. There is significance ($p<0.01$) increase in the level of catalase at group 3 when compared with group 2. There is significance ($P<0.0001$) increase level of catalase at group 4, 5 when compared with group 2. There is significance ($P<0.0001$) decrease in the level of GSH at group 2 when compared to group 1. There is significance ($P<0.001$) increase in the level of GSH at group 3 when compared to group 2. There is significance ($P<0.0001$) increase level of GSH at group 4, 5 when compared with group 2.There is significance ($P<0.01$) decrease in the level of SOD at group 2 when compared with group 1.there is no significance increase in the level of group 3 when compared with group 2. There is significance ($P<0.01$) increase in the level of SOD at group 4 when compared with group 2. There is significance ($P<0.0001$) increase in the level of SOD at group 5 when compared with group 2. There is significance ($P<0.0001$) increase in the level of TBARS at group 2 when compared with group1. There is significance ($P<0.01$) decrease in the level of TBARS at group 3, 4, and5 when compared with group 2.

Table no: 4 Tissue levels of Catalase, GSH, SOD, and TBARS

Treatment Group	TISSUE PARAMETERS			
	CATALAS EIU/dl	GSH µg/g	SOD IU/dl	TBARS nmol/gm
Group 1	47.7±7.53	360.7±14.0	2.5±0.06	10.8±0.7
Group 2	4.41±1.0[a]	168.9±8.8[a]	0.2±0.05[b]	25.2±1.2[a]
Group 3	22.1±3.0*	347.2±46.8**	0.5±0.1	15.2±0.7*
Group 4	45.3±2.3***	386.5±23.8***	2.3±0.6*	15.0±1.1*
Group 5	46.5±4.2***	411.4±39.2***	4.0±0.9***	15.6±1.4*

All values expressed as mean±SEM. One Way Anova followed by Dennett's Multiple Comparison Test. a $P<0.0001$ vs Group 1; b – $P<0.01$ vs Group 1; *** - $P<0.0001$ vs Group 2; * - $P<0.01$ vs group 2;**-$p<0.001$vs group2

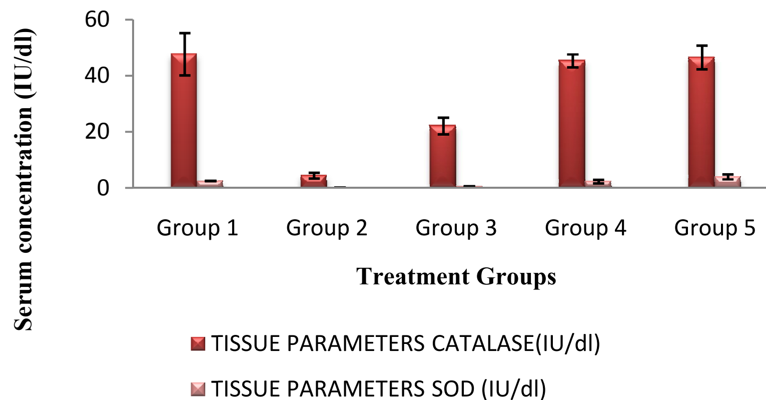
Group 1– normal control rats were treated with vehicle (distilled water 10 ml/kg bwp.o.)

Group 2– toxic control rats were treated with CCl_4 at a single dose 0.1 ml/100 g bwp.o.)

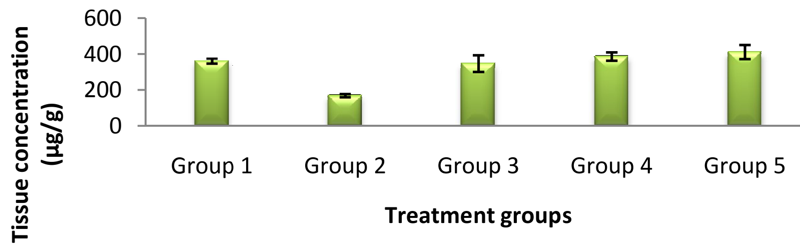
Group 3– rats were treated with silymarin 2 mg/100 g body weight p.o

Group 4– rats were treated with *C. sebestena* 200 mg/kg body weight p.o.

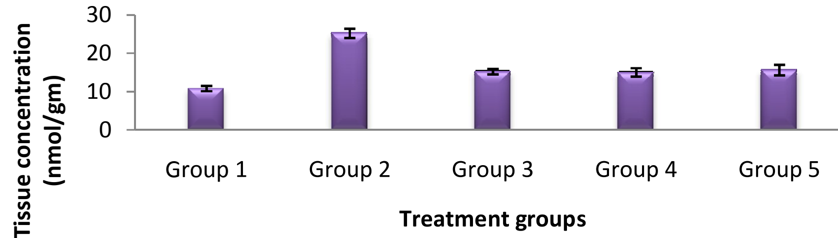
Group 5– rats were treated with *C. sebestena* 400 mg/kg body weight p.o



Graph no.3 Tissue level of Catalase, SOD,



Graph no. 4 Tissue level of GSH



Graph no. 5 Tissue level of TBARS

Effect of *C. sebestena*on histopathology of liver

Light microscopical structure of liver from control rat showing normal architectures of hepatocytes with central vein

Light microscopical structure of liver from CCl_4 treated rat showing extensive of neurotic damage of hepatocytes complete damage of central vein with inflammation and haemorrhage

Light microscopically structure of liver from Silymarin and plant extracts treated rats showing with mild neurotic damage and with minimal inflammation at control vein

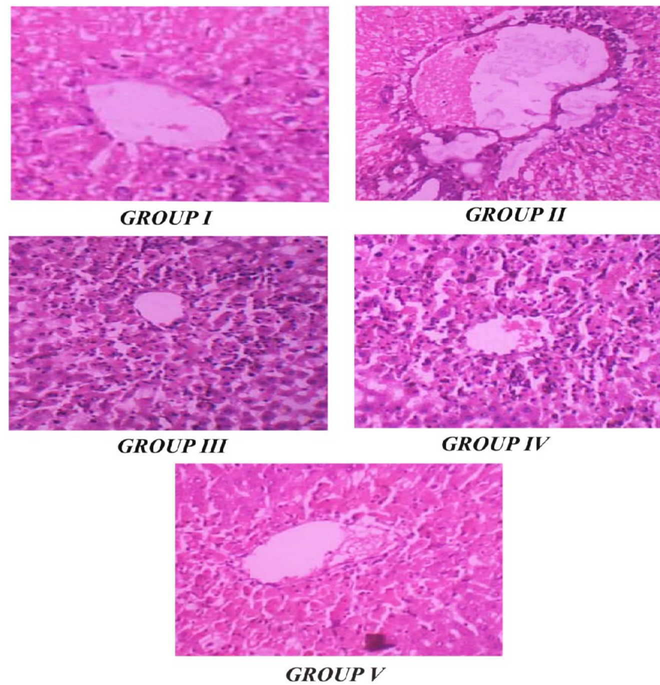


Figure No.3 Histopathology of liver

Group 1– normal control rats were treated with vehicle (distilled water 10 ml/kg bwp.o.)
Group 2– toxic control rats were treated with CCl_4 at a single dose 0.1 ml/100 g bwp.o.)
Group 3– rats were treated with silymarin 2 mg/100 g body weight p.o
Group 4– rats were treated with *C. sebestena* 200 mg/kg body weight p.o.
Group 5– rats were treated with *C. sebestena* 400 mg/kg body weight p.o

CONCLUSION:

The present study concluded that *Cordia sebestina* shows potent hepatoprotective activity through antioxidant mechanism due to the presence of active principle flavonoids.

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