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ORIGINAL ARTICLE

Hepatoprotective activity of stems of *Cuscuta reflexa* Roxb. against carbon tetrachloride induced toxicity: A Biochemical and Histopathological Study

Trushal V. Chorage[1]*, Ravindra Y. Patil[2]

1. PDEA's Seth Govind Raghunath Sable College of Pharmacy, Saswad, Pune – 412 301 (India)
2. PDEA's Shankarrao Ursal College of Pharmaceutical Sciences and Research Centre, Kharadi, Pune – 411 014 (India)

Corresponding Address's E-mail: trushalchorage83@gmail.com

ABSTRACT

Hepatotoxicity in one of the major parameters need to be consider in drug therapy, because most of the drugs in single at low/high doses for a prolonged period or multiple drug therapy causes liver damage. The present study was aimed to investigates the possible hepatoprotective activity of Cucuta reflexa Roxb. against carbon tetrachloride (CCl_4) induced hepatotoxicity in albino Wistar rats. Methanolic extract of Cuscuta reflexa Roxb. (CRM) stems was used in the study for determination of hepatoprotective activity against carbon tetrachloride (CCl_4) induced hepatotoxicity in albino Wistar rats. A comparison was also made between the actions of C. reflexa Roxb. stem extracts and a known hepatoprotective drug silymarin. Five groups of rats (n=6) where Group I (normal control), Group II (CCl4 control, 1 ml/kg BW p.o), Group III (Standard treated with silymarin 100 mg/kg BW p.o), Group IV and V served as methanolic extract of Cuscuta reflexa Roxb. (200 mg/kg and 400 mg/kg BW p.o respectively) for 9 days followed by induction of hepatotoxicity using carbon tetrachloride on 7th and 8th day. Blood samples and livers were collected, biochemical parameters like SGOT, SGPT, total bilirubin and total albumin along with histopathology study were carried out to evaluate the hepatoprotective activity. Showed CRM extract found to have significant hepatoprotective activity against carbon tetrachloride, as indicated by reduction in the elevated levels of SGOT, SGPT, Total bilirubin and Total albumin. Histopathology showed regeneration of hepatocytes to normal with the stem extract further confirmed the hepatoprotective activity of extract of C. reflexa Roxb. These findings suggest that more research on the potential benefits of Cuscuta reflexa Roxb. stem extracts for hepatotoxicity is worthwhile.

Keywords: Cucuta reflexa, silymarin, CCl_4, SGOT, SGPT

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INTRODUCTION

Liver is the primary visceral organ in human body, responsible for the synthesis, excretion, metabolism, and detoxification of several endogenous and foreign chemicals, including medications [1]. The primary symptom of liver diseases, which are a major global health concern, is hepatocyte death. Diseases that impair liver function, chemicals (ethanol, CCl4, thioacetamide, D-galactosamine, environmental pollutants), and drugs (like paracetamol) are a few examples of causative agents [2]. Increased biochemical parameter levels such as SGOT/AST, SGPT/ALT, TA, and TB are always associated with hepatocyte necrosis in cases of liver injury or failure [3, 4].

Globally, there is a rise in the amount of drugs that cause liver damage when inhaled or consumed. Conventional medications used to treat drug-induced liver damage are often inadequate and have significant side effects. Over 50% of acute liver damage is said to be brought on by medications, according to the US Acute Liver Failure Study Group [5]. Approximately 13% of hospital admissions are related to drug-induced liver damage which is caused by a paracetamol overdose. [6].

Despite enormous advancements in modern medicine, there are very few trustworthy treatments which can prevent damage to the liver and/or facilitate hepatic cell regeneration [7].

The use of some plants and the consumption of a variety of fruits and vegetables are significant aspects of human health care. Approximately 80% of people worldwide receive their medical treatment from traditional medicine, which is mostly based on botanical elements [8]. It has been demonstrated that about 160 phytoconstituents from 101 plant species demonstrate hepatoprotective properties [9]. Few efficient natural medications are available in the market that can be used to improve liver function, prevent liver damage, may promote the liver cell's ability to regenerate, despite advancements in modern medicine [10]. Thus, there is ongoing interest in finding safe and effective treatments for liver diseases.

Cuscuta reflexa Roxb. is an annual stem parasite that twines over other plants with leafless, thread-like orange, red, or yellow stems. Common throughout India, plentiful on the plains of Bengal, Ceylon, and growing as high as 2800 meters in several regions of Himachal Pradesh. It is known as akaswel or amarbel because it only develops as a parasitic thread on other plants and lacks a root system [11].

The glabrous, pale greenish yellow stems are branching, very long, thick, densely twinning, and occasionally spotted with crimson. Bracts are 1.5 mm long, oval-oblong, and fleshy; flowers are solitary, with short racems. Pedicels are usually bent, short, and glabrous. Broadly oval, slightly uneven, 3 mm lobes, calyx divided almost to the base. White corolla with a tube measuring 6-8 by 4 mm and almost cylindrical lobes measuring 2.5-3 mm long, big, and acutely deltoid. The corolla tube's neck contains stamens. Ovarian oval, style short, thick, and simple; two separate, ovoid, long stigmas. glabrous, curcumscissile capsules, 6–8 mm in diameter, toward the base.

The current work aims to explore the hepatoprotective properties of *C. reflexa* Roxb. stem methanol extract against hepatotoxicity induced by carbon tetrachloride in rats for the purpose of validating its ethno medicinal uses.

MATERIAL AND METHODS

Plant Collection and extract preparation

The stems of *Cuscuta reflexa* Roxb., family Convolvulaceae, were collected from western ghat, Satara, Maharashtra. The Joint Director, Botanical Survey of India, Western Regional Centre, Pune, India, verified the authenticity of the plant sample (No.: BSI/WRC/IDEN.CER./2020/H3/90) as well as the voucher specimen being kept in the department library. (TVC-01) for further references. Coarsely powdered stems that had been shade dried were first defatted for four hours at 60–80°C using petroleum ether and then they were extracted for ten to twelve hours using 95% methanol in a soxhlet apparatus. Extract was concentrated to dryness in to rotary vacuum at a lower pressure after being filtered with Whatman filter paper (No. 1). After solidification, the extract was labeled and percentage yield of methanolic extract of *Cuscuta reflexa* Roxb. (CRM) (34 % w/v) was reported. The extracts were stored for later use in a refrigerator. The presence of alkaloids, glycosides, flavonoids, tannins, phytosterols, carbohydrates, carotenoids, fixed oils, and saponins in the extracts was determined by preliminary phytochemical analysis. [12, 13].

Drugs and chemicals

The following substances were used: We procured silymarin (a standard drug), carbon tetrachloride, methanol, formalin, biochemical parameters examination kits from Thermosil Fine Chem Industries (Pune, India). The study utilized analytical-grade chemicals and solvents for all other purposes.

Animal Preparation

Animals were used in this investigation, consisting adult wistar rats of both sex (180–220 g) were purchased from Global Bioresearch Solution Pvt. Ltd., Pune. (Maharashtra, India). They were kept in typical lab cages in 12-hour light/dark cycle room that had consistent temp. (22 ± 1°C) and a moderate humidity (50 ± 5%). Throughout the trial, food and water were available to all animals without restriction. Following a week of acclimation, the animals were divided up into five different groups, each with six members: Groups I, II, III, IV, and V. Every experiment was run from 9:00 to 16:00 hours during the light period. CPCSEA committee of "JSPM's Rajarshi Shahu College of Pharmacy and Research, Pune, India–411033" was approved the experiment protocol. The Institutional Animal Ethics Committee's regulations were properly followed during conducting experiments (Ethics approval: IAEC/2023/04).

Hepatoprotective activity

1. Carbon tetrachloride induced hepatotoxicity in rats.

Five groups, each with six adult wistar rats of each sex, were created. Group I was the vehicle control group, Group II was the CCl4 control group, which was given carbon tetrachloride treatment (1 ml/kg BW p.o.), Group III was standard treated group, which was served as silymarin (100 mg/kg BW p.o.), Group IV and Group V were served CRM (200 mg/kg and 400 mg/kg BW p.o resp.). Except for Group I, every group

fasted for twenty-four hours before receiving carbon tetrachloride treatment. The drug or vehicle was taken orally for nine days straight, with the seventh and eighth days seeing the simultaneous administration of carbon tetrachloride. Throughout the study period, The rats had unrestricted access to water and a regular diet. Under light ether anesthesia, blood was drawn via cardiac puncture on the tenth day, and the serum was separated for analysis of biochemical indicators. The animal livers have been removed and stored in 10% formalin solution for histological examinations [14].

Collection of blood

Under light ether anesthesia, blood was drawn by cardiac puncture to assess the biochemical indicators. After that, the serum was separated using centrifugation for 15 minutes at 3000 rpm.

Biochemical analysis

The UV Kinetic technique was utilized to determine the levels of SGPT and SGOT in the serum [15,16]. A process of SGOT/AST estimation involves the catalytic transfer of an amino group from L-aspartate to alpha-ketoglutarate, which results in the production of L-glutamate and oxaloacetate. The indicator reaction, performed by malate dehydrogenase (MDH), reduces the oxaloacetate while concurrently oxidizing NADH to NAD. The rate at which absorbance at 340 nm decreases as a result is directly correlated with AST activity. In order to estimate SGPT/ALT, ALT in the sample must catalyze the transfer of an amino group from L-alanine to α-ketoglutarate, which results in the creation of pyruvate and L-glutamate. L-lactate is formed when pyruvate is degraded in the presence of NADH and lactate dehydrogenase (LDH). NADH is oxidized to NAD+ in this process. The rate at which the absorbance at 240 nm decreases as a result of NADH being converted to NAD+ is used to track the process. Total bilirubin (TB) was estimated by measuring the color of the azo molecule, which is produced when bilirubin reacts with diazotized sulphanic acid at a wavelength of 546 nm. Total albumin (TA) is measured by measuring the change in the yellow BCG dye's absorbance caused by its binding to bromocresol green (BCG) at pH 4.2. The blue-green colour that results from photometric measurements between 580 and 630 nm, with maximal absorbance at 625 nm, is directly related to the amount of albumin present.

Histological investigation (For Liver)

Hematoxylin and eosin (H&E) staining and wax embedding were done after the rats' livers had been removed, washed, and kept in a 10% neutral formalin buffered solution for microscopic histological examination [17]. Under a microscope, the stained tissue slices were randomly examined without taking the animal or group into account.

Statistical Analysis

The means ± standard error of the mean will be used to present the data (SEM) (n = 6). A one-way ANOVA will be used to assess any significant differences in group means, and then the Dunnett's t-test will be performed for multiple comparisons test. When *p < 0.05, the data will be defined as the statistical significance. With Graph Pad Prism 10, the statistical analysis and figures will be generated.

RESULTS AND DISCUSSION:

The purpose of this study was to assess phytochemical composition of *Cuscuta reflexa* Roxb. and its capacity to protect rats from the toxicity and damage to the liver caused by CCl4. An effective and well-known toxin, carbon tetrachloride(CCl4) caused liver damage by causing reactive oxidative stress. The dangerous reactive trichloromethyl peroxyl (CCl*3) radical is created during the bioactivation of the cytochrome P-450 system, which is the cause of CCl4 toxicity. Additionally, this radical damages membrane lipids, setting off a series of events that result in membrane lipid peroxidation, hepatocellular damage, and malignancy [18,19,20].

According to the current investigation, rats that were intoxicated with CCl4 had higher levels of AST, ALT, TA, and TB. Liver damage also analyzed with the help of histopathological observation.

Cuscuta reflexa Roxb. has also undergone phytochemical evaluation, confirming the presence of tannins, flavonoids, phytosterols, triterpenoids, glycosides, carbohydrates, fixed oils as well.

Previous studies have demonstrated the presence of a variety of bioactive substances, including tannins, lutein, lycopene, kaempferol-3-O-glucoside, stigmasterol, quercetin, caffeic acid, mannitol, flavonol, dulcitol, kaempferol, cuscutamine, and quinic acid. Cuscutalin, isorhamnetin-3-O-neohesperidoside, amarbelin, leuteolin, quercetin-3-Oglucoside, alphaamyrin, coccinoside, alphaamyrin and apigenin-7-O-glucoside etc. among other polyphenols [20,21,22,23]. These compounds are said to have many health benefits and strong antioxidant activity [24, 25, 26]. The *C. reflexa* Roxb. stem extract showed a significant levels of flavonoids and phenolic. Plants that contain phenolic components like tannins, phenolic acids, and flavonoids are the main source of their antioxidant behavior. Research has demonstrated that these compounds derived from plants exhibit number of therapeutic properties viz.

anti-inflammatory, anti-atherosclerotic and anti-tumor characteristics etc. The existence of these secondary metabolites indicates that Cuscuta reflexa Roxb. possesses strong antioxidant properties and may have had a role in hepatoprotective actions by preventing oxidative stress.

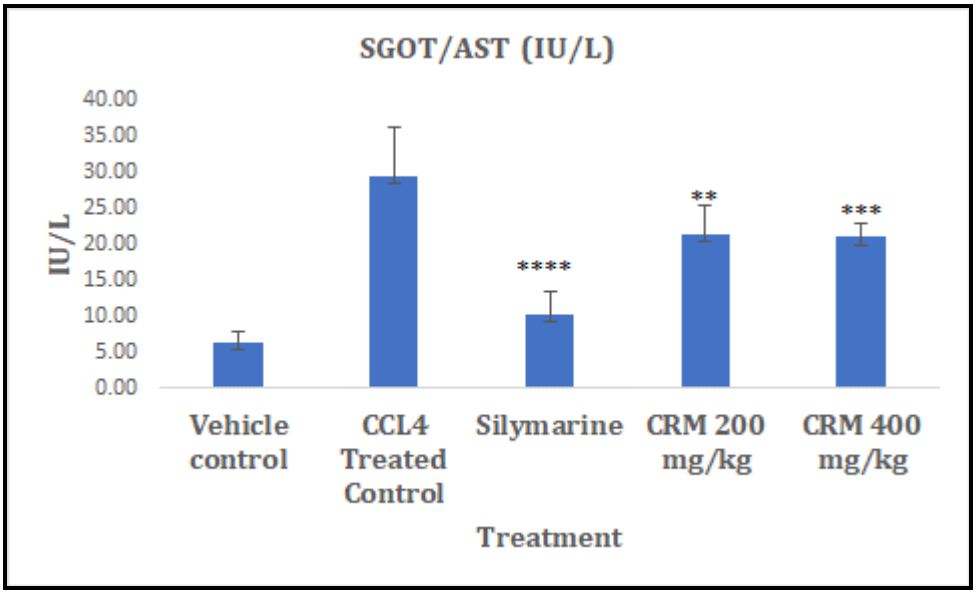
When determining liver damage, two important biochemical markers are measured: SGOT/AST and SGPT/ALT. In normal control rats, the serum concentrations of SGOT, SGPT, TA, and TB are within the normal range. As compared to normal rats, CCl4-induced rats serum enzyme levels increased significantly, indicating liver injury [27]. The methanolic extract of *Cuscuta reflexa* Roxb. was shown to be harmless up to a dose of 4 g/kg BW and didn't result in any type of alterations in the tested animals behavior or mortality. The hepatoprotective effect of methanolic extract of stems of *Cuscuta reflexa* Roxb appears in Table 1., wherein the extract, administered at two different doses of 200 mg/kg BW and 400 mg/kg BW, significantly lowered high levels of SGOT, SGPT, TA, and TB. This revealed that the methanolic extract of stems of *Cuscuta reflexa* Roxb potentially restore the damage caused by CCl4.

Biochemical study requires the support of histopathological examinations [28]. Severe hepatotoxicity has been shown by rats given CCl4 alone, as evidenced by hepatocyte destruction, lipid alterations, and the development of centrilobular necrosis. The control rats that were given normal saline revealed clear central veins, a prominent nucleus, and normal cytoplasm. Animal liver slices that had been previously treated with methanolic extract of stems of *Cuscuta reflexa* Roxb. reveals hepatoprotective activity. A small number of hepatocytes showed almost little damage. Fig. 2 displayed representative photos of the histopathological alterations that showed how the test material affected animals given CCl4.

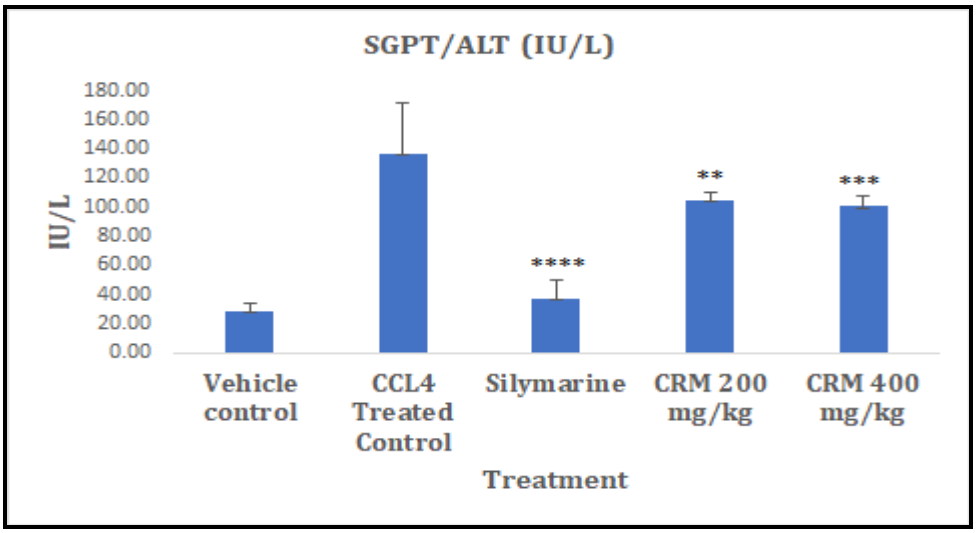
Table 1: Effect of CRM extract on different biochemical parameters.

Group	Treatment	SGOT/AST (IU/L)	SGPT/ALT (IU/L)	T B (mg/dL)	T A (g/dL)
I	Vehicle control	6.32 ± 1.52	28.97 ± 4.56	1.20 ± 0.14	3.02 ± 0.44
II	CCl_4 Treated Control (1 ml/kg)	29.44 ± 6.85	137.02 ± 34.06	6.26 ± 1.46	8.99 ± 2.09
III	Silymarine (100mg/kg)	10.22**** ± 3.31	37.55*** ± 12.36	1.65**** ± 0.59	3.38**** ± 0.32
IV	CRM 200 mg/kg	21.38** ± 3.84	104.39** ± 5.52	4.80** ± 0.68	6.93*** ± 0.42
V	CRM 400 mg/kg	20.91*** ± 2.02	100.39*** ± 7.75	3.01*** ± 0.38	6.77*** ± 0.30

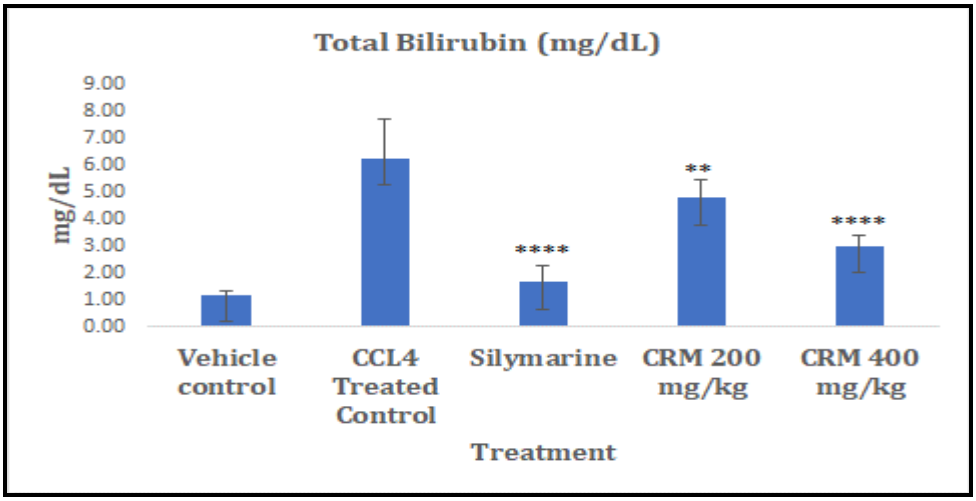
n=6, Values are expressed as Mean±S.E.M.; *= p < 0.05, ** = p < 0.01, *** = p < 0.001, **** = p < 0.0001 when compared to control group. Statistically analyzed by One Way ANOVA followed by Dunnett test.



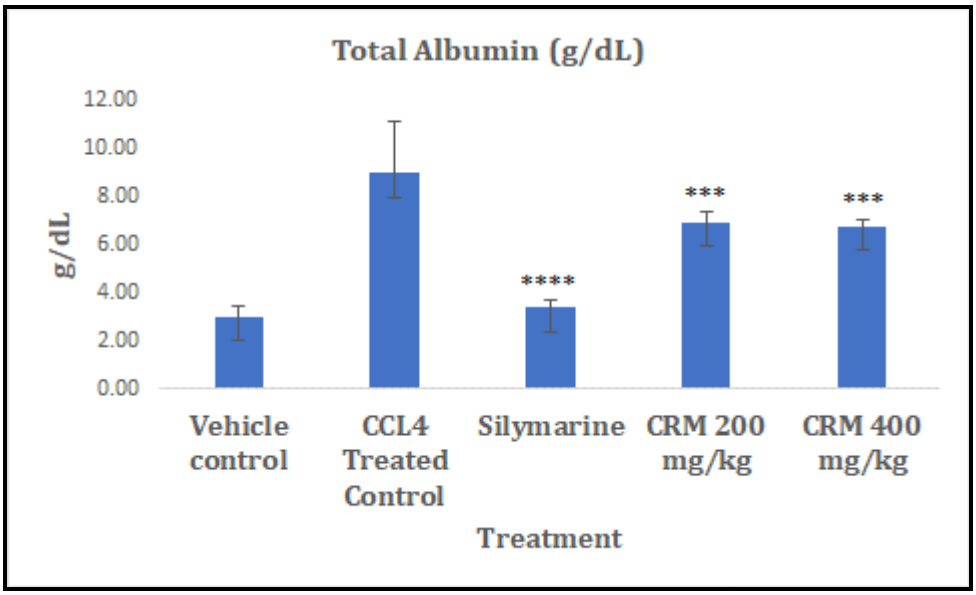
A: SGOT



B: SGPT

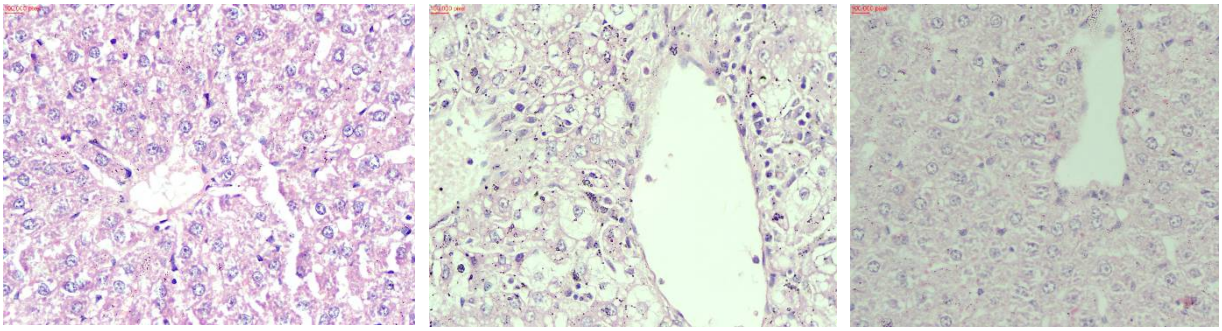


C: Total Bilirubin (TB)

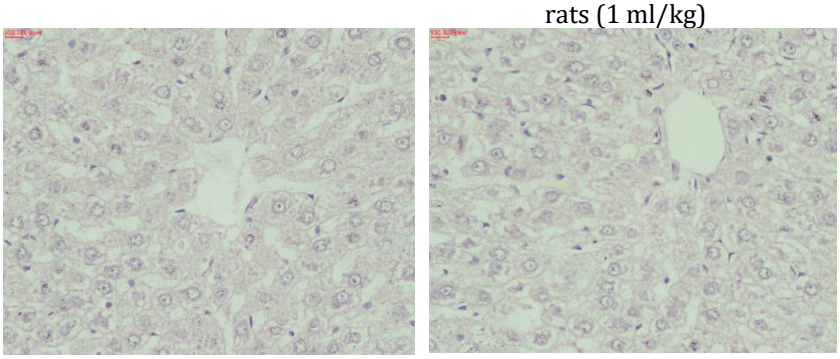


D: Total Albumin (TA)

Fig. 1. Effect of CRM on different biochemical parameters (A: SGOT, B: SGPT, C: TB and D: TA)



Normal control | Carbon tetrachloride intoxicated rats (1 ml/kg) | CCl4 + Silymarin (100 mg/kg)



CRM (200 mg/kg) | CRM (400 mg/kg)

Fig. 2. Representative images of histopathological studies showing effect of the test material on the rats intoxicated with carbon tetrachloride.

CONCLUSION

When methanolic extract of stems of *Cuscuta reflexa* Roxb. were administered, the adverse effects of CCl4 were reversed. The hepatoprotective action of drugs was further established by histopathological studies of liver sections, which showed that hepatotoxin toxicity altered the normal liver structure. The normal cellular structure was preserved in liver sections of the rats served with methanolic extract (200 and 400 mg/kg) in comparison with silymarin confirming protective action of the *Cuscuta reflexa* Roxb extracts. Flavonoids, which have hepatoprotective properties, may be the root cause of *Cuscuta reflexa* Roxb.'s hepatoprotective action. These findings suggest that more research on the potential benefits of *Cuscuta reflexa* Roxb. stem extracts for hepatotoxicity is worthwhile.

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CONFLICT OF INTEREST

No conflicts of interest are disclosed by the authors.

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