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Research Article

Hepatoprotective Activity of Whole Part of the Plant *Cuscuta reflexa* Roxb. (Convolvulaceae) in Chloroform, Ethanol and Paracetamol Induced Hepatotoxic Rat Models

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ABSTRACT
Cuscuta reflexa Roxb. is a rootless, leafless perennial parasitic twining herb belonging to the family Convolvulaceae and is commonly known as Akashvalli or Dodder. The *Cuscuta reflexa* is reported to possess antitumor, antimicrobial, hepatoprotective, antioxidant anticonvulsant, and induced alopecia activities. The research work is focused on phytochemical screening of whole part of the plant *Cuscuta reflexa* after each successive extraction with petroleum ether, chloroform, methanol and water respectively followed by its hepatoprotective activity study. *In vivo* hepatoprotective activity of the *Cuscuta reflexa* whole plant extract was carried out using carbon tetrachloride, ethanol and paracetamol induced hepatotoxic rat models and compared with silymarin (20 mg/kg) as reference standard. Preliminary phytochemical screening revealed that the aqueous extracts of *Cuscuta reflexa* whole plant contains carbohydrates, reducing sugars, phenolic compounds, tannins, flavonoids and saponins. Two way analysis of variance study of the estimated biochemical parameters for instance, aspartate aminotransferase, alanine amino transferase and alkaline phosphatase were concluded that there is significant difference (p-value < 0.001) exists between the different treatment groups. Furthermore, least significant difference test of various biochemical parameters have indicated the highest dose of aqueous extract (200 mg/kg) was having comparable hepatoprotective activity to that of standard silymarin, which was also evident from the histopathological study of liver sections. However, activity of aqueous extracts of *Cuscuta reflexa* whole plant (100 mg/kg and 200 mg/kg) were statistically not comparable (p-value < 0.05) with their respective SL treated standard groups.

Keywords: Alanine amino transferase, alkaline phosphatase, aspartate aminotransferase, *Cuscuta reflexa*, least significant difference, phytochemical screening, silymarin

INTRODUCTION

Hepatotoxicity may result from direct toxicity of the primary compound and/or from a reactive metabolite or from an immunologically-mediated response affecting hepatocytes, biliary epithelial cells and/or liver vasculature.[1] Hepatotoxicants are exogenous compounds of clinical relevance and may include overdoses of certain medicinal drugs, industrial chemicals and natural chemicals like microcystins, herbal remedies and dietary supplements.[2] Certain drugs may cause liver injury when introduced even within the therapeutic ranges. The hepatotoxic response elicited by a chemical agent depends on the concentration of the toxicant which may be either parent compound or toxic metabolite, differential expression of enzymes and concentration gradient of cofactors in blood across the acinus.[3] Hepatotoxic response is expressed in the form of characteristic patterns of cytolethality in specific zones of the acinus. Hepatotoxicity related symptoms may include a jaundice or icterus appearance causing yellowing of the skin, eyes and mucous membranes due to high level of bilirubin in the extracellular fluid, pruritus, severe abdominal pain, nausea or vomiting, weakness, severe fatigue, continuous bleeding, skin rashes, generalized itching, swelling of the feet and/or legs, abnormal and rapid weight gain in a short period of time, dark urine and light colored stool.[4] *Cuscuta reflexa* Roxb. is a rootless, leafless perennial parasitic twining herb of Convolvulaceae family, commonly known as Akashvalli or Dodder. The plant is distributed worldwide and in India about 6 species are found. It has no chlorophyll and cannot make its own food by photosynthesis. It grows on thorny or other shrubs, sometimes completely covering the bushes and trees.[5] It spread from one host to another, and on each victim, they twine and cling tightly with special branching organs called haustorium. Haustorium penetrate the host and connect to the host xylem as well as to the host phloem and absorb from it both water and elaborated food stuffs such

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Table 1- Experimental design for hepatoprotective activity of CR

Group code	Group name	Treatment
A	Normal	Normal saline (2 mg/kg, i.p) once daily
B1	Control CT	CT (2 ml/kg, i.p.) once daily for 3 days
B2	Control ET	ET (2 ml/kg, i.p. once daily for 3 days
B3	Control PT	PT (200 mg/kg, i.p.) once daily for 3 days
C1	Standard SL-CT	SL (20 mg/kg, orally) twice daily for 7 days followed by CT (2 ml/kg, i.p.) on the 8th day
C2	Standard SL-ET	SL (20 mg/kg, orally) twice daily for 7 days followed by ET (2 ml/kg, i.p.) on the 8th day
C3	Standard SL-PT	SL (20 mg/kg, orally) twice daily for 7 days followed by PT (200 mg/kg, i.p.) on the 8th day
D1	Test CT-CR 50	Aqueous extract of CM (50 mg/kg, orally) twice daily for 7 days followed by CT (2 ml/kg, i.p.) on the 8th day
D2	Test CT-CR 100	Aqueous extract of CM (100 mg/kg, orally) twice daily for 7 days followed by CT (2 ml/kg, i.p.) on the 8th day
D3	Test CT-CR 200	Aqueous extract of CM (200 mg/kg, orally) twice daily for 7 days followed by CT (2 ml/kg, i.p.) on the 8th day
E1	Test ET-CR 50	Aqueous extract of CM (50 mg/kg, orally) twice daily for 7 days followed by ET (2 ml/kg, i.p.) on the 8th day
E2	Test ET-CR 100	Aqueous extract of CM (100 mg/kg, orally) twice daily for 7 days followed by ET (2 ml/kg, i.p.) on the 8th day
E3	Test ET-CR 200	Aqueous extract of CM (200 mg/kg, orally) twice daily for 7 days followed by ET (2 ml/kg, i.p.) on the 8th day
F1	Test PT-CR 50	Aqueous extract of CM (50 mg/kg, orally) twice daily for 7 days followed by PT (200 mg/kg, i.p.) on the 8th day
F2	Test PT-CR 100	Aqueous extract of CM (100 mg/kg, orally) twice daily for 7 days followed by PT (200 mg/kg, i.p.) on the 8th day
F3	Test PT-CR 200	Aqueous extract of CM (200 mg/kg, orally) twice daily for 7 days followed by PT (200 mg/kg, i.p.) on the 8th day

Table 2- Serum enzyme levels of different biochemical parameters

Group code	Treatment	Serum Enzyme levels in IU/L (Mean ± SEM)		
		SGOT	SGOT	SGOT
A	Saline 2 ml/kg i.p	18.89 ± 0.3	19.8 ± 0.19	81.7 ± 0.05
B1	CT 2ml/kg	86.73 ± 0.65	181.44 ± 0.4	442.5 ± 0.62
C1	SL 20mg/kg	21.6 ± 0.03	24.65 ± 0.07	87.9 ± 0.02
D1	CR 50 mg/kg	30.4 ± 0.03	45.28 ± 0.04	118.3 ± 0.94
D2	CR 100 mg/kg	22.3 ± 0.65	32.3 ± 0.66	84.7 ± 0.25
D3	CR 200 mg/kg	22.8 ± 0.02	22.97 ± 0.02	91.97 ± 0.02
B2	ET 2ml/kg	83.7 ± 0.33	163.35 ± 0.05	373.7 ± 0.05
C2	SL 20mg/kg	21.95 ± 0.63	24.31 ± 0.37	90.23 ± 0.6
E1	CR 50 mg/kg	33.8 ± 0.2	41.0 ± 0.1	279.4 ± 0.65
E2	CR 100 mg/kg	25.2 ± 0.72	27.05 ± 0.04	89.3 ± 0.65
E3	CR 200 mg/kg	28.3 ± 0.65	27 ± 0.35	131.5 ± 0.72
B3	PT 200 mg/kg	65.3±0.65	131.7±0.25	340.6±0.35
C3	SL 20mg/kg	21.62 ± 0.63	24.32 ± 0.37	88.4 ± 0.6
F1	CR 50 mg/kg	32.7±0.25	42.2±0.65	293.1±0.8
F2	CR 100 mg/kg	26.7±0.25	27.5±0.35	180.4±0.55
F3	CR 200 mg/kg	25±0.3	27.3±0.65	127.5±0.7

as sugar and amino acid. The connection (haustorium) at the contact site is established through the secretion of enzymes and sticky substances consisting mainly of de-esterified pectins.[6] The *Cuscuta reflexa* is investigated for antitumor, antimicrobial,[7] hepatoprotective,[8] anticonvulsant,[9] antioxidant,[10] induced alopecia[11] activities. Many chemical constituents have been isolated from *Cuscuta reflexa* such as cuscutin, amarbelin, beta-sterol, stigmasterol, myricetin, qurecetin, cuscutamine, luteolin, bergenin etc.[12] The present investigation is designed in order to study the *in vivo* hepatoprotective activity of whole part of the plant *Cuscuta reflexa* Roxb. (Convolvulaceae) after its successive extraction with petroleum ether, chloroform, methanol and water respectively. Carbon tetrachloride (CT), ethanol (ET) and paracetamol (PT) induced hepatotoxic rat models were chosen to examine the hepatoprotective activity of *Cuscuta reflexa* (CR) and compared with silymarin (SL) as

reference standard. The whole plant extracts of CR was also subjected to preliminary phytochemical screening after each successive extraction.

MATERIALS AND METHODS

Plant Materials and Chemicals: Whole part of the plant *Cuscuta reflexa* (CR) were collected from Balasore district, Odisha and authenticated by Dr. N. K. Dhal, Taxonomist, IMMT, Bhubaneswar, Odisha. Paracetamol (PT), obtained as a gift sample from Cadila Pharmaceuticals, Ahmedabad, Gujurat, India. Silymarin (SL), SGOT (Serum Glutamate Oxaloacetate Transaminase) Kit, SGPT (Serum Glutamate Pyruvate Transaminase) Kit and ALP (Alkaline Phosphatase) Kits were purchased from Scientific Corporation, Rasulgarh, Bhubaneswar, Odisha. Petroleum ether, chloroform, methanol, carbon tetrachloride and ethanol were purchased from Merck. All the reagents used were of analytical grade and were used as received.

Animals: Albino rats of Wistar strain weighing 100-150 g of either sex were purchased from M/s Ghosh Enterprises, Kolkata and used for the study. The animals were housed individually in polypropylene cages at a temperature of 27 ± 2°C and 50 – 60 % RH with food and an unlimited supply of drinking water. Animals were kept on a standard light / dark cycle (12 hr/12 hr) with lights on at 7:00 AM. The animals were randomly selected, marked to permit individual identification, and kept in their cages for 7 days prior to dosing to allow for acclimatisation to the laboratory conditions. They were fed with Amrut Laboratory Animal Feed (Nay Maharashtra Chakan Oil Mills Ltd, Pune).

Extraction of plant materials: Whole part of the plant *Cuscuta reflexa* (CR) were washed thoroughly in water, dried for a week (35-40^{0}C) and pulverized in an electric grinder. The powder obtained was successively extracted in petroleum ether (60-80^{0}C), chloroform, methanol and distilled water. The extracts were then dried by a rotary

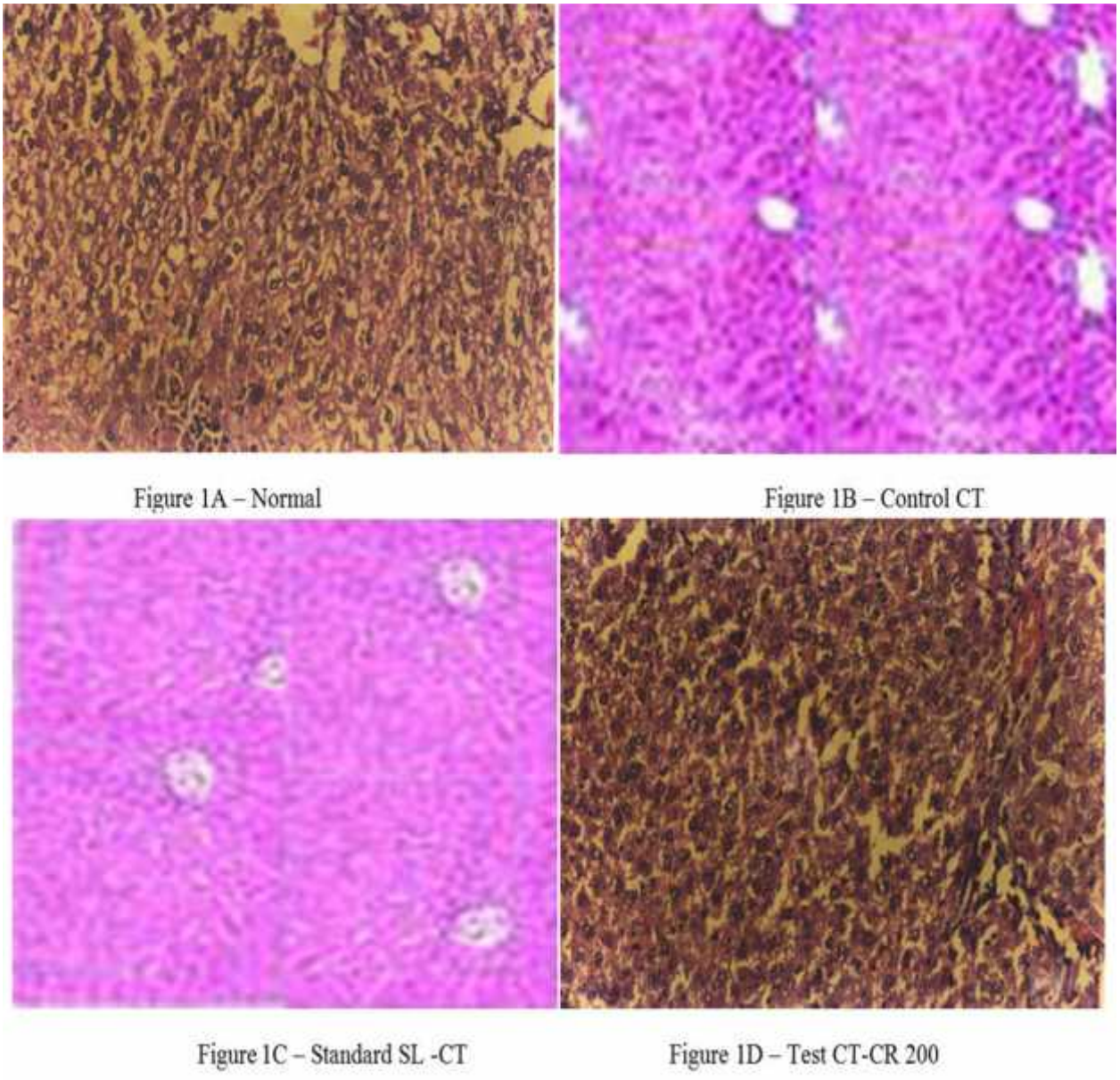

Figure 1- Representative microphotographs for histopathological study of CT induced hepatotoxicity

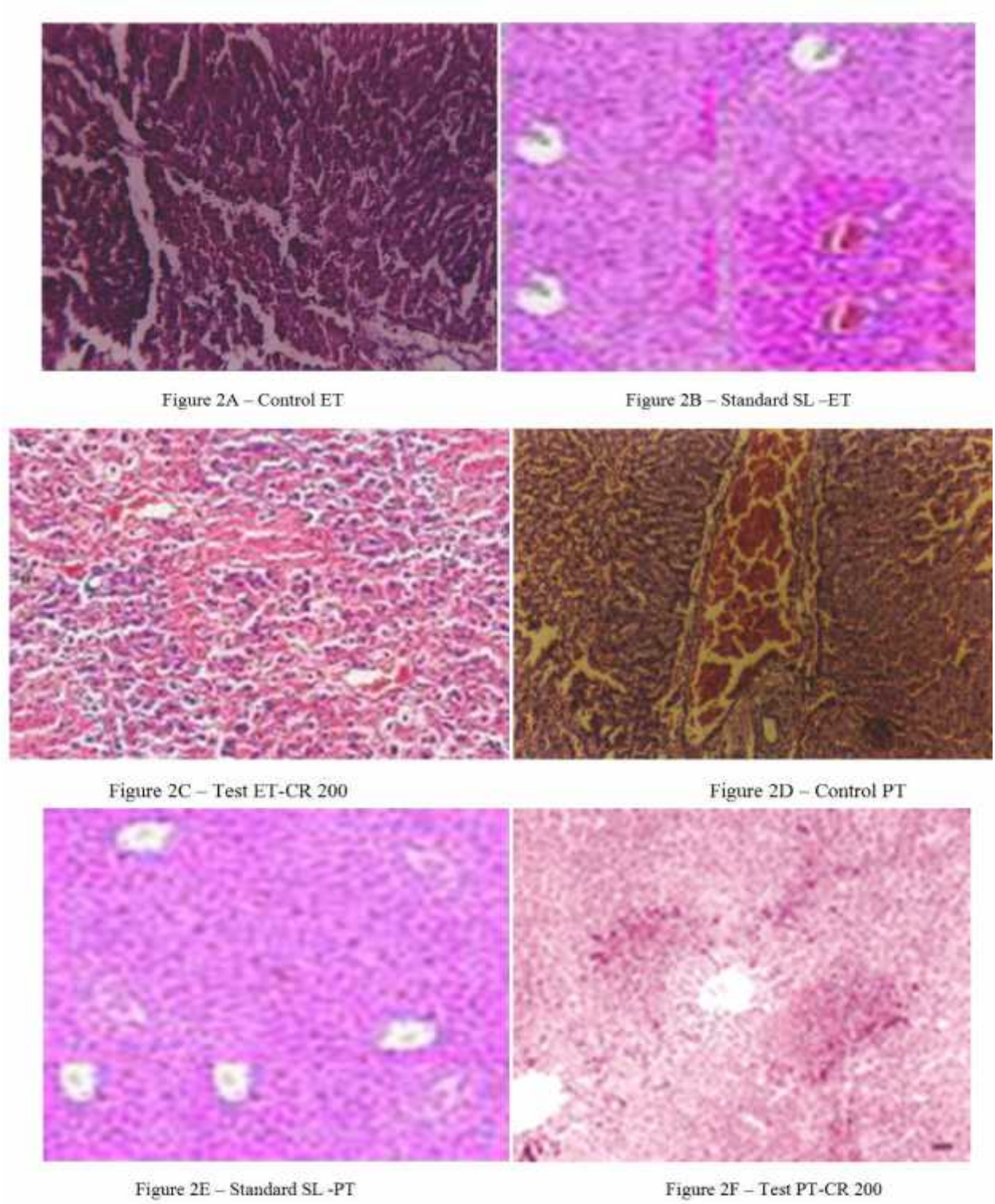

Figure 2A – Control ET

Figure 2B – Standard SL –ET

Figure 2C – Test ET-CR 200

Figure 2D – Control PT

Figure 2E – Standard SL -PT

Figure 2F – Test PT-CR 200

Figure 2- Representative microphotographs for histopathological study of ET and PT induced hepatotoxicity

evaporator under reduced pressure. Accordingly, powdered aqueous extract of this plant was prepared in sufficient quantity and stored in a well closed tight container for further use.

Preliminary phytochemical screening: The whole plant part extracts of CR obtained after each successive steps were subjected to qualitative chemical testing for preliminary screening of phytoconstituents. Phytochemical screening were performed using standard procedures.[13, 14] Phytochemical screening of CR extracts include test for alkaloids, saponins, glycosides and sugar, phenolic compounds and tannins, flavonoids and flavones, coumarin and its derivatives and triterpenoids.

Experimental design of *in vivo* hepatoprotective activity: In vivo hepatoprotective activity was evaluated on the basis of CT (2 ml/kg, i.p.), ET (2 ml/kg, i.p.) and PT (200 mg/kg, i.p.) induced liver toxicity in rats. Total 96 rats were divided in to 16 groups (each group consists of 6 animals). Three different doses of aqueous extracts of CR (50 mg/kg, 100 mg/kg and 200 mg/kg, orally) were chosen

for the screening of hepatoprotective activity and compared with standard SL (20 mg/kg, orally). Detail of the experimental design was given in Table 1. After 36 hours of CT/ET/PT/normal saline administration, blood was collected from all the groups of rats by cardiac puncture. Serum was separated by centrifugation at 2500 rpm at 37^0C for 15 min and analysed for various biochemical parameters like SGOT, SGPT and ALP using commercially available test kits and UV-Visible spectrophotometer (SHIMADZU 1700-JAPAN). The experimental protocol was approved by Institutional Animals Ethics Committee (CPCSEA Regd. No. – CPCSEA/C/990/2005).

Histolopathological study of liver: Immediately after sacrificing the rats, small pieces of liver tissues were fixed in 10% formalin and proceed for embedding in paraffin sections. Serial sections of 5 µm thickness were made, stained with hematotoxyline and eosin followed by examination under microscope for histopathological changes which include all necrosis fatty changes and infiltration of kuffer cell and lymphocytes. One normal group, all the three control groups, all the three standard SL treated groups and three CR treated groups (only 200 mg/kg doses) were subjected to liver section microscopic study in order to compare the standard SL with highest dose of aqueous CR extract.

Statistical analyses: All values were expressed as mean ± SEM (standard error about mean), where n = 6. Data from *in vivo* experiments for hepatoprotective activity were treated by two-way analysis of variance (ANOVA) and followed by least significant difference (LSD) test from the following formula.

$$LSD = t_{df,\ 0025}\sqrt{\frac{2\ MSN}{N}}$$

Where $t_{df,\ 0.025}$ = table value of t at degrees of freedom (df) of error term at 0.025 level of significance, MSE = Mean square error value, N= number of observations. Any difference between the mean values of different treatment groups exceeding the LSD is considered to be statistically significant.[15]

RESULTS AND DISCUSSION

Preliminary phytochemical screening: Chemical tests with petroleum ether extract of whole part of the plant *Cuscuta reflexa* (CR) resulted that there is presence of flavonoids, whereas triterpenoids and flavonoids were found in chloroform extract of CR. Methanolic extract of CR had shown presence of flavonoids and saponins. Lastly, aqueous extracts of CR revealed that there is presence of carbohydrates, reducing sugars, phenolic compounds, tannins, flavonoids and saponins.

In vivo hepatoprotective activity: Administration of aqueous extracts of whole plant of CR had resulted significant reduction (p-value < 0.0001) in various biochemical parameters like SGOT, SGPT and ALP as compared to their respective control groups. Two way ANOVA study of the *in vivo* data revealed that there is significant difference (p-value < 0.0001) exists between the different treatment groups. LSD values for SGOT, SGPT and ALP were 3.98, 5.11 and 9.08 respectively for CT induced hepatotoxicity. Difference between mean of control and any respective treatment value exceeds their corresponding LSD values, thus indicating statistical significant effect (p-value < 0.05) of the CR aqueous extracts at different doses. Test CT-CR 200 and Test CT-CR 100 groups had shown statistically equivalent activity (p-value > 0.05) which was comparable to that of standard SL treatment group (p-value > 0.05) in case of SGOT and ALP, whereas in case of SGPT only Test CT-CR 200 had shown statistically equivalent activity (p-value > 0.05) which was comparable to that of standard SL treatment group. There exists statistically significant difference (p-value < 0.001) between all other treatments and the CT control groups as far as SGOT, SGPT and ALP values were considered. LSD values for SGOT, SGPT and ALP were 3.53, 8.08 and 18.41 respectively for ET induced hepatotoxicity. LSD values for ET induced hepatotoxicity depicted that different treatment values were differ significantly from their respective control values (p-value < 0.001), but Test ET-CR 100 and Test ET-CR 200 groups had shown statistically equivalent activity (p-value > 0.05) as compared to standard SL treatment group in the context of biochemical parameter estimation, for instance, SGPT and ALP. LSD values for SGOT, SGPT and ALP were 4.12, 4.73 and 7.29 respectively in case of PT induced hepatotoxicity. PT induced hepatotoxicity depicted that different treatment values were differ significantly from their respective control values (p-value < 0.001). Test PT-CR 100 and Test PT-CR 200 were found to be statistically equivalent (p-value > 0.05) with their respective SL treated groups in the context of SGPT values. All other treatments were having significant difference from their respective control groups (p-value < 0.001). The aqueous extracts of CR having dose at 100 mg/kg and 200 mg/kg were statistically not comparable (p-value < 0.05) with their respective SL treated standard groups in the context of estimation of SGOT and ALP, though these values were reducing significantly. Serum enzyme levels of different biochemical parameters (SGOT, SGPT and ALP) were shown in Table 2 as mean ± SEM.

Histolopathological study of liver: Liver section of normal control rats had shown in Figure 1A, which indicated normal hepatic cell with well preserved cytoplasm, prominent nuclei, nucleoli and normal position of hepatic artery portal vein and bile ducts. CT induced fatty degeneration with severe liver necrosis of parenchyma cell mostly seen in central lobular region, sinusoidal congestion, broad infiltration of kupffer cells and loss of boundaries were observed in the liver section of control CT groups as observed from Figure 1B. It was well portrayed by Figure 1C that the hepatic cells radiate outwardly from the central vein and constitute the parenchyma of the lobules. The portal tract consists of hepatic artery, portal vein and bile ducts. It is observed in Figure 1D that the severe hepatic lesions induced by CT were remarkably lowered after the administration of CR 200 mg/kg which is also supported by the results of the biochemical analysis and is comparable to the liver section of standard SL treated groups (Figure 1C). Control ET groups had shown

fatty degeneration with severe liver necrosis of parenchyma cell mostly seen in central lobular region, infiltration of kupffer cells and loss of boundaries (Figure 2A), whereas control PT revealed necrosis of parenchyma cell seen in central lobular region, sinusoidal congestion and broad infiltration of kupffer cells (Figure 2D). It was clearly indicated by Figure 2B and 2E that the hepatic cells radiate outwardly from the central vein and constitute the parenchyma of the lobules. Moreover, it was also observed that severe hepatic lesions induced by ET and PT were remarkably lowered by the administration of CR 200 mg/kg to their respective groups (Figure 2C and 2F), which was also proven by the results of the biochemical analysis and is comparable to the liver section of standard SL treated groups (Figure 2B and 2E).

CONCLUSIONS

In this research work successive extraction method was successfully adopted for whole part of the plant *Cuscuta reflexa* Roxb. (Convolvulaceae) and its hepatoprotective activity was studied against carbon tetrachloride, ethanol and paracetamol induced liver damage. Aqueous extracts of *Cuscuta reflexa* had shown the presence of carbohydrates, reducing sugars, phenolic compounds, tannins, flavonoids and saponins. Estimation of biochemical parameters like aspartate aminotransferase, alanine amino transferase and alkaline phosphatase were depicted that highest dose (200 mg/kg) of aqueous extract of *Cuscuta reflexa* whole plant possess hepatoprotective activity comparable to that of standard silymarin at a dose of 20 mg/kg against carbon tetrachloride, ethanol and paracetamol induced hepatotoxicity. Equivalent hepatoprotective activity of *Cuscuta reflexa* whole plant with standard silymarin had also been supported by histopathological study of liver sections. Severe hepatic lesions induced by chloroform, ethanol and paracetamol were remarkably lowered after the administration of CR 200 mg/kg to the respective control groups which is also proven by the results of the biochemical analysis and was comparable to the liver section of standard SL treated groups without any statistical significant difference. Though aqueous extracts of *Cuscuta reflexa* whole plant (100 mg/kg and 200 mg/kg) had shown reducing effect on SGOT and ALP in case of paracetamol induced hepatotoxicity, on the other hand were statistically not comparable (p-value < 0.05) with their respective SL treated standard groups in the same context.

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REFERENCES

1. Deng X, Luyendyk JP, Ganey PE, Roth RA. Inflammatory stress and idiosyncratic hepatotoxicity: Hints from animal models. *Pharmacol Rev* 2009; 61: 262-282.
2. Papay JI, Clines D, Rafi R, Yuen N, Britt SD, et al. Drug-induced liver injury following positive drug rechallenge. *Regul Toxicol Pharmacol* 2009; 54: 84-90.
3. Kedderis GL. Biochemical basis of hepatocellular injury. *Toxicol Pathol* 1996; 24: 77-83.
4. Bleibel W, Kim S, D'Silva K, Lemmer ER. Drug-induced liver injury: Review Article. *Dig Dis Sci* 2007; 52: 2463-2471.
5. Catterjee D, Sahu RK, Jha A, Dwivedi J: Evaluation of Antitumor Activity of *Cuscuta reflexa* Roxb (Cuscutaceae) against Ehrlich Ascites Carcinoma in Swiss Albino Mice. *Trop J Pharm Res* 2011; 10: 448-453.
6. Vaughn KC. Attachment of the parasitic weed dodder to the host. *Protoplasma* 2002; 219:227-237.
7. Inamdar FB, Oswal RJ, Chorage TV, Garje K, In vitro antimicrobial activity of *Cuscuta reflexa* Roxb. *International Research Journal of Pharmacy* 2011; 2 (4): 214-216.
8. Balakrishnam BR, Sangameswaran B and Bhaskar VH. Effect of methanol of *Cuscuta reflexa* aerial parts on hepatotoxicity induced by antitubercular drugs in rats. I*nternational Journal of Applied Research in Natural Products* 2010; 3: 18-22.
9. Borole SP, Oswal RJ, Antre RV, Kshirsagar SS and Bagul YR. Evaluation of anti-epileptic activity of *Cuscuta reflexa* Roxb. *Research Journal of Pharmaceutical Biological and Chemical Sciences* 2011; 2: 657-663.
10. Sharma S, Hullatti KK and Kumar S. Comparative antioxidant activity of *Cuscuta reflexa* and *Cassytha filiformis. Journal of Pharmacy Research* 2012; 5: 441-443.
11. Pandit S, Chauhan NS and Dixit VK. Effect of *Cuscuta reflexa* Roxb on androgen induced alopecia. *Journal of Cosmetic Dermatology* 2008; 7: 199-204.
12. Patel S, Sharma V, Chauhan NS and Dixit VK. An updated review on the parasitic herb of *Cuscuta reflexa* Roxb. *Journal of Chinese Integrative Medicine* 2012; 10: 249-255.
13. Sofowora A. Screening plants for bioactive agents. In: Medicinal Plants and Traditional Medicinal in Africa. 2nd ed., Spectrum Books Ltd., Ibadan, Nigeria, 1993, 134-156.
14. Trease GE, Evans WC. Pharmacognosy. 15th ed., Saunders Publishers, London, 2002, 42-393.
15. Bolton S. Statistical applications in the pharmaceutical sciences. In: Lachman L, Lieberman HA, Kanig JL, editors. The theory and practice of industrial pharmacy. 3 rd ed.,Varghese Publishing House, Bombay, 1990, 269-276.