

“*IN-VIVO* ESTIMATION OF HEPATOPROTECTIVE PROPERTIES OF METHANOLIC EXTRACT OF STEM OF *CUSCUTTA REFLEXA*”

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Summary

The methanolic extract of the stem of *Cuscutta reflexa* Roxb. was evaluated for hepatoprotective activity by observing its effects on carbon tetrachloride (CCl₄) induced hepatotoxicity in liver histoarchitecture and alteration in certain biochemical parameters. The biochemical parameters studied were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin and total protein. The crude extract was administered through the intraperitoneal route in two dose groups, a lower dose group receiving 5mg/kg body weight/day and a higher dose group receiving 10mg/kg body weight/day. The study was carried out using Swiss albino mice of either sex, the weight of which ranged from 20-40 gm. The results that were compared with Silymarin, which was used as a standard to ascertain the presence of hepatoprotective activity in the methanolic extract of the stem of the said plant.

The crude extract administration led to reversal of the altered biochemical parameters in the group receiving the higher dose. Also, significant alterations of CCl₄-induced changes in the histoarchitecture of the liver cells were observed in the same. Both the doses of test drug i.e. methanolic extract (direct) of *Cuscutta reflexa* (Roxb.) were effective in lowering different elevated serum parameters like AST, ALT, ALP, total protein and bilirubin.

In comparison with the standard drug Silymarin, the higher test group (TGB) i.e. the group receiving the extract at a dose of 10mg/kg/day intraperitoneally showed better efficacy in lowering the aforesaid parameters with respect to the group receiving the test drug at a lower dose (TGA) of 5mg/kg.

Key words: *Cuscutta reflexa*, Hepatoprotective activity, Carbon tetrachloride, Silymarin, Hepatotoxicity

Introduction

Cuscuta reflexa also known as Swaranlata, Devils guts, Hair weed and Love-wine.⁽¹⁾ It is a deciduous perennial that prefer many types of soil with a pH ranging from acid to alkaline and partial to full sun with moderate moisture. Stem is slender pale-yellow or greenish-yellow glabrous with adhesive disc at the point of attachment on host plant.^(2,3) Flowers waxy, white, solitary or in umbellate clusters of 2-4 in short racemes; pedicels short, glabrous usually curved; bracts 1.5 mm. long, ovate-oblong, obtuse, fleshy.⁽⁴⁾

It is distributed throughout India ascending upto 2600 m. and in Cylone-Malaya along with other tropical and sub-tropical regions around the world^(5,6)

Traditionally the stem is used as a purgative. It is given in combination with other purgative decoction. Varalians of the dodder are highly useful in piles.^(7,8) According to the traditional Chinese healers, cuscute seeds are neutral nature and have a pungent, sweet taste.⁽⁹⁾ They are associated with the diseases liver and kidneys and are used in formulas that help both yin and yang deficiencies, depending on the patients condition.^(10,11) The plant is mixed with the twigs of *Vitex negundo* L. and is warmed and applied as fomentation on the abdomen of children in kwashiorkor.^(12,13) According to Carter an infusion made from the plant is used to wash sores in Lakhimpur.⁽¹⁴⁾ Inhabitants of Pithoragarh district in Uttar Pradesh use the juice of the plant for diphtheria. It is also used to check fall of hairs.^(11, 14) The Tharu tribes of the Uttar Pradesh, the plant is tied on the neck of the patient and also spread on the bed, to cure jaundice. The water vapor of this plant is inhaled for treating jaundice and warm paste is applied in rheumatism and gout and other affected parts of the body, and the paste of whole plant is applied for relieving head ache.⁽¹⁴⁾ Gaddi tribes of the Western Himalaya in Himanchal Pradesh, use the whole plant paste in the treatment of swelling of the tusticle and in headache.^(15, 16)

Methods

The stems of *Cuscuta reflexa roxb.* was collected from the surrounding areas of Dibrugarh University campus and was identified by Dr. M. Islam, Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India. The voucher specimen herbarium sheet of the said plant has been deposited in the departmental museum of Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India.

The plant is subjected to shed drying for about three to four weeks to remove the resident moisture. After this these were cut to small pieces and about 20 gm. were packed in Soxhlet apparatus and extracted with methanol for 12 hours after which the solvent was recovered and the extract was dried using rotary vacuum evaporator. The yield of the methanolic extract was used as a fine suspension in 3.0% Tween 80 for experimentation.

The hepatoprotective activity of methanolic extract of the stem of *Cuscuta reflexa roxb.* were studied by *in vivo* method.

Swiss albino mice of either sex weighing between 20-40 gm obtained from the departmental animal house were used in the experimentation. They were fed on standard laboratory diet with water *ad libitum* and housed in plastic cage at room temperature and were exposed to natural day and night cycles.

The animals were divided into five groups, each groups contain six animals.

1. Group 1- it served as the control, and received the vehicle (3.0% tween 80 in distilled water) at a dose of 0.1ml/100g body weight/day/intraperitoneal (i.p.) for 14 days.⁽¹⁷⁾
2. Group 2 – it served as the toxicant group, and received carbon tetrachloride (CCl₄) prepared in the vehicle, at a dose of 0.1ml/100g body weight/ twice a week/i.p. The second dose of the toxicant was given 36hrs. after administering the first dose.^(17,18)
3. Group 3 – it served as test group with lower dose, it received the methanolic extract at a dose of 5mg/1000g body weight/day/i.p. for 7 days. 24 hrs after the final extract dose the animals were intoxicated with CCl₄ (0.1ml/100g body weight/twice a week/i.p.) and 36-48 hours after the second CCl₄ injection the animals were sacrificed. Liver was dissected out weighed and preserved in Bouin's solution.^(17,18,19)
4. Group 4 – it served as test group with higher dose, received the direct methanolic extract at a dose of 10mg/1000g body weight/day/i.p. for 7 days. 24 hrs after the final extract dose the animals were intoxicated with CCl₄ (0.1ml/100g body weight/twice a week/i.p.) and 36-48 hours after the second CCl₄ injection the animals were sacrificed. Liver was dissected out and preserved in Bouin's solution.^(17,18,19)
5. Group 5 – it served as the standard control group, received silymarin at a dose of 100mg/1000g body weight/day for 7 days/i.p followed by toxicant, CCl₄ (0.1ml/100g body weight/twice a week/i.p.).⁽²⁰⁾

Blood was collected by heart puncture method and allowed to stand for 20min, and then centrifuged for 15-20 minutes at 2000 rpm to separate the serum and the latter was used for biochemical estimations of parameters like Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Bilirubin and Total protein.

All the above biochemical parameters were assayed using standard laboratory kit from Crest Biosystems; namely: Alkaline Phosphatase Kit, Bilirubin Kit, Total Protein Kit, SGOT (ASAT) Kit and SGPT (ALAT) Kit.

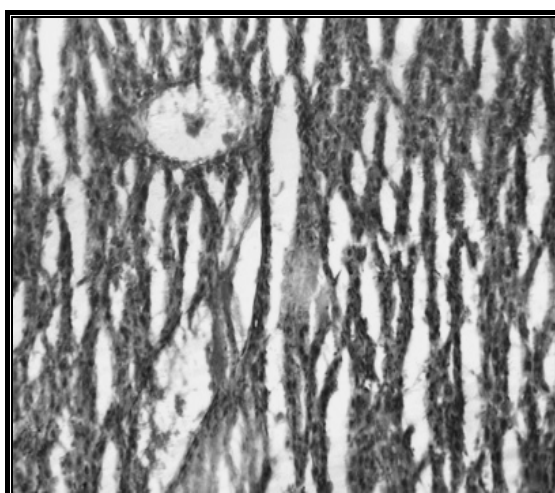
Statistical analysis of the data obtained was carried out by employing Student's 't' test for unpaired data using Origin 6.1, a statistical analysis software, at P<0.05.

Liver samples of each of the animals were dissected out and kept in Bouin's solution and preserved at a temperature of 2-3⁰C in the refrigerator. For the performance of histopathological examination samples were prepared using Rapid process: liver samples were first cleared by washing with acetone and allowed to stand in acetone for half hour. Three washings with acetone were done. The samples were dehydrated by keeping them in benzene for half hour. Three such washings of each sample were done. Then paraffin was melted and liver samples were embedded into it and cut into thin ribbon using microtome and strips placed in glass slides. These slides were then incubated overnight at a temperature of 37⁰C. Then the slides were melted and washed with xylol, then with absolute alcohol, 90% and 70% alcohol for 3-5min. and finally with water for 20min. Then the slides were stained with haematoxylin and allowed to stand for 3-5 min. and excess stain washed off with water. The slides were then treated with acid-alcohol (1% HCl in absolute alcohol) and washed with water and treated with eosin, after which it is fixed and observed under microscope.

Table 1. Analysis of Different Serum Parameters with respect to Each Group

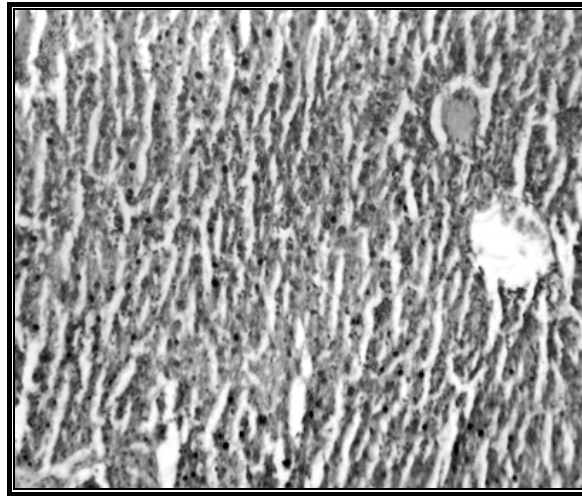
Number of animals per group (n) = 6

Treatment	AST ^f (U/ml)	ALT ^g (U/ml)	ALP ^h (K.A. unit)	Bilirubin (mg/dl)	Total Protein (g/dl)
Vehicle ^a (control)	102.67±3.09 ⁱ	142.5±6.65	4.42±0.43	3.19±0.31	7.99±0.66
TA ^b +CCl ₄	131.42±2.48	164.27±3.92	8.26±0.50	5.01±5.21	9.14±0.11
TB ^d +CCl ₄	125±2.71	158.15±2.52	7.87±3.48	4.96±0.1	8.67±0.08
Silymarin ^c +CCl ₄	113.75±2.61	150.27±8.39	6.75±1.92	4.42±0.71	8.26±1.14
CCl ₄ ^c (toxicant)	139.5±3.79	179.75±4.07	18.37±1.05	6.09±2.73	13.89±0.57

^a Vehicle, 3% tween 80; dose 0.1ml/100g/day/i.p. for 14 days^b TA, test drug lower dose, methanol direct extract; dose 5mg/kg/day/i.p. for 7days, 24hrs after the final dose the animals were intoxicated with CCl₄ (0.1ml/100g/twice a week/i.p.) till 14th day; 36-48hrs after the 2nd CCl₄ administration the animals were sacrificed.^c CCl₄, toxicant, carbon tetrachloride; dose 0.1ml/100g/twice a week/i.p. till 14th day; 36-48hrs after the 2nd CCl₄ administration the animals were sacrificed.^d TB, test drug higher dose, methanol direct extract; dose 10mg/kg/day/i.p. for 7days, 24hrs after the final dose the animals were intoxicated with CCl₄ (0.1ml/100g/twice a week/i.p.) till 14th day; 36-48hrs after the 2nd CCl₄ administration the animals were sacrificed.^e Silymarin, dose 100mg/kg/day/i.p. for 14 days;^f AST, Aspartate amino transferase^g ALT, Alanine amino transferase^h ALP, Alkaline phosphataseⁱ Values represent the mean ± standard error of six animals in each group**Figure 1** Photomicrograph (Leica photomicroscope-DM 1000) of section of liver from TX (toxicant, CCl₄) mice group.

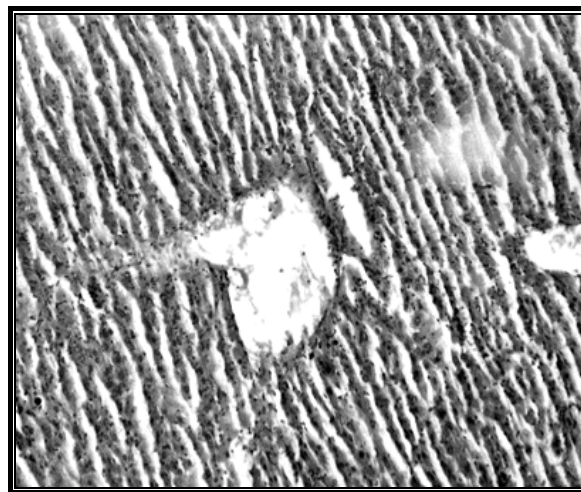
Periportal fibrosis, acute inflammatory change, centrilobular necrosis, vacuolization of cells are observed.

Figure 2. Photomicrograph (Leica photomicroscope-DM 1000) of section of liver from **TB** (test group receiving higher dose) mice group.



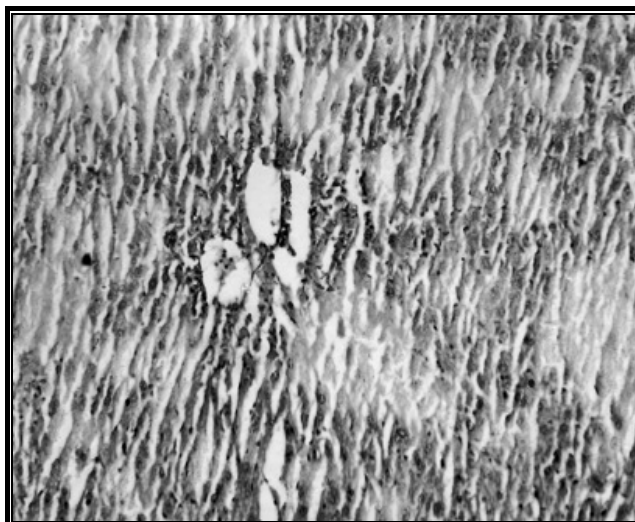
Liver cells mostly normal with localized areas of sub capsular inflammation;

Figure 3. Photomicrograph (Leica photomicroscope-DM 1000) of section of liver from **SC** (Silymarin + CCl₄) mice group



Mostly normal hepatocytes are seen with very less fatty change along with areas of regeneration.

Figure 4. Photomicrograph (Leica photomicroscope-DM 1000) of section of liver from TA (lower dose group) mice group



No significant hepatocellular damage, localized fatty changes seen in some areas along with diffused cloudy swelling.

Results

Serum Parameters:

It can be noted from Table 1 that the levels of all the enzyme parameters of serum i.e. AST, ALT, ALP, total protein and bilirubin were found to be significantly increased in the CCl₄ treated mice group (TX).

Treatment with lower dose of methanolic extract of stem of *C. reflexa* showed decreased activities of serum transaminase along with total protein and bilirubin levels.

However, the significantly increased activities of serum ALT, AST in CCl₄ treated mice groups were predominantly reversed by the higher dose of methanolic extract of *C. reflexa* receiving group (TB) the effects of which is comparable with silymarin; which was used as standard.

At P<0.05 levels it showed maximum recouplement in serum enzyme activities and bilirubin and protein levels with extract treatment at a higher dose of 10mg/kg in the current study.

Histopathological changes:

Liver of mice treated with CCl₄ showed extensive signs of degeneration. Vacuolization of hepatocytes were very common, peripheral fibrosis, centrilobular necrosis and fatty degenerations were also observed (figure1). Administration of extract at dose of 10mg/kg showed nearly well maintained histoarchitecture (figure 2). There were no perinuclear vacuolation in the hepatocytes and were comparable to silymarin standard (figure 3). Nuclei of hepatocytes were also well maintained. The lower dose group getting 5mg/kg dose also showed less hepatocellular damage, in some areas localized fatty changes along with diffused cloudy swellings are seen (figure 4).

Discussion

Raised activity of serum transaminases in intoxicated mice as found in the present study can be attributed to the damaged structural integrity of the liver, because these are cytoplasmic in location and are released into the circulation after cellular damage.⁽²¹⁾

Many fold increase of enzyme leakage as demonstrated by an increased level of serum enzymes ALT, AST and ALP was noted indicating liver damage by CCl₄.⁽²²⁾ The methanolic extract of *Cuscutta reflexa* has notably prevented the leakage of these enzymes and restoring the activity of enzymatic variables.

The response to silymarin and methanolic extract of *Cuscutta reflexa* is comparable in most parameters and the differences observed are largely quantitative. Furthermore, the reversal in activities of hepatic enzymes like ALT was higher in higher dose receiving group than with silymarin. The increased levels of other parameters in serum were significantly reversed by silymarin and the effects were comparable with the group receiving the higher dose i.e 10mg/kg body weight of the methanolic extract of *Cuscutta reflexa*.

Histopathological studies demonstrated degenerative lesion, vacuolation, periportal fibrosis, fatty degeneration, sub-capsular inflammation in the hepatocytes induced by CCl₄. These findings were further supported by earlier reports^(23,24) showing degeneration in hepatocytes and hepatic chords. Focal and periportal area degeneration, localized acute inflammatory changes, cloudy swelling were seen. Significant normalization in the histoarchitecture was seen with the higher dose administration at 10mg/kg, however close to normal with periportal area degeneration along with localized cloudy swelling was observed, in lower dose mice group receiving dose at 5mg/kg. The observations were based upon comparison with the standard drug i.e. silymarin.

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