

**Research Article****Investigations on hepatoprotective effect of *Cuscuta reflexa* extract in Paracetamol induced toxicity**

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**Abstract**

**Objective:** The present study was carried out to provide evidence to the traditional use of *Cuscuta reflexa* in the treatment of hepatotoxic effect. **Material and Methods:** The plant was extracted with chloroform and ethanol. Hepatotoxicity were induced by single dose of Paracetamol (Acetaminophen) by i.p. route. In test group 50% ethanolic extract of *C. reflexa* at a dose 84.1mg/kg of body weight was given orally on every alternate day for 1 month. Weight of the animal on initial and after 1 month were taken and weight variation was calculated. After 1 month the animal was dissected and histopathological investigation of liver were carried out and the reduce in GSH level in blood as well as liver was calculated. **Results and Conclusion:** Results were found that 50% ethanolic extract of *Cuscuta reflexa* showed hepatoprotective activity. In histopathological investigation it was observed that the 50% ethanolic extract of *Cuscuta reflexa* has less necrosis and ballooning (swelling of hepatocytes) than the control group. When the animal received single application of paracetamol at the dose of 500mg/kg and *Cuscuta reflexa* 50% ethanolic extract at the dose of 84mg/kg body weight for 30 days showed increase in reduced glutathione level in liver whereas decreased was observed in blood of mice. It has been observed in other studies also the decrease level of glutathione in blood level but increase in liver.

**Keywords:** Glutathione Stimulating Hormones (GSH), *Cuscuta reflexa*, acetaminophen

**Introduction**

Hepatotoxicity implies chemical-driven liver damage. The liver plays a central role in transforming and clearing chemicals and is susceptible to the toxicity from these agents. Certain medicinal agents when taken in overdoses and sometimes even when introduced within therapeutic ranges may injure the organ. Other chemical agents such as those used in laboratories and industries, natural chemicals (e.g. microcystins) and herbal remedies can also induce hepatotoxicity. Chemicals that cause liver injury are called hepatotoxins (Ghosh, 1984).

More than 900 drugs have been implicated in causing liver injury and it is the most common reason for a drug to be withdrawn from the market. Chemicals often cause subclinical injury to liver which manifests only as abnormal liver enzyme tests. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of

all acute liver failures (Patel, 1998).

Drugs continue to be taken off the market due to late discovery of hepatotoxicity. Due to its unique metabolism and close relationship with the gastrointestinal tract, the liver is susceptible to injury from drugs and other substances. 75% of blood coming to the liver arrives directly from gastrointestinal organs and then spleen via portal veins which bring drugs and xenobiotics in concentrated form. (Lui et al., 2004) Several mechanisms are responsible for either inducing hepatic injury or worsening the damage process. Many chemicals damage mitochondria, an intracellular organelle that produces energy. Its dysfunction releases excessive amount of oxidants which in turn injures hepatic cells. (Shetty et al., 2013). Activation of some enzymes in the cytochrome P-450 system such as CYP2E1 also lead to oxidative stress. Injury to hepatocyte and bile duct cells lead to accumulation of bile acid inside liver. This promotes further liver damage. Non-parenchymal cells such as Kupffer cells, fat storing stellate cells and leukocytes (i.e. neutrophil and monocyte) also have role in the mechanism (Martinon, 2002).

IL-1 $\beta$  is a very potent proinflammatory cytokine, and IL-1 $\beta$

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levels are known to be increased during APAP hepatotoxicity. In addition, signaling through the IL-1 receptor (IL-1R) was recently shown to be important in APAP-induced hepatotoxicity. The mechanisms by which IL-1 $\beta$  is upregulated during a sterile inflammatory response are not known. There are, however, extensive data on IL-1 upregulation by a variety of pathogens. Activation of TLRs by pathogen-associated molecular patterns (PAMPs) results in upregulation of pro-IL-1 $\beta$  via a MyD88/NF- $\beta$  B pathway. Analogous to other potent inflammatory steps, production of IL-1 $\beta$  requires a second signal resulting in caspase-1-mediated cleavage of pro-IL-1 $\beta$  to release the active molecule (Lynch and Price, 2007).

*Cuscuta* (Dodder) is a genus of about 100-170 species of yellow, orange or red parasitic plants. Formerly treated as the only genus in the family Cuscutaceae, recent genetic research by the Angiosperm phylogeny group has shown that it is correctly placed in the family Convolvulaceae (Mashkar et al., 2000). The genus is found throughout the temperate to tropical region of the world, with the greatest species diversity in subtropical and tropical regions, the genus becomes rare in cool temperate climate, with e.g. only four species native to northern Europe.

*Cuscuta reflexa* is leafless and rootless. Initially the starter plant would have some roots. Within a few days of germination, the plant which is touch sensitive, finds a host or dies. After establishing itself on the host body, it draws nutrition from the host as a stem parasite and the roots wither away. The twining stem develops Haustoria which are root like and penetrate the host stem to draw water and nourishment (Dawson, 1990).

The flowers are small, white, having a perfect bell shape and a fresh calyx, attached directly to the stem node. Although a few species are reported to have medicinal use, the rampant Dodder plant is a voracious and destructive vine which usually will overgrown and kill the host. It also is a cause of transmission of different viruses' diseases such as citrus mosaic and purple blotch to field crops and trees. Its seeds can remain dormant for five year and control of dodder is an important issue for crops and forests. Plants lover and conservationists enjoying this site should note the in current Science Magazine. It has been identified as an emerging threat to the plant diversity in the valley of flowers. While this flower appears like a perfect lustrous bell, the experts advise that wherever an unwanted dodder vine is growing, all its stem pieces should be removed (Preferably before seeding) and burnt. It has very low level of chlorophyll, some species such as *Cuscuta reflexa* can photosynthesis slightly, while other such as *C. europeans* nutrition (Chado & Zetsche, 1990).

The major constituent present in *C. reflexa* are 21-Hydroxy odoroside H, Odoroside H, Neritaloside, Strosposide, Gitoxigenin, Ursolic acid, B-Sitosterol glucoside, 4-O-p-

Coumaroyl-O-glycoside, Methyl cinnamate and Dihydroajugapitin. (Versani and Muhammad, 1997).

## Material and Methods

### Collection and identification of plant material

According to opinion of the local traditional practitioner, this plant is being used for the treatment of cancer. The compiled plant therefore is subjected to identification and then literature survey to conform that the plant has not been previously investigated for the anticarcinogenic activity. So, the selection of the medicinal plant for anticarcinogenicity has been undertaken. (Kokate, 2003).

The herb were dried under shade, and then powered with mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further studies (Rajan et al., 2001).

### Extraction of plant material

The herb was collected from the local forest and dried for few days in shade. Then powder was made with the help of grinder, the *Cuscuta reflexa*. 50gm of powder was taken in a separating funnel and added 50% ethanol, then mixed gently. After every 24 hrs. extract was collected in a beaker till the solvent appears colourless. In same way chloroform extract was prepared with 100% chloroform in place of 50% ethanol. The extract was dried into powder by water bath at 60°C and hot air oven at 45°C. The total weight of extract powder was measured and calculated % yield of each extract. On the day of experimentation, the desired amount of powder was suspended in to double distilled water (DDW) for the final administration (Khandelwal, 2005).

### Hepatoprotective activity

We had taken 8 swiss albino mice of weight  $28 \pm 2$  gm., and divided into 2 groups (Test & Control) having 4 mice in each. A single dose of Paracetamol (Acetaminophen) at 500mg/kg of body weight was given to each animal in each group by i.p. route. In test group 50% ethanolic extract of *C. reflexa* at a dose 84.1mg/kg of body weight was given orally on every alternate day for 1 month. Weight of the animal on initial and after 1 month were taken and weight variation was calculated. After 1 month the animal was

**Table 1.** Experimental protocol of Glutathione activity

S. No.	Group of Animals	No. of Animals
1.	Paracetamol (500mg/kg) + <i>C. reflexa</i> (84.1mg/kg)	04
2.	Paracetamol alone (500mg/kg)	04
3.	Fresh (Untreated)	04

dissected and histopathological investigation of liver were carried out. And the reduce in GSH level in blood as well as liver was calculated (Ostapowicz et al., 2002).

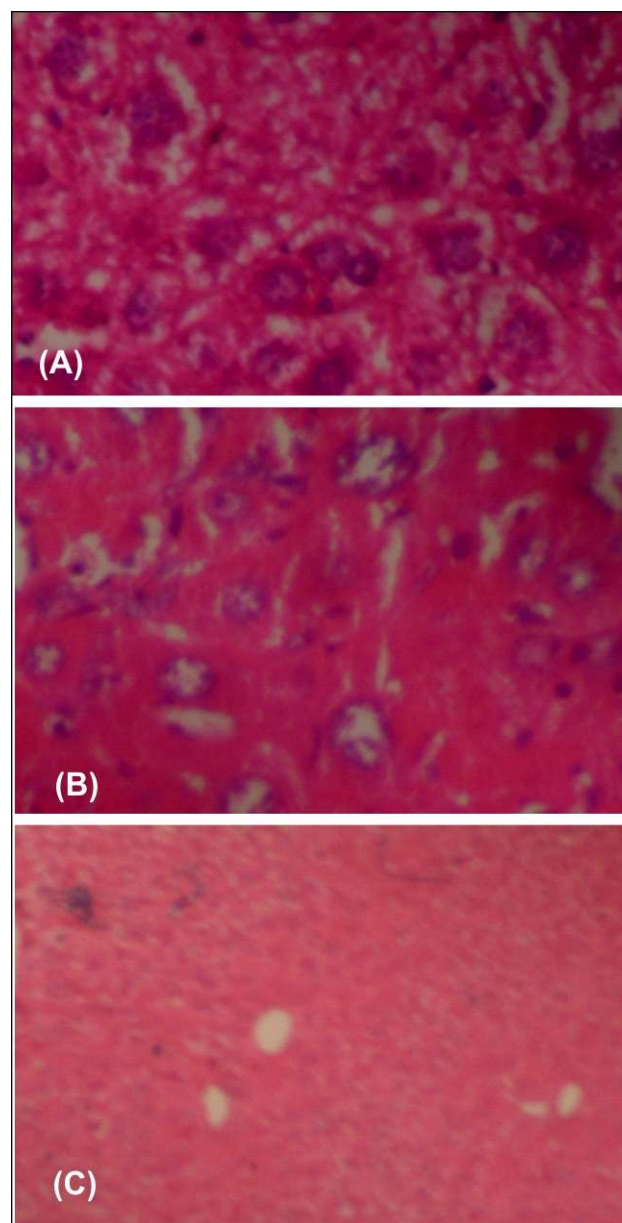
### Glutathione activity

A dose of *Cuscuta reflexa* 50% ethanolic extract (85mg/kg body wt.) by i.p. was given to the animals. Each group containing four animals. Firstly 0.2ml blood was taken from eye by orbital puncture method, and added 1.8ml of double distilled water on sample. Taken 2ml double distilled water in another test tube, used as a blank. Then mice were killed by cervical dislocation and liver was separated (Martinon, 2002). Weight of the liver was taken & centrifuged with the help of homogenizer. Then in both test tube (blood & liver) add 3ml of ppt. solution was added, and left it for 10min, then filtered out by whattman filter paper. In the filtrate and blank, added 8ml buffer solution and 1ml DTNB solution. Then wrapped the all test tubes very carefully with the help of alluminium foil, and left it for 30min. for incubation (Jessica et al., 2003). After that, optical density was measured with the help of spectrophotometer at 412nm. Calculation was done with the help of ANOVA and t-test (Table 2).

### Results

Results showed (Table 2) that all mice have reported to loss their weight (approx 8-10 gm) in the duration of experiment, but the weight loss was less in the *Cuscuta reflexa* extract treated group. The animals which was given single dose of paracetamol at the dose of 500mg/kg body weight. followed by *Cuscuta reflexa* 50% ethanolic extract at the dose of 84.1mg/kg body weight by oral route was sacrificed after 30 days of treatment.

Results showed that, when the animal received single application of paracetamol at the dose of 500mg/kg and *Cuscuta reflexa* 50%



**Figure 1.** Histopathology of liver in different groups of mice: (A) Fresh (B) Control (C) Treated

**Table 2.** Weight losses mice of different groups

S.No.	Groups	Dose	Weight loss
1.	Test	Paracetamol 500mg/kg + <i>C.reflexa</i> extract 84.1mg/kg	2.1 ± 1.2
2.	Control	Paracetamol 500mg/kg	5.2 ± 0.32

**Table 3.** Percentage of reduced GSH in blood and liver of different groups.

S. No.	Groups	Dose	% of reduced GSH in Blood	% of reduced GSH in Liver
1.	Test	Paracetamol 500mg/kg + <i>C.reflexa</i> 84.1mg/kg	36.15 ± 17.34	555.03 ± 204.18
2.	Control	Paracetamol 500mg/kg	64.58 ± 19.49	458.82 ± 94.15
3.	Fresh	-	49.44 ± 7.85	730.56 ± 27.81

ethanolic extract at the dose of 84mg/kg body weight for 30 days showed increase in reduced glutathione level in liver whereas decreased was observed in blood of mice. It has been observed in other studies also the decrease level of glutathione in blood level but increase in liver. All mice have lost their weight approx 8-10 gm in the duration of experiment. Autopsy results. We have killed 3 animals which was given single dose of paracetamol at the dose of 500mg/kg body weight. followed by *Cuscuta reflexa* ethanolic extract at the dose of 500mg/kg body weight by oral route. After 30 days of treatment we have killed the animals, we observed that (Shown in figure): (a) The liver of 2 animals were normal and one animal showed abscess formation. (b) The stomach of 2 animals showed some node

like projections, which were preserved in 10% formaline for histopatho examination.

### Discussion

In our experiment it was found that 50% ethanolic extract of *Cuscuta reflexa* has a hepatoprotective activity. In histopathological investigation it was observed that the 50% ethanolic extract of *Cuscuta reflexa* has less necrosis and ballooning (swelling of hepatocytes) than the control group.

Acetaminophen (*N*-acetyl-para-aminophenol [APAP]) hepatotoxicity is the most common cause of death due to acute liver failure in the developed world and is increasingly recognized as a significant public health problem. The initial event in APAP-induced hepatotoxicity is a toxic-metabolic injury leading to hepatocyte death by necrosis and apoptosis. This results in secondary activation of the innate immune response involving upregulation of inflammatory cytokines with activation of NK cells, NKT cells, and neutrophils. The molecular pathways for innate immune activation after hepatocyte death are of great interest, as they are likely common to sterile inflammation (Keplowitz, 2004).

Glutathione is widely found in all form of life and plays an essential role in the health of organisms, particularly aerobics organisms. In animals, including humans and in plants, glutathione is the predominant, non-protein thiole and functions as a redox buffer, keeping with its own SH groups those of protein in a reduced condition, among other anti-oxidant activities.

When the animal received single application of paracetamol at the dose of 500mg/kg and *Cuscuta reflexa* 50% ethanolic extract at the dose of 84mg/kg body weight for 30 days showed increase in reduced glutathione level in liver whereas decreased was observed in blood of mice. It has been observed in other studies also the decrease level of glutathione in blood level but increase in liver. It may be due to the process of carcinogenesis itself because it has been reported that cancer cells need a lot of glutathione for proliferation, but in liver it may be due to the detoxification mechanism.

Glutathione is present in tissue in concentrations as high as one millimolar. Cystine, the business residue of glutathione, neither has the solubility nor activity of glutathione at physiological pH. It appears that nature has built the cystine molecule into the glutathione tri-peptide to make the amino acid more soluble and allow it to have redox buffering activity in a living tissue environment. Glutathione also plays role in the catalysis, metabolism, signal transduction, gene expression and apoptosis. It is a co-factor for glutathione S-transferases, enzymes which are involved in the detoxification of xenobiotics including carcinogenic genotoxification peroxidase, crucial selenium containing antioxidants enzyme, it is also involved in the

regeneration of the ascorbate from its oxidized form, dehydroascorcorbate.

**Conflicts of interest:** Not declared.

### References

- Ghosh MN. 1984. Toxicity studies. In Fundamentals of Experimental Pharmacology 153–158.
- Jessica R. Oesterheld, Kelly L. Cozza, Armstrong Scott. 2003. Concise Guide to Drug Interaction Principles for Medical Practice: Cytochrome P450s, Ugt's, P-Glycoproteins. Washington, DC: American Psychiatric Association, 167–396.
- Kaplowitz, N. 2004. Acetaminophen hepatotoxicity: what do we know, what don't we know, and what do we do next? *Hepatology* 40:23–26.
- Khandelwal KR. 2005. Practical Pharmacognosy. 1st ed. Pune: Nirali Prakashan 27-35.
- Kokate CK. 2003. Practical Pharmacognosy. 4th edition New Delhi 56-59.
- Liu ZX, Govindarajan S, Kaplowitz N. 2004. Innate immune system plays a critical role in determining the progression and severity of acetaminophen hepatotoxicity. *Gastroenterology* 127:1760–1774.
- Lynch T, Price A. 2007. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. *American Family Physician* 76(3):391–6.
- Machado MA, Zetsche K. 1990. Structural, functional and molecular analysis of plastids of the holoparasites *Cuscuta reflexa* & *Cuscuta europeae*. *Planta* 181:91-96.
- Martinon F, Burns K, Tschopp J. 2002. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of ProIL-Beta. *Molecular Cell* 10:417-426.
- Mhaskar KS, Blatter E, Caius JF. 2000. Indian Medicinal Plants, Shri Satguru Publication, Indian Book Center, New Delhi, 3<sup>rd</sup> Ed.; Vol. 8, 2400.
- Ostapowicz G, Fontana RJ, Schiødt FV. 2002. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Annals of Internal Medicine* 137(12):947–54.
- Patel T, Roberts LR, Jones BA, Gores GJ. 1998. Dysregulation of apoptosis as a mechanism of liver disease: an overview. *Seminars in Liver Disease* 18(2):105–14.
- Rajan S, Baburaj DS, Sethuraman M, Parimala S. 2001. Stem and stem bark used medicinally by Tribals Irulas and Paniyas of Nilgiri District, Tamil Nadu.

Ethnobotany 6:19-24.

Shetty J, Shyamjith, D, Alwar MC. 2007. Acute toxicity studies and determination. *Current Science* 93:9-17.

Versani G, Muhammad Ali. 1997. Studies in the Chemical Constituents of *Bombax Ceiba* & *Cuscuta Reflexa*. PhD Thesis, Under University of Karachi, Pakistan 191-197.