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# Antifibrotic effect of *Cleome viscosa* Linn on Carbon tetra chloride ($CCl_4$) induced liver fibrosis

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## Abstract

Liver fibrosis is the excessive accumulation of extracellular matrix proteins including collagen that occurs in most types of chronic liver diseases. This study was carried out to evaluate the antifibrotic effect of ethanolic extract of whole plant of *Cleome viscosa* Linn. Liver fibrosis was induced by carbon tetra chloride ($CCl_4$) administration in rats. The extent of liver fibrosis was assessed by measuring the level of liver hydroxyproline, thiobarbituric acid and serum enzymes levels. Following $CCl_4$ administration, hydroxyproline, thiobarbituric acid levels were significantly increased and total platelet was decreased and serum enzymes levels were elevated. Treatment with two different doses of the ethanolic extract of *Cleome viscosa* Linn reduces hydroxy proline, thiobarbituric acid and also the serum enzyme levels. The liver weight that increased following $CCl_4$ administration due to deposition of collagen was reduced by the ethanolic extract *Cleome viscosa* Linn. Thus the results showed that the ethanolic extract of whole plant of *Cleome viscosa* Linn found to possesses Antifibrotic effect and that evidenced by the biochemical parameters.

**Keywords:** Fibrosis, Carbon tetra chloride ($CCl_4$), Antifibrotic effect, *Cleome viscosa*, Hydroxy proline.

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## Introduction

Liver represents the largest gland of the body and is the remarkable organ that is able to regenerate [1]. It performs a number of functions. While looking into the anatomy, six types of cells are classified, viz hepatocytes, bile duct epithelial cells (collagiocytes) kupffer cells, hepatic stellate cells, sinusoidal endothelium and pit cells. The majority of cells in the liver are hepatocytes, which constitute 2/3$^{rd}$ of mass of liver. In hepatitis, inflammation of liver cell occurs resulting in injury or destruction [2]. Liver can be harmed by viruses as in hepatitis & by

parasites causing amoebiasis & schistosomiasis. Long term use of certain drugs can also damage liver.

Certain HCV (Hepatitis C-virus) proteins can induce derangement of lipid metabolism or alter signal transduction of infected hepatocytes which leads to the production of reactive oxygen radical & profibrinogenic mediators in particular TGF beta-1 [3]. TGF beta-1 plays a pivotal role in the pathogenesis of post inflammatory liver scarring [4]. Not only HCV, HIV also is connected with liver fibrosis.

HIV infection modifies HCV & accelerates rate of fibrosis. A low CD4 cell count was independently associated with disease which suggests that early antiretroviral therapy may be of benefit in slowing HCV progression in co infected patients [5]. Hepatitis C infection alters the AST/ALT ratio of >1 which along with a platelet count of <1,50,000 can predict advanced stage of fibrosis and cirrhosis [6].

Among the 6 types of cells of liver, Hepatic stellate cells (HSC) are closely associated with fibrosis. Normally, HSC store vitamin-c and on activation become the major producers of extracellular matrix including collagen type-I & II. Therefore stellate cells are responsible for hepatic fibrogenesis & development of cirrhosis. Under the direction of growth factor, oxidants & additional stimuli released from injured hepatocytes, bile duct epithelia, kupffer cells or other inflammatory cells cirrhosis can progress to end stage liver disease & give rise to liver cancer.

Liver fibrosis is scar tissue formation as a result of chronic inflammation. The scar occurs when liver tries to repair repeatedly the damaged tissues. The scar results from excessive secretion of matrix proteins by HSC which proliferate during fibrotic liver injury [7]. When fibrosis becomes widespread and progresses to the point that the internal structure of liver has become abnormal, it results in cirrhosis.

Liver fibrosis is a significant cause of death. The development of anti-fibrotic drug is still at infancy for the simple fact that long term consumption of the drug should be free from toxic effects. Hence plants are being investigated. One such plant is *Cleome viscosa*. Various methods have been adopted to induce liver fibrosis. Bile duct ligation is a common technique for induction. However various chemicals can harm the liver leading to fibrosis. One such chemical is $CCl_4$ [8].

Hence in this method, the protective effect of *Cleome viscosa* was investigated using $CCl_4$ induced liver fibrosis models.

## Results and Discussion

***Biochemical Parameters:***

The liver fibrosis is characterized by proliferation of HSC & excessive deposition of extra cellular matrix (ECM). The extent of liver fibrosis was assessed in terms of biochemical parameters, hydroxy proline (HP) content, thiobarbituric acid (TBA) and hematological parameters. The statistically significant increase in biochemical parameter viz AST, ALT, ALP,

GGTP, TBA, TBL and decrease in total platelet count were observed after CCl4 treatment. Following treatment with the extract at two different dose levels (100 & 200mg/kg), there was significant fall in these biochemical parameters & TPC restored to normal value (**Table-1 & 2**).

**Table – 1: Effects of Ethanolic Extract of *Cleome viscosa* on Biochemical Parameter**

| TREATMENT | AST(IU/L) | ALT(IU/L) | ALP(IU/L) | TBL(mg/dl) | GGTP(IU/L) |
|---|---|---|---|---|---|
| NORMAL CONTROL(G1) | 98.62 ±1.92 | 29.1±0.52 | 110.08±2.55 | 0.602±0.008 | 84.07±1.12 |
| EXTRACT ALONE(G2) | 97.33±4.06 | 28.23±0.89 | 104.96±3.17 | 0.4977±0.012 | 83.82±1.34 |
| $CCl_4$ ALONE(G3) | 181.94±55.67*a | 48.95±0.45*a | 222.91±4.21*a | 1.623±0.026*a | 154±0.47*a |
| $CCl_4$+EXTRACT (100mg/kg)(G4) | 107.53±3.98*b | 31.05±1.174*b | 119.00±2.32*b | 0.7620±0.016*b | 92.6±5.19*b |
| $CCl_4$+EXTRACT (200mg/kg)(G5) | 99.25±1.76*b | 29.5±0.34*b | 110.9±2.35*b | 0.6182±0.008*b | 84.8±1.32*b |

**Table – 2:**

| TREATMENT | HP (µg of liver) | TPC (lakhs/cu.mm) | TBA (p.mol/mg protein) |
|---|---|---|---|
| NORMAL CONTROL(G1) | 252±4.98 | 3.10±0.015 | 194±2.32 |
| EXTRACT ALONE(G2) | 240±2.61 | 2.99±0.021 | 189±0.82 |
| $CCl_4$ ALONE(G3) | 524±6.79*a | 1.60±0.009*a | 372±1.22*a |
| $CCl_4$+EXTRACT (100mg/kg)(G4) | 360±5.30*b | 2.52±0.012*b | 241±0.89*b |
| $CCl_4$+EXTRACT (200mg/kg)(G5) | 288±1.40*b | 3.00±0.014*b | 206±0.72*b |

Values are represented as Mean ± SEM (n=6), Newman keul's multiple range test ($p<0.05$) is used, *a values significantly different from Normal control, *b values significantly different from Toxic control

A considerable reduction in body weight was noted in $CCl_4$ treated rat. Simultaneous treatment with the extract at two different doses (100 & 200mg/kg) restored the body weight. The weight of liver in $CCl_4$ treated rat was increased, which was reduced by extract treatment at both the doses (**Table- 3**).

**Table – 3: Effects of Ethanolic Extract of *Cleome viscosa* on Liver & Body Weight**

| TREATMENT | BODY WEIGHT (gm) DAY 1 | BODY WEIGHT (gm) DAY 28 | LIVER WEIGHT (gm) ON DAY 28 |
|---|---|---|---|
| NORMAL CONTROL(G1) | 160.11±4.89 | 161.33±1.44 | 2.79±1423 |
| EXTRACT ALONE(G2) | 160.82±1.56 | 163.22±1.79 | 2.624±0.155 |
| $CCl_4$ ALONE(G3) | 163.05±1.34 | 157.45±0.46 | 3.263±0.2018*a |
| $CCl_4$+EXTRACT (100mg/kg)(G4) | 165.52±1.32 | 161.13±1.12 | 2.557±0.2276*b |
| $CCl_4$+EXTRACT(200mg/kg)(G5) | 166.4±1.33 | 164.62±1.04 | 2.191±0.172*b |

Values are represented as Mean ± SEM (n=6), Newman keul's multiple range test ($p<0.05$) is used, *a values significantly different from Normal control, *b values significantly different from Toxic control

Elevation of enzymes AST, ALT, ALP, GGTP, TBA, TBL & HP content of liver following $CCl_4$ administration indicate the hepatic injury & fibrosis caused by toxicant. This is further substantiated by increase in liver weight which might be due to deposition of collagen.

***Histopathological Studies:***
G1 & G2 section showed structure of liver with normal hepatocytes & parenchyma, whereas in G3, the section showed liver parenchyma with sheets of hepatocytes showing hydropic & fatty changes. Severe necrosis occurs. There was dense periportal inflammatory infiltrate & periportal fibrosis indicating severe hepatic injury.

G4 & G5: sinusoids show dilatation, Necrosis is mild but showing hydropic change & mild fatty change in hepatocytes.

The most widely used method for inducing the fibrosis is $CCl_4$ administration. $CCl_4$ undergoes bioactivation by CYP450 to a reactive metabolite trichloromethyl radical. The free radical thus generated cause hepatic injury by initiating peroxidation. Simultaneous treatment with ethanolic extract reduces the degree of hepatocellular injury as evidenced by improved biochemical parameters & the hydroxyl proline content. The reason for this improvement may be that whole plant of *Cleome viscosa* contains flavonoids, which might have scavenged free radical offering hepatoprotection. The extract at higher dose 200mg/kg is more hepatoprotective & antifibrotic. Purification of extract & identification of active principle might yield a good hepatoprotective drug.

***Statistics:***
The results were expressed as Mean ± SEM. The data was evaluated using one way Anova followed by Newman- keuls multiple range test & differences below $p<0.05$ are considered as significant.

# Materials and Methods

Male albino wistar rats (150-180gm) of good health were selected. They were given standard pellet diet. Throughout the experiment, the animals were housed 2 or 3 per cage maintained at room temperature under 12 h light / dark cycles. The animals were kept in these facilities for at least one week before the experiment. The study has been approved by institutional animal ethical committee.

***Preparation of Ethanolic Extract from Whole Plant of Cleome viscosa:***
The fresh leaves were collected in & around Madurai, Tamilnadu. Multiple biological properties have been described for the herb including antidiarrhoeal, analgesic, and antiseptic. The seeds & leaves are mainly used for medicinal purposes. The whole plant of *Cleome viscosa* Linn was collected & identified, cut into small pieces, shade dried & powdered. About 1000gm of dry powder was extracted with petroleum ether & ethanol at $60^{0}$-$70^{0}$c by continuous hot percolation using soxhlet apparatus, extraction continued for 72hrs. The petroleum ether & ethanol extract was filtered & concentrated to dry mass by using vacuum distillation. The yield for 1000gm of drug were 15gm (petroleum ether extract) & 20gm (ether extract). The ethanol extract was

purified using glass column of 2.4cm diameter packed with activated silica gel in the form of slurry. The column was packed to height of 65cm in order to establish a column of 300ml. The column was then developed with a series of solvent starting with n-hexane, petroleum ether, benzene, acetone, ethyl acetate, ethanol & methanol.

***Induction of Liver Fibrosis by $CCl_4$:***

$CCl_4$ was administered to rats orally1ml/kg of body weight, mixed with equal volume of corn oil twice a week for 28 days [9]. Simultaneously the animals were treated with ethanol extract (100 & 200mg/kg) of whole plant of *Cleome viscosa* Linn for same period [10]. One group of rats was just treated with extract only, another group which was neither treated with $CCl_4$ & extract was kept as normal control. On day 28, the animals were sacrificed under ether anesthesia and blood & liver samples were collected. The level of serum aspartate aminotransferase (AST), alanine aminotranferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGTP), total bilurubin (TBL), hydroxy proline (HP), thiobarbituric acid (TBA) and total platelet count (TPC) are estimated. The liver samples were used for histopathological studies.

# Conclusion

The results of these studies imply that the ethanolic extract of *Cleome viscosa* Linn is having protective effect against carbon tetra chloride ($CCl_4$) induced liver fibrosis.

# References

[1] C.P. Chung, J.B. Park, K. Bae, *Chang. Gung. Med. J*., **2003**, 26(3), 193-198.
[2] Mary F Lipscomb, In: L. Stanley Robine, S. Ramzi Cotran, Vinay kumar (Ed.), Basic Pathology, W.B. Saunders Company, USA, **1987**, 582-597.
[3] D. Schuppar, A. Krebs, M. Bauer, *Cell Death Differ*., **2003**, 10(1), 56-67.
[4] H. Taniguchi, N. Kato, M. Otuska, *J. Med. Virol*., **2004**, 72 (1), 52 - 59.
[5] A.H. Mohsen, P.J. Easter brook, C. Taylor, *Gut*., **2003**, 52 (7), 1035 – 1040.
[6] N. Klokhar, *J. Pak. Med. Assoc*., **2003**, 53 (3), 101 – 104.
[7] J.P. Iredale, R.C. Benyan, J. Pickering, *J. Clin. Invest*., **1998**, 102 (2), 538 –549.
[8] M. Bickel, E. Baeder, Brocks, *Gut*., **2001**, 52, 234 -238.
[9] G. Achliya, S. Wadodker, A. Dorle, *Indian. J. Pharm. Sci*., **2004**, 66(2), 252 -254.
[10] B. Parimala Devi, R. Boominathan, S.C. Mandal, *Fitoterapia*., **2003,** 74 (3), 262 -266.