

HEPATOPROTECTIVE EFFICACY OF MOLLUGO CERVIANA LINN AGAINST CARBON TETRACHLORIDE INDUCED LIVER DAMAGE IN RATS

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Abstract

Liver damage caused by pathogen as well as chemical reagents are of similar nature and a proper treatment regime is absent for both. Most of the hepato toxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages. Lack of allopathic medicine for the cure of hepatic injury exhorted the scientist to explore herbal medicine. Thus we have attempted to establish the hepatoprotective potency if *Mollugo cerviana*. In the present investigation we examined the hepato protective activity alcoholic extract of *Mollugo cerviana* on CCl₄ induced hepato toxicity. The results revealed that all the enzymes were significantly increased in rats treated with CCl₄ compared with control rats. Silymarin (25 mg / kg) was used as positive control. *Mollugo cerviana* treated rats also showed a marked increase in activities of antioxidant enzymes. The increased activities of these enzymes antioxidant defenses and hence increased scavenging of free radicals and decreased lipid peroxidation. The presence of flavonoids compound in the extract may be responsible for the significant hepatoprotective property.

Introduction

Liver plays a major role in detoxification and excretion of many endogenous any exogenous compounds, and injury to it or impairment of its function may lead to many complications on one's health¹. There is no rational therapy available for treating liver disorder and is still a challenge to the modern medicine. Modern medicines have little to offer for alleviation of hepatic ailments whereas most important representatives are of phyto constituents.

The liver injury is caused by hepato toxins, microbial infections and excessive alcohol ingestion or during certain therapy². Most of the injury is caused due to the oxidative damage by free radical generation, which leads to the formation of lipid per oxidation products³. Traditional medicines are very effective in curing many liver disorders. Among them *Mollugo cerviana* is also are of the traditionally used medicines, which commonly practiced in many villages of India due to its various beneficial effects. It is considered as a good stomachic, aperient and anti bacterial⁴. Our present study has been designed with an aim to evaluate the hepato protective activity of crude methanol extract of the plant and the salient features are reported after observing the bio chemical parameters in blood and histopathological parameters in liver.

Material and Methods

Collection of Plant Material

The fresh whole plants of *Mollugo cerviana* were collected from Tirunelveli, Tamilnadu, India. They were authenticated by Dr. V. Chelladurai, Department of Botany, Central Siddha Research Unit, Tirunelveli. The sample voucher was preserved for further reference.

Preparation of Extract

The dried and powdered whole plant was extracted with methanol for 48 hrs by using soxhlet apparatus. The extract was filtered and the filtrate was evaporated to dryness under reduced pressure using rotary evaporator to get semisolid mass and used for further investigation.

Preliminary Phyto chemical Screening of the Methanolic Extract

Preliminary phyto chemical screening of the crude methanolic extract was done by thin layer chromatography and the qualitative chemical tests was conducted for the detection of flavonoids, triterpenoids, saponins and tannins.

Chemicals

All chemicals were of the high quality from BDH Chemicals and Sigma Aldrich Chemical Co. All other solutions were prepared in double distilled deionised water.

Animals

Adult Wister albino rats of either sex (200 – 250 gms) were procured for the study. They were kept under standard laboratory conditions and were fed with commercial rat pellets and drinking water *ad libitum*. The animals were fasted over night before performing the experiment. The experiment protocol was duly approved by Institutional Animal Ethics Committee. (Registration No. 265/ CPCSEA)

Acute Toxicity Studies

Acute toxicity study was carried out as “up and down” or “star case” method. The maximum non lethal dose was found to be 2000 mg / kg body weight; hence 1/10th of the dose was taken as an efficient dose (200 mg / kg body weight).

CCl₄ Induced Hepato toxicity in Rats

The experimental animals were dividing into 5 groups of rats each. The animals in Group I served as the control. The group II, III, IV & V received CCl₄ (0.5 ml / kg, i.p). The group III, IV received aqueous and methanolic extracts (200 mg / kg, p.o) respectively. Group V received silymarin (25mg / kg, p.o). Duration of treatment has been carried out for 14 days. All the animals were sacrificed on 15th day. Blood samples were collected from carotid artery and allowed to coagulate for 30 min at 37^oc.

Clear serum was separated and used for the estimation of total bilirubin, total protein and marker enzymes (SGOT, SGPT, AST and ALP). Then the liver was carefully isolated and cleaned off extraneous tissue and preserved in 10% neutral formalin and then subjected to histopathological studies.

Estimation of Biochemical Parameters

Blood samples were collected from carotid artery and serum was separated for estimation of biochemical parameters. Total protein and bilirubin was estimated by Biuret method⁵⁻⁶. The marker enzymes (Ast and Alp) were studied by Reitzmen method and Bessey method⁷⁻⁸ respectively.

Histopathological Studies

The livers were excised from the experimental animals of each group after collecting the blood sample and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin and then with bovine solution. They were processed for paraffin embedding following the microtome technique⁹. The sections were processed in alcohol xylene series and were stained with haemotoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes.

Statistical Analysis

Values were represented as mean ± SEM. Data were analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test¹⁰. P < 0.01 was considered significant.

Results

The preliminary phyto chemical screening showed the presence of essential phyto constituents such as flavonoids, saponins, terpenoids and tannins.

From the acute toxicity studies, the LD₅₀ dose was found to be 2000 mg / kg body weight. 1 /10th of the LD₅₀ dose were taken as an effective dose (200 mg / kg body weight). CCl₄ intoxication in normal rats elevated the serum levels and bilirubin significantly. The rats treated with alcoholic and aqueous extracts of *Mollugo cerviana* linn. showed a significant reduction in all biochemical parameters elevated by CCl₄ (Table 1).

Discussion

Results indicated that the plant of *Mollugo cerviana* provides significant protection against the toxic effect of CCl₄ on liver. In CCl₄ induced toxic hepatitis, toxicity begins with the changes in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures¹¹. The toxic metabolite CCl₃ radical produced by microsomal oxidase system, binds covalently to the macromolecule and causes peroxidation degradation of lipid membranes of the adipose tissues. The blood samples of the treated group animals showed drastic increase in the levels of serum total bilirubin, SGOT, SGPT, AST and ALP. On the contrary, the total protein level was decreased as compared to the control. Accumulation of higher concentration of bilirubin and lower level of total protein confirms the depth and intensity of liver damage. The rapid elevation in the levels of serum aspartate transaminase indicates the extent of liver necrosis.

Administration of aqueous and methanolic extracts of *Mollugo cerviana* showed recovery against the toxic effects of CCl₄ of shown in the Table 1. Among these significant hepato protective was noticed in the group IV animals treated with methanolic extract of *Mollugo cerviana*, while the aqueous extract treated animals exhibited less hepato protective activity.

Histo pathological profile of the methanolic extract treated animals should the recovery against the CCl₄ induced necrosis in their manual compact arrangement of hepatic cells (Fig .3) as compared to the controls. Where as, the section of the animals treated with aqueous extracts showed moderate accumulation of fatty lobules around the vein (Fig .4) but the extent of liver damage was lesser in magnitude as compared to the CCl₄ treated animals.

Conclusion

CCl₄ produces the dose dependent hepato toxicity by directly affecting the liver causing lipid peroxidation. The mechanism of action of CCl₄ is complex multi factorial and not completely understood. When administrated CCl₄ accumulates in hepatic parenchyma cells, which is metabolized to CCl₃ free radicals as (CCl₄ as *CCl₃ + Cl). The radicals react with molecular oxygen to produce peroxy radicals (H₂O₂, O₂ and *OH due to incomplete reduction of molecular oxygen) thereby causing the oxidative destruction of poly unsaturated fatty acids.

The biochemical studies in albino rats revealed that CCl₄ induced hepatic injury was inhibited significantly (P < 0.001) by alcoholic and aqueous extract of the whole plant of *Mollugo cerviana*. All the results can be comparable with the standard drug silymarin (25 mg / kg body weight).

The alcoholic extract has shown significant hepatoprotective activity when compared to aqueous extract. In support the histopathological reports also revealed that there is a regenerative activity with the liver cells.

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References

- [1] Tripathy K.D, Pharmaco dynamics, In Essential of Medical Pharmacology, New Delhi, India: Jaypee Brothers medical Publishers; 1999, 64.
- [2] Bowman W.C, Rand M.J. The liver Hepatic Function and drugs Effecting the Liver, Text book of Pharmacology, London: Blackwell Scientific Publication; 1984, 26-28.
- [3] Feher J, Cosmos G, Verecke A, In Free radical reactions in Medicine, Springer verley Berlin ed. 1986, 107-122.

- [4] Kim SS, Kim Jy, Lee NH, Hyun CG. *J. Gen. Appl. Microbiol.*, 2008, 54 (2), 101-106.
 [5] Mallory H.T and Evelyn E.A: The determination of bilirubin with photo electric colorimeter, *J. Biol. Chem.*, 1937, 119-485.
 [6] Kingsley S.R: The determination of serum total protein, albumin and globulin by the Biuret reaction, *J. Biol. Chem.*, 1939, 131-197.
 [7] Reitzman S. and Frankel S: A Colorimetric method for the determination of serum glutamic oxalo acetic acid, glutamine pyruvic transaminase, *Amer. J. Clin. Path.*, 1957 28, 56-63.
 [8] Bessey O.A, Lowery D.M and Brock M.J: A Method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *J. Biol. Chem.*, 1964, 164, 321-329.
 [9] Galigher A.E and Kayloff E.N: Essential of Practical Micro techniques, Lea and Febiger, Philadelphia, 1971, 77.
 [10] Dunnet C.W: Biometrics, 1964, 20,482.
 [11] Reznagel R.O: Carbon tetrachloride hepato toxicity status QUO and further prospects, *Trends Pharmacol. Sci.*, 1983, 4,129-131.
 [12] Hawk P.B, Oser B.L and Summurson W.H, In: Hawk P.B., Ed. Bile and Liver Functions- Practical Physiological Chemistry, Mc Graw Hill Press, NewYork, 1954, 408.
 [13] Gebhardt R, Oxidative Stress- Plant derived antioxidants liver fibrosis, *Planta Medica*, 2002, 68,289-296.

Table1: Effect of Aqueous and Alcoholic Extracts of *M.Cerviana* on CCl₄ Induced Hepato toxicity in Rats

Group	Total Bilirubin mg/dl	Total protein gm %	SGPT IU/L	SGOT IU/L	AST IU/L	ALP IU/L
Control	0.39±0.04	9.25 ±0.12	35.08±2.2	97.3±6.6	52.04±3.284	15.92±1.2
CCl ₄	0.89±0.03	5.96±0.30	136.9±7.6	186.7±5.2	1386.24±29.10	98.3±4.3
CCl ₄ + Aqueous extract	0.72±0.06*	8.246±0.32*	95.21±3.3*	154.57±4.7*	95.54±7.692*	61.25±2.6*
CCl ₄ + Alcoholic extract	0.66±0.05**	8.012±0.212*	78.54±2.3*	138.2±8.4*	202.72±32.40*	49.25±2.7**
Silymarin + CCl ₄	0.57±0.02*	9.824±0.292*	49.4±2.3**	105.3±3.5**	51.25±1.52*	43.6±1.7*

Data are expressed as Mean ± SEM, n=6

*P <0.01 Vs Control

**P <0.001 Vs Control