

HEPATOPROTECTIVE EFFECT OF *AVERRHOA BILIMBI* LINN. AGAINST CARBON TETRACHLORIDE INDUCED HEPATIC DAMAGE IN RATS.

Dnyaneshwar M. Nagmoti, Shekhar B. Yeshwante, Shaijesh S. Wankhede, Archana R. Juvekar*

Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology (ICT), Matunga, Mumbai-400 019, (MH), India.

* Corresponding Author:

Dr. (Mrs.) Archana Juvekar

Professor in Pharmacology and Physiology

Department of Pharmaceutical Sciences and Technology,

Institute of Chemical Technology (ICT),

Matunga, Mumbai-400 019, (MH), India.

Email: arj04@rediffmail.com, dmnagmoti@gmail.com

Ph. No.: 02233611111 Ext. 2215

Summary

The present study examined the hepatoprotective activity of methanolic extract of *Averrhoa bilimbi* (leaves) in carbon tetrachloride intoxicated rats. Liver toxicity was induced by intraperitoneal administration of carbon tetrachloride (CCl₄) at the dose of 1 ml/kg with a gap of 72 hrs for 10 days in wistar rats. The hepatoprotective activity of methanolic extract of *Averrhoa bilimbi* was evaluated by measuring levels of serum marker enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and alkaline phosphatase (ALP), total protein and bilirubin. Administration of methanolic extract of *Averrhoa bilimbi* (250 and 500 mg/kg, p.o.) significantly (p< 0.01) prevented CCl₄-induced elevation of levels of serum GPT, GOT, ALP, and bilirubin. The total protein level was decreased due to hepatic damage induced by CCl₄ and it was found to be increased in methanolic extract of *Averrhoa bilimbi* treated group. Treatment of rats with CCl₄ led to a marked increase in lipid peroxidation as measured by malondialdehyde (MDA) which was associated with a significant reduction of the hepatic antioxidant system like reduced glutathione (GSH). These biochemical alterations resulting from CCl₄ administration were significantly (p< 0.01) inhibited by treatment with methanolic extract of *Averrhoa bilimbi*. The results are comparable with standard drug Silymarin (100 mg/kg). These data suggest that the methanolic extract of *Averrhoa bilimbi* may act as a hepatoprotective and antioxidant agent.

Keywords: *Averrhoa bilimbi*, carbon tetrachloride, hepatoprotective, Silymarin

Introduction

Liver diseases remain one of the serious health problems due to the absence of reliable liver protective drugs in allopathic medical practices, herbs play a role in the management of various liver disorders. A number of medicinal plants and their formulations are used for liver disorders in ethnomedical practice as well as traditional system of medicine in India ¹.

Averrhoa bilimbi Linn.(Oxalidaceae, common name: Bilimbi), a common plant in Asia, is a small-sized tree growing up to 15 m tall and 30 cm diameter. The chemical constituents of *A.bilimbi* that have been identified include amino acids, citric acid, cyanidin-3-O- β -D-glucoside, phenolics, potassium ion, sugars and vitamin A². The fruits and leaves are valued in medicine and used as antibacterial, antiscorbutic, anti-inflammatory, astringent; post-partum protective medicine; treatment of fever, mumps, pimples, inflammation of the rectum and diabetes (decoction of the leaves); treatment of itches, boils, rheumatism, cough and syphilis (paste of leaves); treatment of scurvy, bilious colic, whooping cough, hypertension and as a cooling drink (juice of preserved fruits); treatment of children's cough (syrup of flowers); treatment of stomach ache (fruits). In French Guiana, it is prescribed in inflammatory conditions especially in hepatitis, diarrhoea and bilious colic³. Preview of the literature revealed that though this plant is known for its hepatoprotective activity by the tribal groups it has not subjected to scientific evaluation, hence the present study aims to evaluate the hepatoprotective potency of this plant against CCL₄ induced hepatic damage in albino wistar rats.

Material and Methods

Drugs and Chemicals

Carbon tetrachloride (CCl₄) was obtained from S.D. Fine Chem. Ltd., Mumbai. Silymarin was obtained from Micro labs, Bangalore. Thiobarbituric acid (TBA), 5-5'-Dithio-bis 2-Nitrobenzoic acid (DTNB) and Glutathione (GSH) were obtained from Sigma Chemical Company, St. Louis, MO, USA. All other chemicals were obtained from local sources and were of analytical grade.

Plant material

The fresh leaves of *Averrhoa Bilimbi* were collected from gardens of Institute of science, Mumbai-32. The leaves were then authenticated by Dr. T. Srinivasu, Botanist at Institute of Science, Mumbai. Leaves were shade dried and defatted with petroleum ether. The defatted material was extracted with 95% methanol and then vacuum dried.

Animals

Wistar rats (150-200g) of either sex were used. They were allowed to acclimatize one week before experimentation; in the departmental animal house. The animals were maintained under standardized environmental conditions (25-30°C, 12 hr dark/light cycle) and fed with standard rat feed (Amrut India Ltd. Pune) and water *ad libitum*. The Institutional Animal Ethics Committee of UICT, Matunga, Mumbai, approved the experimental protocol in accordance with the guidelines provided by committee for the purpose of Control and supervision of Experiments on animals with registration no. 87/1999/CPCSEA.

Acute toxicity study

Oral acute toxicity study in wistar rats were carried out for methanolic extract of *Averrhoa bilimbi* in accordance with OECD guideline no.423 and LD50 of the extract was found to be 5000 mg/kg b.w. One tenth of this (i.e. 500 mg/kg b.w.) was selected as maximum dose for the evaluation of antihepatotoxic activity.

Experimental procedure:

Wistar albino rats of either sex (150-200g) were selected and divided into five groups of six animals each. The animals were treated as follows: Group I animals served as normal control and received olive oil (vehicle) 1.0 ml/kg body wt intraperitoneal (*i.p.*). Group II animals constituted the hepatotoxic group, which received 30% CCl₄ suspended (in olive oil 1.0 ml/kg body wt *i.p.*) after every 72 hr for 10 days. Group III and IV received methanolic extract of *Averrhoa bilimbi* (250 and 500 mg/kg b wt/day) and Group V received silymarin (100 mg/kg b wt/day) for 10 days and CCl₄ was given as in group II rats.

Statistical analysis:

Results were expressed as mean \pm S.D. from six rats in each group. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnet's test. P values < 0.01 were considered significant.

Results

The effect of methanolic extract of *Averrhoa bilimbi* on serum transaminases, alkaline phosphatase, bilirubin and total protein levels in CCl₄ intoxicated rats are summarized in Table 1. There was a significant increase in serum GPT, GOT, ALP and bilirubin levels. The total protein levels were significantly decreased in CCl₄ intoxicated rats. The methanolic extract of *Averrhoa bilimbi* at the doses of 250 and 500 mg/kg orally significantly ($p < 0.01$) decreased the elevated serum marker enzymes and reversed the altered total protein to almost normal.

The effect of methanol extract of *Averrhoa bilimbi* on lipid peroxidation (expressed in terms Thiobarbituric acid reactive substances (TBARS)) and glutathione levels in rat liver tissue are shown in Table 2. Lipid peroxidation levels were significantly increased and glutathione levels were significantly decreased in CCl₄ treated rats when compared with that of the normal animals. Treatment with methanolic extract of *Averrhoa bilimbi* at the doses of 250 and 500 mg/kg orally significantly decreased ($p < 0.01$) the elevated lipid peroxide levels and the altered glutathione levels restored to the near normal which are comparable to the standard drug (Silymarin) in CCl₄ intoxicated rats.

From the tables, it is clear that methanolic extract of *Averrhoa bilimbi* showed dose-dependent hepatoprotective activity. However, methanolic extract of *Averrhoa bilimbi* (500 mg/kg, p.o.) exhibited relatively higher protective action, which is comparable with the standard drug, Silymarin (100 mg/kg, p.o.).

Table No. 1: Effect of *Averrhoa Bilimbi* methanolic extract on liver marker enzymes in the serum of control and experimental animals.

Values are Mean±SD for 6 animals in each group

$P \# < 0.01$ considered significant as compared to normal control.

$P^{**} < 0.01$ considered significant as compared to negative control (CCl₄ induced).

$P^* < 0.05$ considered significant as compared to negative control.

Parameters	Normal (Group I)	CCl ₄ induced (Group II)	CCl ₄ +ABM (250 mg/kg) (Group III)	CCl ₄ +ABM (500 mg/kg) (Group IV)	CCl ₄ +Silymarin (100 mg/kg) (Group V)
ALT (U/L)	88.31±23.19	309.55±25.75#	232.94±14.38**	218.97±9.75**	189.73±6.37**
AST(U/L)	98.06±4.56	371.75±5.02#	233.45±24.57**	212.41±15.21**	203.11±14.60**
ALP(U/L)	85.60±8.17	261.09±11.91#	194.60±5.26**	179.31±6.70**	152.32±9.34**
Bilirubin	0.72±0.13	1.18±0.02#	1.12±0.007ns	1.06±0.078*	1.05±0.03*
Total protein	3.04±0.14	1.60±0.093#	2.27±0.09**	2.42±0.28**	2.58±0.08**
Albumin	4.07±0.08	1.36±0.04#	2.08±0.24**	2.12±0.19**	2.85±0.38**

[ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase.]

Table No. 2: Effect of *Averrhoa Bilimbi* methanolic extract on lipid peroxidation and reduced glutathione levels in the liver tissue of control and experimental animals.

Parameters	Normal (Group I)	CCl ₄ induced (Group II)	CCl ₄ +ABM (250 mg/kg) (Group III)	CCl ₄ +ABM (500 mg/kg) (Group IV)	CCl ₄ +Silymarin (100 mg/kg) (Group V)
Lipid peroxidation	0.18±0.01	0.63±0.03#	0.47±0.02**	0.38±0.004**	0.32±0.002**
Reduced GSH	7.12±0.34	4.38±0.39#	5.16±0.35**	5.88±0.12**	6.01±0.18**

Values are Mean±SD for 6 animals in each group

P #<0.01 considered significant as compared to normal control.

P **<0.01 considered significant as compared to negative control (CCl₄ induced).

Values are expressed as GSH, glutathione (µmoles/mg protein); lipid peroxidation [µM TBARS formed/ml/mg pr]

Discussion

Carbon tetrachloride is one of the most commonly used liver toxicant in the experimental study of liver diseases and the hepatotoxic effects are largely due to its active metabolite, trichloromethyl radical (CCl₃•) or trichloroperoxy radical (CCl₃O₃•)⁴. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum which produce damage to the membrane⁵. The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in transaminases and alkaline phosphatase was the clear indication of cellular leakage and loss of functional integrity of the cell membrane⁶. The present study revealed a significant increase in the activities of SGOT, SGPT, ALP and serum bilirubin levels on exposure to CCl₄, indicating considerable hepatocellular injury. Administration of methanolic extract of *Averrhoa bilimbi* at two different dose levels attenuated the increased levels of the serum enzymes, produced by CCl₄ and caused a subsequent recovery towards normalization almost like that of Silymarin treatment.

Serum albumin is a marker of synthetic function of the liver⁷. CCl₄ administration alters the protein metabolism by impairing albumin synthesis and causes bilirubin excretion in blood due to obstruction of bile canaliculi^{8,9}. Hence hypoalbuminaemia and hyperbilirubinemia can be deemed as useful index of the severity of cellular dysfunction. The attainment of near normalcy in albumin content and stabilization of plasma bilirubin levels on methanolic extract of *Averrhoa bilimbi* is a clear indication of improvement in the liver function (Table 1). GSH is a critical determinant of tissue susceptibility to oxidative damage and the significant impairment of hepatic GSH associated with a substantial hepatocellular damage induced by CCl₄, suggested the determinant role of hepatic GSH in the development of carbon tetrachloride toxicity¹⁰. Depletion of GSH results in enhanced lipid peroxidation, which in turn causes increased GSH

consumption¹¹. In the present study, a significant decrease ($P<0.01$) in the liver GSH was observed after the administration of CCl_4 compared to normal controls. Methanolic extract of *Averrhoa bilimbi* significantly enhanced capability of the cells to cope up with CCl_4 induced free radical damage by increasing the GSH levels and reducing the TBARS levels (Table 2).

All the effects of methanolic extract of *Averrhoa bilimbi* were comparable with those of silymarin, a proven hepatoprotective agent. The results of our study indicate that administration of methanolic extract of *A. bilimbi* leaves showed significant hepatoprotective and antioxidant activity against CCl_4 induced hepatotoxicity.

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