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**Research Article**

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# Hepatoprotective effect of various extracts of *Bambusa vulgaris* Striata on Carbon tetrachloride-induced liver injuries

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**ABSTRACT**

*Herbal medicines are prescribed commonly for the treatment of hepatic diseases and disorders. The aim of the present study was to investigate the hepatoprotective activity of various extracts of Bambusa vulgaris against carbon tetra chloride ($CCl_4$) induced hepatic damage in rats. The liver marker enzyme such as serum glutamate oxy-transaminase (SGOT), serum glutamate pyurate transaminase (SGPT) and alkaline phosphatise (ALP). The serum biochemical analysis results suggest that the chloroform extract of Bambusa vulgaris at the dose of 250 mg/kg body weight exhibited significant protective effect from hepatic damage in $CCl_4$ induced hepatotoxicity model. Histopathological analysis of the $CCl_4$ induced animal showed severe necrosis, this study also supported the protective effect of the hepatic disorder.*

**Key words:** *Bambusa vulgaris;* carbontetrachloride; histopathology, hepatoprotective, Chloroform extract, necrosis.

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## INTRODUCTION

Liver plays a pivotal role in regulating the body's internal chemical environment. It is involved in several important functions like metabolism, secretion and storage. It has a great capacity to detoxicate the toxic substances, synthesize physiologically vital principles [1].

Liver diseases are worldwide problem. Management of liver diseases has become a critical concern in medical science. Very few drugs available in allopathy system of medicine are not free from side effects. So, there is an enormous scope for the herbs in the management of liver diseases. Search for herbs available locally for treating hepatitis is continuing to reduce the cost of treatment [2][3][4]

*Bambusa vulgaris* is a pantropical species, origin of the species is unknown but most commonly cultivated everywhere, especially the horticultural varieties with yellow culms, the genus of bamboo are *arundinacea, surinamensis, Oxytenanthera, Dendrocalamus tulda* are present throughout the continent under rainfall ranging from 700 to 1500 mm. This is a medium-sized bamboo, not densely tufted with culms 8-20 m tall. Culms in yellow or green stripes, flowering are not common. Internodes 25-35 cm long, 5-10 cm diameter and thickness of wall ranges 7-15 mm. Inflorescence panicle, with many spikelet, no seeds[2][3][4]. Vegetative propagation methods, Culm cuttings, rhizome planting, branch cutting, layering. Because of the monocarpic character, episodic flowering, over-exploitation and bush fires, Thanks to its very easy adaptation to certain favourable ecological conditions, bamboo is often used to fight against water and wind erosions. Bamboo is a multipurpose species and is utilized in various handicraft, building, food and medicine. Although *Bambusa vulgaris* is taxonomically a grass, its habit is tree-like. It

is the most widespread member of its genus, and has long been cultivated across the tropics and subtropics. Vulgaris species are traditionally used for hepatic disorder [5].

The present research was taken up to explore the hepatoprotective property of various extracts of B. vulgaris, which is not reported earlier. Carbon tetrachloride induced hepatotoxicity model in Albino rats was selected for the evaluation of the hepatoprotective activity. Silyamarin was used as a standard for comparison [6].

## MATERIALS AND METHODS

### Plant material

Leaves of *Bambusa vulgaris* were collected in Kangra District of Himachal Pradesh, during the month of March. The plant was authenticated by Forest Research of India, Dehradoon, Uttarakhand. The collected material was dried at room temperature under shade for 15 days, and then it was converted into coarse powder using grinder.

### Preparation of extracts

The air-dried leaves were ground to a fine powder, defatted with Petroleum ether, and macerated with chloroform, ethyl acetate and methanol successively to give chloroform extract (CE), ethyl acetate extract (EAE), and methanol extract (ME). The extracts were collected separately and reduced to a small volume under reduced pressure.

### Phytochemical screening of successive extraction of *B. Vulgaris [7]*

1. Alkaloids

The small portions of the extracts were dissolved in suitable solvent and each extract was stirred separately with few drops of dilute hydrochloric acid and filtered. The filtrate was tested for alkaloids by using the following reagents.

a. Mayer's reagent (Potassium mercuric iodide)
b. Dragendroff's reagent (Potassium bismuth iodide)
c. Wagner's reagent (iodine potassium iodide)
d. Hager's reagent (saturated picric acid)

2. Carbohydrates

A small portion of the extract was dissolved separately in 5ml of water and filtered. The filtrate was subjected to the following test.

a. Molish Test

To a small portion of the filtrate alpha-naphthol in alcohol was added followed by con. $H_2SO_4$ was added through the sides of the test tube.

b. Fehling's solution

To a solution of the substance, a mixture of equal parts of Fehling's solution A and B was added and the test tube was heated on a water bath.

c. Barfoed's test

To a small portion of the substance, Barfoed's solution was added and it was boiled.

d. Benedict's test

To a small portion of the substance, Benedict's solution was added and mixed well and it was boiled. Then it was allowed to cool.

3. Triterpenoids

The extracts were taken and it was added with chloroform and sulphuric acid and the fluorescence of the solution was noted.

4. Proteins and amino acids

a. Million's Test

Extract was added with Million's reagent and it was boiled.

b. Ninhydrin Test

Extract was added with Ninhydrin solution and it was boiled. Then it was allowed to cool.

c. Biuret Test

Extract was added with Biuret reagent and colour was observed.

d. Xanthoprotein Test

To the extract, concentrated nitric acid was added and the change was observed.

5. Phytosterols

The extracts were refluxed separately with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted with distilled water and extracted with ether. The ethereal extract was evaporated and the residue was subjected to Libermann-Burchard test.

a. Liebermann – bur chard test

Extracts were shaken with few drops of dry acetic acid. To this, 3 ml of acetic anhydride was added followed by 3 drops of con. Sulphuric acid.

6. Phenolic compounds and tannins

Small quantities of alcoholic and aqueous extracts were taken separately in water and the presence of Phenolic compounds and tannins were tested by adding with dilute $Fecl_3$ solution (5%) / Lead acetate solutions (10%).

7. Glycosides

a. Baljet's Test: To the extracts, Sodium picrate solution was added.

b. Legal's Test: To the extracts, few ml of pyridine, 2 drops of nitroprusside and a drop of 20% of NaOH solution were added.

c. Borntrager's Test: The extract was mixed with dilute $H_2SO_4$ and filtered. The filtrate was shaken with chloroform and the chloroform layer was separated. To this dilute ammonia was added.

Hepatoprotective activity was studied against Carbon tetra chloride induced hepatotoxicity in rats [8].

**Animals**

Male albino rat's (Wistar Kyoto) weight ranging from 150-250g was chosen. The animals were provided with standard pellet diet with free access to water and libitum [8]. The animals were divided into 6 groups of 6 each. All animal experiment carried out in as per Committee for the Purpose of Control and Supervision of Experimental on Animals (CPCSEA) guidelines after getting approval of the Institute's Animal Ethics Committee. Reg. no. 1751/PO/ac/14/CPCSEA.

**Toxicity Studies**

Acute toxicity of the chloroform extract was determined by using albino rat as per the OECD Guideline 420 (fixed dose method). CE was found to be 250 mg/kg. Doses were selected for further study [8] [9][10][11].

**Preparation of Sample**

| | | |
|---|---|---|
| Normal | : | 10% aqueous Tween 80. |
| Control | : | Carbon tetra chloride 0.5 ml / kg |
| Standard | : | Silymarin 200mg/kg |

**Test drugs:**

| | | |
|---|---|---|
| Methanol extract | : | 250mg/kg in 10% aqueous Tween 80. |
| Chloroform extract | : | 250mg/kg in 10% aqueous Tween 80. |
| Ethyl acetate extract | : | 250mg/kg in 10% aqueous Tween 80. |

**Carbon tetra chloride induced hepatotoxicity**

Group I animal received 10% aqueous Tween 80.
Group II animal received Carbon tetrachloride 0.5ml/kg.
Group III animal received Silymarin 200mg/kg.
Group IV animal received Methanol extract 250mg/kg.
Group V animal received Chloroform extract 250mg/kg.
Group VI animal received Ethyl acetate extract 250mg/kg.

**Biochemical Studies**

The treatment was continued for 10 days, on $10^{th}$ day $CCl_4$ (0.5ml/kg) was injected to the Group II-VI. Then after twenty four hrs, animal was anesthetized, blood sample was collected by direct cardiac puncture, and the serum was separated and used for the assay of marker enzymes (SGOT, SGPT and ALP) at 340 nm wavelength Ultraviolet spectroscopy (Lab India 3000+). The enzyme level was determined by the following formula.

**HISTOPATHOLOGICAL STUDIES**

The animal was sacrificed and the liver was removed and washed in saline, and preserved in 10% formaldehyde solution for histopathological studies. The pieces of liver were processed and embedded in paraffin wax, sections made were 4 – 6 µm in thickness, and they were stained with haematoxylin and eosin and photographed.[11-16].

## RESULTS AND DISCUSSION

### Phytochemical Screening of Bambusa Vulgaris Extracts

**Table 1 Result of Phytochemical screening of successive extraction of *B. Vulgaris***

| Extract | Components | | | | | | |
|---|---|---|---|---|---|---|---|
| | Alkaloids | Carbohydrates | Flavonoids | Phytosterols | Terpenoids | Protein & amino acids | Phenols |
| Chloroform | - | - | - | + | + | - | + |
| Ethyl Acetate | + | + | - | - | + | - | - |
| Methanol | - | + | + | + | - | - | + |
| + *indicates the presence of constituents, - indicates the absence of constituents* | | | | | | | |

### Histopathological studies

The effect exhibited by Group IV & V was comparable with the standard group treated with Silymarin (200 mg/kg b. w). Chloroform extract of *B. vulgaris* shows almost normal histology.

The groups received $CCl_4$ alone, the damage of cells around the central vein were well evident, whereas, the intensity of damage was found lesser in the studies involved pre-treatment of *B. vulgaris*. The results of the histopathological studies supported and well correlated with data obtained from evaluation of the biochemical parameters (Table 2)

**Table 2 Hepatoprotective effect of various extracts of *Bambusa vulgaris***

| Treatment | SGOT level | % activity | SGPT level | % activity | ALP level | % activity |
|---|---|---|---|---|---|---|
| Control | 4678.05 | - | 4761.18 | - | 3518 | - |
| Silymarin | 1987.05 | 100 | 3182.28 | 100 | 2561.45 | 100 |
| Methanol extract | 2376.92 | 83.54 | 3640.21 | 87.42 | 3265.67 | 78.43 |
| Chloroform extract | 2232.85 | 88.99 | 3282.28 | 96.95 | 2752.09 | 93.07 |
| Ethyl acetate extract | 2523.71 | 78.73 | 3429.28 | 92.79 | 3345.21 | 76.57 |
| *SGOT-Serum glutamic oxaloacetic transaminase, SGPT-Serum glutamic pyruvic transaminase, ALP- Alkaline phosphate* | | | | | | |

*Bambusa vulgaris* extract causes hepatoprotective effect in liver cell of albino rats, hepatic cell necrosis caused by metabolic activation and produce free radicals from $CCl_4$. The enzyme level decreases as normal, in case administration of extract of *B. vulgaris* was found protection against $CCl_4$ induced increase enzyme level of SGOT, SGPT and ALP. These enzymes were good indicator for pathology of jaundice. Treatment with 200mg/kg body weight of chloroform extract of *B. vulgaris* reduced the elevation of SGOT, SGPT and ALP.

*Bambusa vulgaris* extract was potential against liver dysfunction found to be 250 mg/kg in albino rats and dose was selected by $LD_{50}$ for hepatoprotective activity. (Fig. 1 and 2)

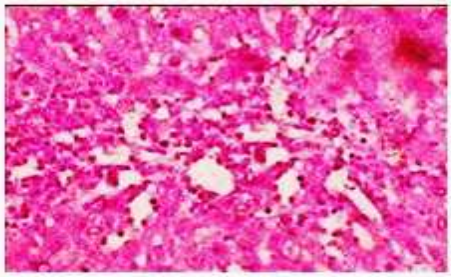


Fig. 1 Liver tissue of normal rats showing normal histology.

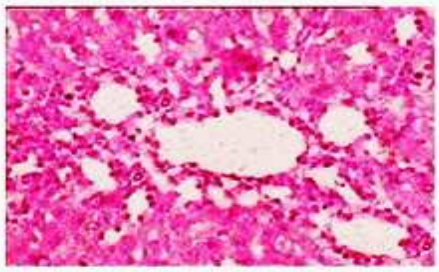


Fig. 2 Liver tissue of rats treated with carbon tetra chloride shows necrosis

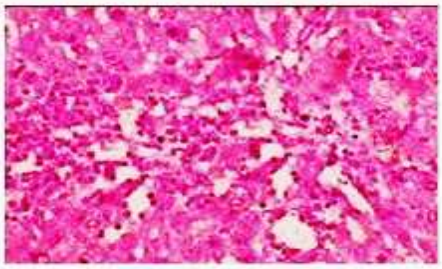


Fig. 3 Liver tissue of rats treated with carbon tetra chloride and silymarin shows almost normal histology

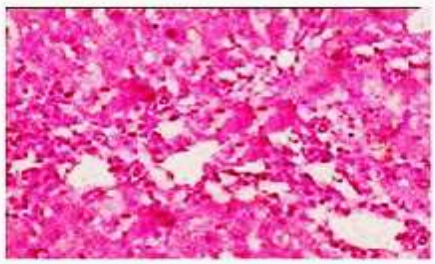


Fig. 4 Liver tissue of rats treated with carbon tetra chloride and methanol extract of *B. Vulgaris* shows almost normal histology

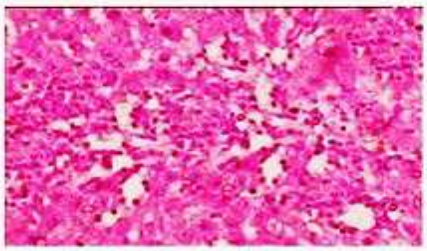


Fig. 5 Liver tissue of rats treated with carbon tetra chloride and Chloroform extract of *B.*

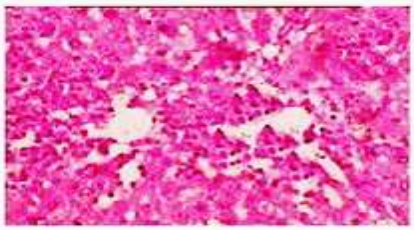
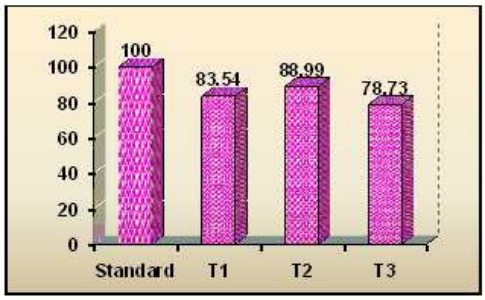


Fig. 6 Liver tissue of rats treated with carbon tetra chloride and ethyl Acetate extract of *B. Vulgaris* shows almost normal histology

**Fig. 1 Histopathology studies of *Bambusa vulgaris* extracts**

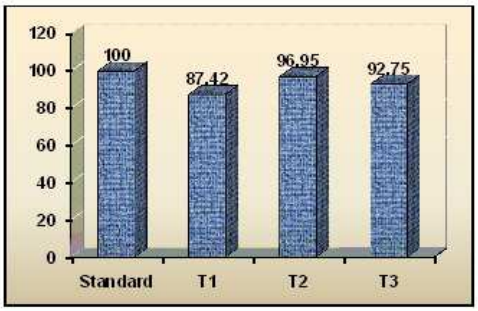
**For SGOT**




Standard - Silymarin

T1 - Methanol extract
T2 - Chloroform extract
T3 - Ethyl acetate extract

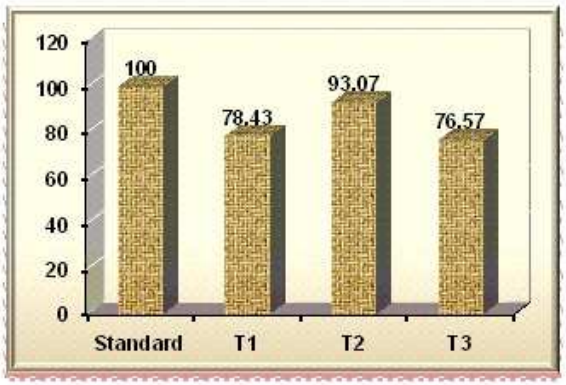
**For SGPT**




Standard - Silymarin

T1 - Methanol extract
T2 - Chloroform extract
T3 - Ethyl acetate extract

**For ALP**




Standard - Silymarin

T1 - Methanol extract
T2 - Chloroform extract
T3 - Ethyl acetate extract

**Fig. 2 Histopathological studies supported and well correlated with data obtained from evaluation of the biochemical parameters**

The toxic group showed excessive formation of connective tissue with nodules and scarred tissue, cell, necrosis, fatty changes. Most effective group *Bambusa vulgaris* and silyamarin had most effective hepatic cytoprotective action with near normal histology

## CONCLUSION

Chloroform extract of *B. vulgaris* shown hepatoprotective activity on $CCl_4$ induced liver injury. It can be concluded that possible mechanism of hepatoprotective activity of *B. vulgaris* may be due to the presence of terpenoid compounds because terpenoid are soluble in chloroform extract. The hepatoprotective effect may be due to its antioxidant potential Biochemical, pharmacological and histopathological parameters indicates the structural and functional integrity of hepatocytes. *B. vulgaris* can be a safe and effective future alternative therapy for the treatment of hepatic disorder.

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## ABBREVIATIONS

The following abbreviations are used in this manuscript:
SGOT: Serum glutamate oxy-transaminase
SGPT: Serum glutamate pyurate transaminase
ALP: Alkaline phosphatise
$CCl_4$: Carbon tetra chloride

## REFFERENCES

[1] Shanmugasundaram, P., Venkataram, S., Hepatoprotective and antioxidant effects of Hygrophila auriculata (K. Schum) Heine Acanthaceae root extract. *J. Ethnopharmacol*. **2006**; 104(1-2): 124-128.
[2] John, D., Soejatmi, D., Elizabeth, W., Renuka, C., Priority species of bamboo and rattan Published IPGRI and INBAR. **1998**.
[3] The Wealth of India: raw materials. New Delhi: NISCAIR, **1976**, 10, 43-44.
[4] Chopra, R. N., Nayar, S. L., Chopra, I. C., Glossary of Indian medicinal plants. New Delhi, Publication and Information Directorate. **1992**, 234.
[5] Guerere, M., Mondon, J. M., Pujaniaye, A. Physicochemical composition of some seeds from plants of the isle of Re-union. Ann Falsif Expert Chim Toxicol. **1985**, 78, 281-286.
[6] Anghore, D., Kulkarni, G. T., Subramanian A. R. S., Preliminary phytochemical investigation of *bambusa vulgaris* var. Striata holtum. *Journal of chronotherapy and drug delivery*, **2014**, 5(1), 27-31.
[7] Kokate, C. K., Purohit, A. P., Gokhale, S. B., Textbook of Pharmacognosy, Nirali prakshan New delhi.
[8] Pradhan, S. C., Girish C., Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine *Indian J Med Res*., **2006**, 124, 491-504.
[9] Tietz, M. N., Rinker, D., Show, L. M., IFCC method for alkaline phosphatase. *J Clin Chem Clin Biochem*., **1983**, 21, 731-748.
[10] Burtis, C. A., Ashwood, E. R., Textbook of Clin Chem, Philadelphia: PA W.B. Saunders Company. **1999**, 3, 1829-1830.
[11] Li, H., Wang, H., Sun, H., Liu, Y., Liu, K., Peng, S., Binding of nitrobenzene to hepatic DNA and hemoglibin at low doses in mice. *Toxicol Lett*. **2003**, 139(1), 25-32.
[12] Triangle Park, N. C., *Holder, J. W.*, Nitrobenzene carcinogenicity in animals and human hazard evaluation. *Toxicol*. *Ind*. *Health*., **1999**, *15, 445–457*.
[13] Mason, R. P., Holtzman, J. L., The mechanism of microsomal and mitochrondrial nitroreductase. Electron *Biochem Biophys Res Commun*., ***1975***, 67, *1267-1274*.
[14] Yamazaki, K., Aiso, S., Matsumoto, M., Arito, H., Nagano, K., Yamamoto S., Matsushima, T., Thirteen week oral toxicity study of 1.4-dichloro-2-nitrobenzene in rats and mice *Ind Health*., **2005,** 43 597-610.
[15] Torel, J., Cilliard, J., Cilliard, P., Antioxidant activity of flavonoids and reactivity with peroxy radical Phytochem., **1986**, 25, 383–385.
[16] Kaviarasan, K., Kalaiarasi., Pugalendi, V. J., Antioxidant efficacy of flavonoid-rich fraction from *Spermacoce hispida* in hyperlipidemic rats, *Appl. Biomed.,* **2008**, 6 , 165– 172.