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Hepatoprotective activity of methanolic shoot extract of *Bambusa bambos* against carbon tetrachloride induce acute liver toxicity in Wistar rats

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ABSTRACT

The young shoots of *Bambusa bambos* are used ayurvedic medicines in India. Young shoots content various chemical components such as cholin, betain, urease, cyanogenetic glucosides, oxalic acid, and benzoic acid. It has antiulcer, antifertility, anti-inflammatory, and antioxidant functions. In the present study, the hepatoprotective activity of methanolic shoot extract of *B. bambos* was tested against carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Female Wistar rats were divided into five groups; Group I served as normal control. Groups II-VI were administered CCl4 mixed with olive oil 1:1 intraperitoneally (1 mL/kg body weight), after every 72 h for 16 days. Group II was CCl_4 negative control. Groups IV and V received methanolic shoot extract mixed with olive oil, 200 mg and 400 mg/kg body weight, respectively. Group III received silymarin 50 mg/kg body weight for 16 days orally once daily. Methanolic extract attenuated the increase in aspartate amino transaminase (AST) and alanine amino transaminase (ALT) as well as alkaline phosphatase (ALP) and total bilirubin that occur during liver injury after CCl_4 injection. Outcome of the present study suggests that treatment with methanolic shoot extract of *B. bambos*-induced reduction in ALT, AST, ALP, and total bilirubin in rats indicating hepatoprotective potential of the extract.

1. INTRODUCTION

Liver plays an important function of processing and destruction of toxic substance which often enters the body. The liver is exposed to many xenobiotics and therapeutic agents. These substances are processed by hepatic drug metabolism enzymes by releasing polar functional groups onto a drug molecule, for example, cytochrome P450 (CYP) enzyme system. CYP belongs to isozymes family which is responsible for the oxidation of organic substance [1]. Various pharmaceutical industries are looking for liver dysfunction and injuries treatment drugs. There are no specific synthetic drugs to treat the liver injury; due to this, they may cause further damages to the liver.

In India, tribal's are using herbal drugs for the treatment of the liver injuries for a long time. Over 80% of the Indians as well as world population show faith on the use of traditional medicine based on plant materials [2]. These ancient traditional and natural healthcare practices as well as Ayurveda, Siddha, and Unani originated from time immemorial and urbanized gradually, to a large extent, without referring any modern scientific principles but only by based on practical experiences [3]. Hepatoprotective plants contain various chemical contents such as phenols, flavonoids, monoterpenes, lignans, glycosides, carotenoids, coumarins, essential oil, alkaloids, organic acids, lipids, and xanthenes. These plants have capacity to speed up the regeneration of liver cells and heal the liver injuries. Many scientists have tested a large number of plants for their active component having the curative property against drug-induced hepatotoxicity model such as *Licorice*, *Solanum xanthocarpum*, and *Melothria heterophylla* [4,5].

Bambusa bambos is well known by its common name Bamboo, Bans, etc., in India. Bamboo is normally distributed throughout the moist region of India. For their growth, some of the species need warm climate, productive soil with rich in water, and some species grow insensibly cold weather. Since ancient time Bamboo has played an important role in human civilization. Apart from all these applications, Bamboo is also known for its medicinal properties. Leaves, roots, shoots, grains, and gums of bamboo are said to be remedy for asthma, cirrhosis, and tumors and have antioxidant as well as antimicrobial properties [6,7]. In many countries, bamboo shoots are consumed as a food, especially young shoots. *B. bambos* is one of those 200 bamboo species which are edible. The young shoot of *B. bambos* is used as a food by rural people of Western Ghats in India. These

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are pinkish brown in color and harvested after growing at particular height. Fresh bamboo shoots are appetizing with high fiber content and known to be healthy. It has been reported that *B. bambos* young shoots content various chemical components such as cholin, betain, urease, cyanogenetic glucosides, oxalic acid, and benzoic acid. The leaves of *B. bambos* have been accounted for its antiulcer, antifertility, anti-inflammatory, and antioxidant activity. There are no reports on hepatoprotective activity of the bamboo shoots [8]. Therefore, the present investigation was undertaken to study hepatoprotective activity of *B. bambos* shoot against CCl_4-induced liver injury in Wistar rats.

2. MATERIALS AND METHODS

2.1. Collection of Plants

Bamboo shoots were collected from Yellapur forest area, Uttar Kannada district, Karnataka. Bamboo shoots were authenticated at Department of Botany, Karnataka University, Dharwad. The shoot was dried under shade, powdered mechanically, and stored in air-tight container.

2.2. Preparation of Methanolic Extract

B. bambos young shoots were air dried at room temperature and powdered. The powdered shoot is then extracted using methanol in Soxhlet apparatus for 72 h at the temperature of 40°C. The extracts were filtered and then evaporated under reduced pressure at 40°C to form solid dark brown mass. Collected methanolic extract was semisolid in nature and dark brown in color.

2.3. Animal

Female Wister rats of 3-month-old weighing between 250 and 350 g, respectively, were acquired from the S.D.M. Medical College Dharwad, North Karnataka, India. They were housed in polypropylene cages and maintained under standard laboratory environmental conditions; temperature 25°C ± 2°C, 12 h light:12 h dark cycle and 55% ± 10% relative humidity with free access to standard pellets and water, *ad libitum*.

2.4. Experimental Design

Wistar rats were divided into six groups as follows, containing five rats in each group. Group I served as normal control with oral administration of olive oil after every 24 h for 16 days. Groups II-VI were treated with CCl4 mixed with olive oil in ratio of 1:1 at a dose of 1.0 mL/kg intraperitoneally every 72 h for 16 days. Group II animals were maintained as CCl4 intoxicated control without any drug treatment. Group III was administered silymarin at a dose of 50 mg/kg of body weight, and Groups VI and V received methanolic bamboo shoot extract 200 and 400 mg/kg body weight in 0.5 mL of olive oil orally once daily for 16 days, respectively, in addition to CCl4 every 72 h as mentioned above.

2.5. Biochemical Assay

Rats were sacrificed on 17th day and blood was collected in the plane tube for aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin estimation. The blood samples were centrifuged at 5000 rpm for 3 min at room temperature. The separated blood serums were collected in fresh tube and proceed for further test. Liver marker enzymes such as AST, ALT, and ALP were estimated.

3. RESULTS

3.1. Methanol Extraction of Bamboo Shoot

Methanol was used for the extraction of phytochemicals from *B. bambos* shoot through Soxhlet apparatus for 72 h at the temperature of 40°C–50°C. Collected methanolic extract (7.3 g) was semisolid in nature and dark brown in color.

3.2. Analysis of Serum Biochemical Parameters

The levels of serum AST, ALT Graph 1, ALP Graph 2, and total bilirubin Graph 3 were considered as marker for hepatotoxicity induced by CCl_4.

3.3. Estimation of AST

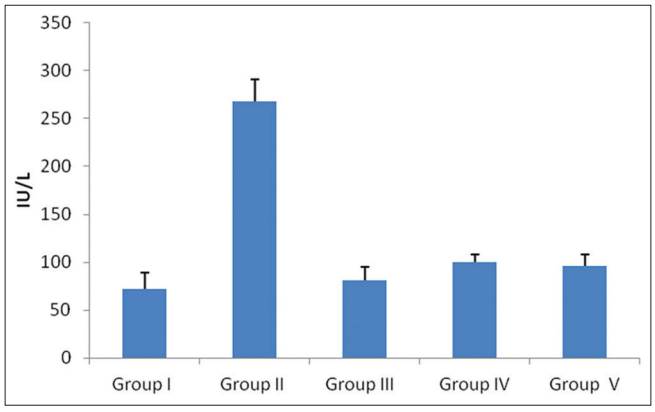
The level of AST elevation in Group II showed more as compared to Group I. In Groups IV and V showed a decreased level of AST compared to Group II. However, Group V has very lower AST level than Group IV which is almost close to Group III [Graph 4].

3.4. Estimation of ALT

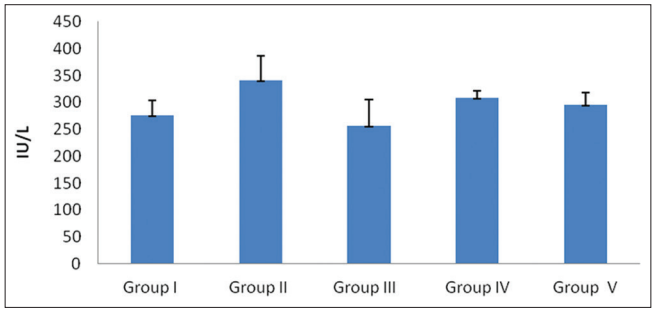
The level of ALT elevation in Group II showed more as compared to Group I. In Groups IV and V showed a decreased level of ALT compared to Group II. However, Group V has very low ALT level than Group IV which is almost close to Group III [Graph 1].

3.5. Estimation of ALP

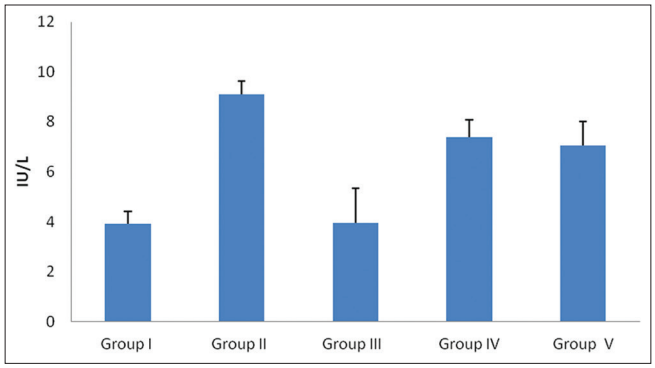
The level of ALP elevation in Group II showed more as compared to Group I. In Groups IV and V showed a decreased level of ALP



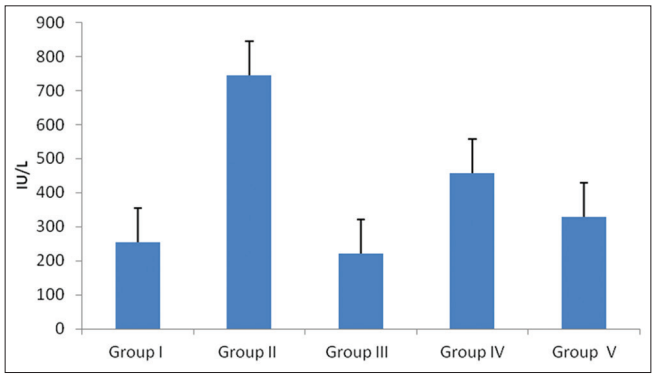
Graph 1: Effects of methanolic shoot extract of *Bambusa bambos* on serum alanine amino transaminase in carbon tetrachloride-treated Wister rats.



Graph 2: Effects of methanolic shoot extract of *Bambusa bambos* on serum alkaline phosphatase in carbon tetrachloride-treated Wister rats.



Graph 3: Effect of methanolic shoot extract of *Bambusa bambos* on total bilirubin in carbon tetrachloride-treated Wister rats.



Graph 4: Effects of methanolic shoot extract of *Bambusa bambos* on serum aspartate amino transaminase in carbon tetrachloride-treated Wistar rats.

compared to Group II. However, Group V has very low ALP level than Group IV which is almost close to Group III [Graph 2].

3.6. Estimation of Total Bilirubin

The level of total bilirubin in Group II showed more as compared to Group I. In Groups IV and V showed a decreased level of total bilirubin compared to Group II. However, Group V has very low total bilirubin level than Group IV which is almost close to Group III [Graph 3].

4. DISCUSSION

Methanol can dissolve more polar compounds from the plants then ethanol. Many studies have investigated that methanol and ethanol can dissolve polar compounds such as amino acid, sugar, glycoside compounds, phenolic compounds with low and medium molecular weights and medium polarity [9,10], anthocyanin, terpenoid, aglycon flavonoid, saponin, tannin, phenone, totarol, quacinoid, xantoxilin, lactone, flavone, and polyphenol [11]. Hence, in the present investigation, carbon tetrachloride is attested for the production free radicals, which influence the cellular permeability of hepatocytes leading to serum enzyme elevation in blood. Serum marker enzymes and total bilirubin were analyzed and compared with control and experimental animals. In Group II (negative control), treated with CCl_4, showed significantly increase ($P < 0.05$) level of enzymes when compared with Group I (normal control). Groups IV and V, treated with methanolic shoot extract of *B. bambos*, showed significantly decline levels of the enzyme ($P < 0.001$) when compared to negative control, but not as low as Group III (positive control) treated with silymarin.

Methanolic shoot extract of *B. bambos* reveals a hepatoprotective effect, showing significant decrease in transaminases, AST and ALT, concentrations in the liver of rats with CCl_4-induced hepatotoxicity [Graphs 1,2,4]. During liver injury, AST and ALT are elevated from the hepatocyte into bloodstream that is used as marker for liver damage. In Group II (negative control), found rise in transaminase concentration as compared to Group I (normal control), this indicates liver injury caused by CCl_4 in Group II. In general, CCl_4 generates a trichloromethyl free radical by cleaving carbon-chlorine bond that reacts rapidly with oxygen producing a trichloromethyl peroxy radical that may induce hepatotoxicity [12].

In positive control treated with CCl_4 along with silymarin has shown rapidly decreased the level of AST and ALT as compared to negative control, which indicates hepatoprotective effect, hence, used as standard drug. Silymarin forms a complex that obstructs the entry of toxins into the interior of liver cells. Silymarin metabolism also activates the RNA biosynthesis of ribosomes to induce protein formation by stimulating hepatic cells [13,14].

In groups treated with CCl_4 along with low dosage (200 mg/kg body weight [bw]) and high dosage (400 mg/kg bw) of *B. bambos* for 16 days exhibiting reduction in AST and ALT concentration when compared with negative control. This reveals the ability of *B. bambos* to protect the hepatocyte from the CCl_4 exposure. The group treated with high dosage of extract has shown considerably low concentration of serum transaminases than lower dosage group which is almost similar to silymarin group. ALP and total bilirubin [Graphs 1 and 3] were also found to be decrease in experimental extract-treated groups when compared with negative group [15].

The comparison of the hepatoprotective ability of *B. bambos* shoot was similar to with other plants such as *Musa sapientum* Linn., Shekwasha (*Citrus depressa*), wild ginseng cambial meristematic cells, and *S. xanthocarpum* [16]. Among these plants, most effective was found to be Shekwasha (*C. depressa*) and *S. xanthocarpum*. There was reduction in AST, ALT, ALP, and total bilirubin in CCl_4-induced hepatotoxicity in rats when treated with *B. bambos* that was comparible to Shekwasha and *S. xanthocarpum*. Shekwasha fruit has the ability to suppress D-galactosamine and protect the liver in D-galactosamine-induced liver injury due to the presence of polymethoxy flavonoids such as citromitin, tangeretin, and specially nobiletin. Whereas, the phytochemical screening of *S. xanthocarpum* reported the presence of flavonoids, steroidal alkaloids, flavonoids, triterpenes, apigenins glycosides, and quercitrin that are involved in hepatoprotection activity [16,17]. In this study, *B. bambos* shows the same characteristic in of suppression CCl_4 – toxicity that may indicate the presence of flavonoids, steroidal alkaloids, etc., which are the major constituents induce the hepatoprotection that are comparable with the control and standard hepatoprotectant (Silymarin).

5. CONCLUSION

In the present study, methanolic shoot extract of *B. bambos* was analyzed for its hepatoprotective activity against model hepatotoxicant CCl_4 in Wistar rats by studying the activity of AST, ALT, ALP, and total bilirubin. The extract showed hepatoprotective activity which was dose-dependent and maximum beneficial effect was observed at a dose of 400 mg/kg. The results were comparable with that of standard drug, silymarin used in the study. The hepatoprotective action is perhaps related to its potent antioxidant activity. Further,

investigations are required to characterize the active hepatoprotective agent and mechanism of action.

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