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## Hepatoprotective activity of methanolic extract of young shoots of *Bambusa arundinaceae* in thioacetamide induced liver injury in rats

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### ABSTRACT

**Background:** Whole plant of *Bambusa arundinaceae* (Bamboo) belonging to family (Graminae) is used in number of disease including liver ailments in various parts of India. The present study is done to evaluate antioxidant and Hepatoprotective activity of methanolic extract of young shoots of *Bambusa arundinaceae* in Thioacetamide induced liver injury in rats. **Material and method:** Antioxidant activity of methanolic extract was evaluated by DPPH, Ferric reducing power assay and Lipid peroxidation. *In vitro* hepatoprotective activity of methanolic extracts (25, 50, 100, 200 and 400 µg/ml) against thioacetamide (100mM) induced cytotoxicity was assessed by monitoring cell viability in HepG2 and Hep3b tumor cell line and also in primary hepatocytes. *In vivo* hepatoprotective activity of methanolic extract (50, 100, 200 mg/kg p.o.) was observed against thioacetamide (100 mg/kg s.c.) induced liver injury in rat by measuring biochemical parameters viz, Aspartate Transaminase, Alanine Transaminase, Alkaline Phosphatase, Total and Direct Bilirubin. The histopathological studies were also performed. **Results:** Methanolic extract of young shoots showed good antioxidant activity. Thioacetamide caused significant reduction in cell viability in *in vitro* studies. Treatment with methanolic extract caused significant increase in cell viability. *In vivo* studies showed that Thioacetamide induced elevation of biochemical parameters in rats were significantly ( $p < 0.0001$ ) decreased with methanolic extract treatment in rats. Histopathology studies also supported the protective effect of methanolic extract. **Conclusion:** The current study reveals that the methanolic extract of *Bambusa arundinaceae* has hepatoprotective activity which may be due to its antioxidant activity.

**Keywords:** Hepatoprotective activity, *Bambusa arundinaceae*, Thioacetamide, HepG2, Hep3b, Primary hepatocytes.

### INTRODUCTION

Liver disease is an important cause of mortality and morbidity worldwide<sup>[1,2]</sup>. Liver is the principal site for clearance and metabolism of xenobiotics which places it in a position to be exposed to potentially toxic, metabolic, microbial, circulatory and neoplastic insults. Dominant primary diseases of liver are alcoholic liver disease, viral hepatitis, and hepatocellular carcinoma. Secondary liver disease may occur due to cardiac decompensation, disseminated cancer, and extra hepatic infections<sup>[3,4]</sup>.

Majority of world's population depends on herbal medicine for their primary care. Herbal preparations or plant derived compounds are used in conjunction with allopathic medicine to treat all types of ailments including liver disease<sup>[5,6]</sup>. The fruits and seeds of Milk thistle have shown hepatoprotective activity in preclinical and clinical studies<sup>[7]</sup>.

Bamboos consist of about 110 genera and 1130-1140 species. *Bambusa arundinaceae* is a native Indian plant cultivated in other regions also and it belongs to the gramineae family<sup>[8]</sup>. *Bambusa arundinaceae* is used in folk medicine as tonic for heart, liver and brain. It fortifies the heart and calms down heart palpitations. It is used in eye inflammations. It soothes stomach troubles of all kinds and its application is also recommended in cases of diarrhoea and chronic liver ailments. It is also used as rejuvenator, hemostatic, antispasmodic and suppresses colds, bronchitis, vomiting convulsions, cough, enuresis, fever, inflammation and gallbladder problems<sup>[9,10]</sup>. It cures various urinary problem such as urodialysis (suppressed urine), uropenia (scanty urine) and urinary tract infection<sup>[11,12]</sup>. It is also a part of many unani and ayurvedic formulation like Qursghafis, Sufuhtabashir, Drakshadichurna which are used in liver ailments<sup>[13,14]</sup>.

*Bambusa arundinaceae* contains phytochemicals like resin, lignin, alkaloids, glucoside, silica, uronic acid, galactose, glucose, arabinose, mannose, xylose, Choline, betain<sup>[15-16]</sup>. It also contains urease, nuclease, proteolytic enzymes, diastatic and emulsifying enzyme<sup>[16]</sup>. flavanoids like orientin, homoorientin, vitexin and isovitexin are present along with phyosterols like stigmasterol and

$\beta$ -sitosterol, Stigmast-5, 22-dien-3 $\beta$ -ol, Stigmast-5-en-3 $\beta$ -ol- $\beta$ -D glucopyranoside, triterpenes and steroidal glycosides, 17, 20, 20-tri demethyl-20 $\alpha$ -isopranyloleanane, eicosanyldicarboxylic acid,  $\alpha$ -amyrin acetate, urs-12-en-3 $\beta$ -ol- $\beta$ -D-glucopyranoside<sup>[17-19]</sup>.

*Bambusa arundinaceae* is reported to possess antifertility, anti-inflammatory, antiulcer, antihyperglycemic, antiarthritic, anthelmintic and antihyperlipidemic activity<sup>[20-27]</sup>. It shows good antioxidant and antimicrobial potential in *in vitro* studies<sup>[28]</sup>.

In light of the aforementioned properties, we hypothesized that *Bambusa arundinaceae* may overcome the hepatotoxicity mediated through oxidative stress. Hence, we evaluated the antioxidant and hepatoprotective effect by *in vitro* studies. We have also evaluated *in vivo* hepatoprotective effect of methanolic extract on thioacetamide induced acute liver injury in rats.

## MATERIALS AND METHOD

### Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH), FBS, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) were purchased from Hi-media. TCA (trichloroacetic acid), TBA (thiobarbituric acid), Thioacetamide, Ascorbic acid was purchased from Sigma. SGOT, SGPT, ALP and Bilirubin kits were brought from span diagnostics and all other chemicals were of analytical grade.

### Plant Collection and preparation of extract

Young shoots of *Bambusa arundinaceae* were collected in the month of August from Assam. They were dried under shed at room temperature (25-30°C). After drying the shoots (10 Kg) were powdered with dry grinder and sieved through sieve 40 mesh. The powder was packed in Soxhlet apparatus and defatted with petroleum ether and then extracted with methanol to obtain methanolic extract. The percentage yield was found to be 15.5% (w/w)

### Primary phytochemical screening of methanolic extract<sup>[29,30]</sup>

#### Test for terpenes

To 5 ml of the extract add 2 ml of chloroform and 3 ml of concentrated H<sub>2</sub>SO<sub>4</sub>. Formation of a reddish brown ring confirms the presence of terpenes.

#### Test for flavonoids

A few drops of concentrated hydrochloric acid were added to a small amount of the extracts of the plant material. Immediate development of a red colour was taken as an indication of the presence of flavonoids

#### Test for saponins

Frothing test: Exactly 0.5 g of the extract was dissolved in distilled water in a test tube. Frothing which persisted on warming was taken as preliminary evidence for saponins.

#### Test for steroids

Liebermann–Burchard reaction: Add 2 ml of acetic anhydride and 2 ml conc. H<sub>2</sub>SO<sub>4</sub> to 5 ml of the extract. Change of colour from violet to blue confirms the presence of steroids.

#### Test for cardiac glycosides

To 2 ml of extract 2 ml of glacial acetic acid containing 1 drop of ferric chloride solution and 1 ml of conc. H<sub>2</sub>SO<sub>4</sub> was added. Appearance of a brown ring indicates the presence of cardiac glycosides

#### Test for proteins

Biuret test: 4% of NaOH and few drops of 1% CuSO<sub>4</sub> solution were added to 3 ml of the extract. Formation of violet or pink colour indicates the presence of proteins.

#### Test for carbohydrates

Monosaccharide. Barfoed's test: Equal volumes of Barfoed's reagent and the extract solution were mixed and heated for 1–2 min in a boiling water bath and cooled. Red colour was observed

#### Test for reducing sugars.

Fehling test: 1 ml of Fehling's A and Fehling's B solutions was boiled with equal volume of extract for 5–10 min. First a yellow then a brick red precipitates were observed

Molisch test: To 2–3 ml of the extract, add two drops of alpha naphthol solution in alcohol shake and add conc. H<sub>2</sub>SO<sub>4</sub> from the sides of test tube. Violet ring is formed.

### Quantitative Determination of Phytochemicals<sup>[31]</sup>

#### Total phenolic content

An aliquot of 0.1 mL Methanolic extract standard was combined with 2.8 mL 10% sodium carbonate and 0.1 mL of 0.2 mol/L Folin-Ciocalteu reagent. After 40 min absorbance at 725 nm was checked by UV-visible spectrophotometer. Total phenolics were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample using the standard calibration curve constructed for different concentrations of gallic acid. Results were expressed in GAE mg/g.

#### Total flavanoid content

50  $\mu$ L of Methanolic extract were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% NaNO<sub>2</sub> solution. 0.3 mL of 10% AlCl<sub>3</sub> solution was added after 5 min of incubation, and the mixture was allowed to stand for 6 min. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was measured at 510 nm. The total flavanoid content was calculated from a calibration curve, and the result was expressed as mg Quercetin equivalent per g dry weigh

## Animals

All experiments were carried out after the approval of Institutional Animal Ethics Committee (IAEC) with protocol number KBRCPC 2013/06/07 in accordance with the guidelines laid by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. Male wistar rats (200-250g) were used in these experiments. All the animals were housed in standard cages (48 cm×35 cm×22 cm) at room temperature (20±2 °C) relative humidity (55±5%) on a 12-h light–dark cycle. Animals had access to standard pellet diet (certified Amrut brand rodent feed, Pranav Agro Industries, Pune, India) and filtered tap water *ad libitum*.

## Antioxidant activity

### Free radical scavenging activity by DPPH method<sup>[32,33]</sup>

Different concentrations of methanolic extract (100, 200, 400, 800, 1600µg/mL) and standards were prepared. To this 3 ml of methanolic solution of DPPH was added, this mixture was incubated at 37°C for 30 min. A blank was prepared in the similar way and the absorbance was measured at 517nm. Scavenging activity was expressed as the percentage inhibition calculated by using the following formulae

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

### Anti-lipid peroxidation effect<sup>[34]</sup>

The 0.5mL of liver homogenate was taken and to it 1mL of 0.15M KCL and 0.5ml of methanolic extract and standard (Ascorbic acid) at different concentrations (100, 200, 400, 800, 1600µg/mL) were added. Lipid peroxidation was initiated by adding 100µL of 1mM ferric chloride. The reaction was stopped by adding 2ml of ice cold 0.25N HCL containing 15% TCA, 0.38% TBA, and 0.2 ml of 0.05% butylated hydroxyl toluene. These reaction mixtures were heated for 60 min at 80°C then cooled and centrifuged at 6900 rpm for 15min. The absorbance of supernatant was measured at 532nm against blank, which contained all reagents except liver homogenate and drug. Same experiments were performed to determine the normal (without drug and FeCl<sub>3</sub>) and induced (without drug) lipid peroxidation level in the tissue. The percentage of anti-lipid per oxidation effect (%ALP) was calculated by the following formula.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

### Reducing power assay<sup>[35]</sup>

Reducing power of methanolic extracts was determined on the ability of anti oxidants to form colored complex with potassium ferricyanide. Different concentration of the extract and standard (Ascorbic acid) (25-250, µg/mL) were mixed with 2.5mL phosphate buffer (pH 6.6) and 2.5ml potassium ferricyanide (1%). The mixture was incubated at 50°C for 20min. 2.5ml TCA (10%) was added to it and centrifuged at 3000rpm for 10min. 2.5ml of supernatant was mixed with 2.5ml of water and 0.5ml of FeCl<sub>3</sub> (0.1%) were added to it and absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power.

## *In vitro* hepatoprotective activity on HepG2, Hep3b, primary hepatocytes<sup>[36-41]</sup>

### Primary hepatocyte isolation and culture

6 week old rat was anesthetized with an intraperitoneal injection (ketamine 100 mg/kg body and xylazine 5 mg/kg). Abdominal cavity of animal was opened the liver of rat was rinsed and digested by the use of the inverse perfusion method improved on Seglen two-step perfusion *in situ*. After washing three times with Hank's balanced salt solution, the liver was put into MEM culture medium to end the digestion and extract cells gently with tweezers. The hepatocytes were mixed fully with MEM after filtration and centrifugation (1300 rpm, 7 minutes). The number and survival rate of hepatocytes were calculated after a trypan blue dyeing experiment. The hepatocytes were seeded to a culture flask under the conditions of 5% CO<sub>2</sub> and 37°C for 24 hours, and then the nonadherent hepatocytes were discarded along with the culture fluid. The culture medium was replaced with a culture medium. Return the cells to the incubator to continue culturing until required.

Hep3B and HepG2 cell line and primary hepatocyte were grown at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in sterile filtered MEM containing 10% FBS with 1 mM sodium pyruvate and 2.2 g/l sodium bicarbonate. After 24 h incubation, the cells were treated with the extract (400, 200, 100, 50 µg/ml), 4 fractions (200, 100, 50, 25 µg/ml), 5 subfractions (100, 50, 25, 12.5 µg/ml) and standard (sylimarin (200, 100, 50, 25 µg/ml), together with Toxicant (Thioacetamide (100 mMol/ml)) and incubated again for 24h. After 24 hr 20µl of MTT (5mg/ml) solution was added & the plate was Incubated at 37 °C for 3 hr. After 3 hour media was aspirated from each well and 100µl of DMSO was added to each well and the readings were noted in ELISA Plate reader at 570nm.

### Thioacetamide induced acute liver injury<sup>[41]</sup>

#### Experimental design

Thirty six male wistar rats were divided into six groups. First group served as normal control and received saline by oral route, second group served as toxicant control. Third group served as standard and treated with sylimarin (25 mg/kg i.p.). Fourth, fifth and sixth group served as treatment groups and received methanolic extract in 50, 100, 200 mg/kg of methanolic extract respectively daily by oral route. On seventh day all group except normal control group were given single dose of thioacetamide (100mg/kg s.c.). After 48 hours of thioacetamide administration blood sample was collected by retro-orbital plexus for biochemical estimation and the rats were sacrificed under light ether anaesthesia and the liver samples of all group were preserved in 10% neutral buffered formalin and sent for histopathology studies.

#### Biochemical estimation

SGOT (Serum glutamate Oxaloacetate transaminase), SGPT (Serum glutamate pyruvate transaminase), ALP (Alkaline Phosphatase), T.Bilirubin (Total bilirubin) and D.Bilirubin(Direct Bilirubin) were assessed by using commercial kits of span diagnostic by biochem analyzer Robonik.

Histopathology studies

The liver samples were sectioned and stained with haematoxylin-eosin (H&E) and subsequently examined under a light microscope (Olympus, Japan) for general histopathology examination.

Statistical Analysis

The IC<sub>50</sub> values were calculated by regression analysis Results are expressed as mean ± S.D. Total variation, present in a set of data were estimated by one way analysis of variance (ANOVA), followed by Dunnett’s test. A p value lower than 0.05 was considered to be statistically significant.

RESULTS

Phytochemical screening

Preliminary phytochemical screening of methanolic extract of young shoots of *Bambusa arundinaceae* shows presence of terpenes, flavanoids, saponins, steroids, proteins, carbohydrates, tannins and phenolic compound.

Total phenolic and Flavanoid content

The total phenolic content of methanolic extract of young shoots of *Bambusa arundinaceae*, calculated from the calibration curve, was 25.5±2.2 gallic acid equivalents/gDW, and the total flavonoid content was 36 ± 3.7 Quercetin equivalents/g DW.

Antioxidant activity

Free radical scavenging activity by DPPH method

Methanolic extract of young shoots of *Bambusa arundinaceae* showed appreciable free radical scavenging activities (IC<sub>50</sub>=257.15) comparable with standard (IC<sub>50</sub>=170.03) (Figure 1).

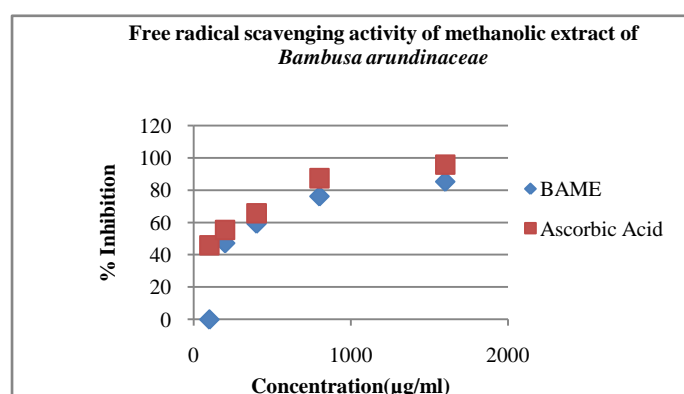


Figure 1: Free radical scavenging activity of methanolic extract of *Bambusa arundinaceae*. BAME= *Bambusa arundinaceae* methanolic extract

Anti-lipid peroxidation effect

Methanolic extract of young shoots of *Bambusa arundinaceae* showed appreciable ant lipid peroxidation effect (IC<sub>50</sub>=197.91) comparable with standard (IC<sub>50</sub>=180.12) (Figure 2).

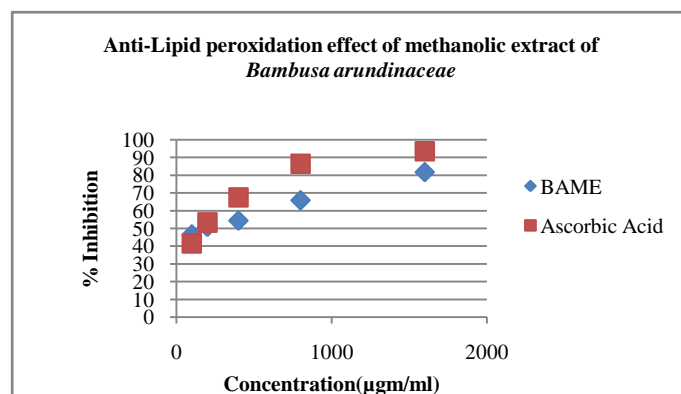


Figure 2: Anti-Lipid peroxidation effect of methanolic extract of *Bambusa arundinaceae*. BAME= *Bambusa arundinaceae* methanolic extract

Reducing power assay

Reducing power has been used as one of important antioxidant capability for medicinal herbs. Increased absorbance of the reaction mixture indicated increased reducing power. Methanolic extract of young shoots of *Bambusa arundinaceae* showed good reducing power comparable with standard (Figure 3).

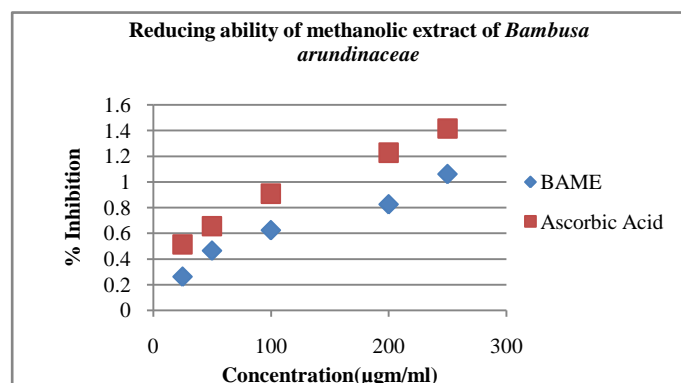


Figure 3: Reducing ability of methanolic extract of *Bambusa arundinaceae*. BAME= *Bambusa arundinaceae* methanolic extract

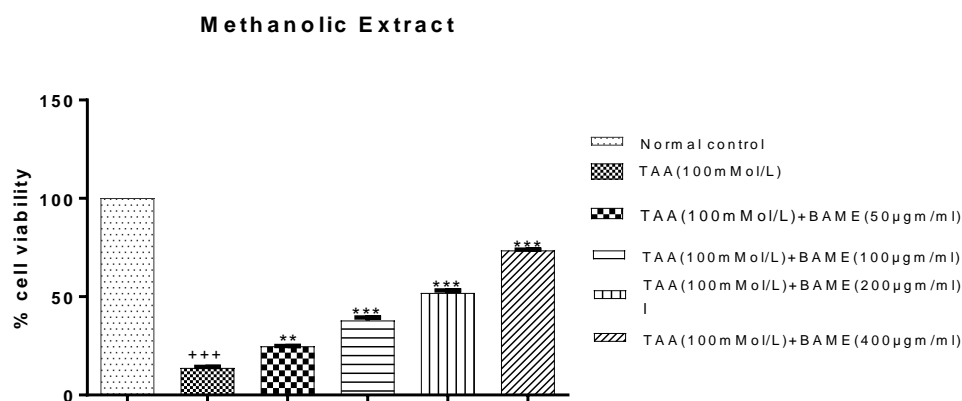
In vitro hepatoprotective activity

Effect of methanolic extract and Thioacetamide on HepG2 and Hep3b Cell Line

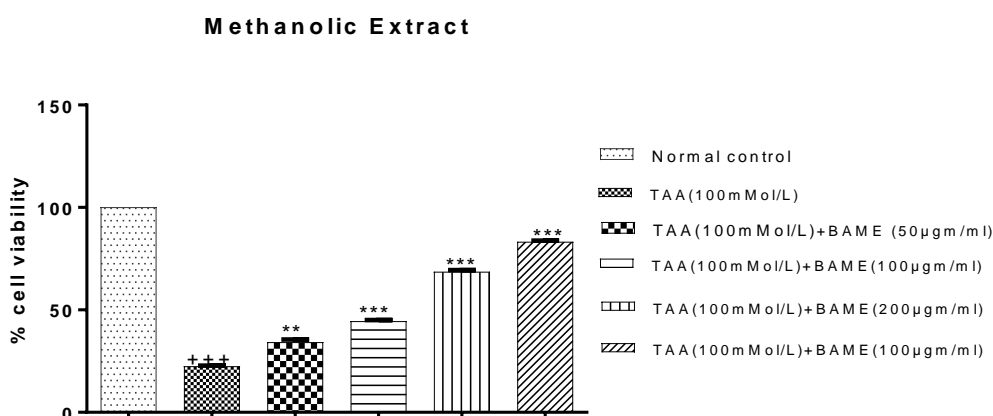
Thioacetamide exposed HepG2 cells and Hep3b showed a percentage viability of 22% and 13.9% respectively. These exposed cells, when treated with different concentrations of the methanolic extract of *Bambusa arundinaceae*, showed a dose-dependent increase in percentage viability and the results were highly significant (p < 0.001), compared to Thioacetamide intoxicated group (Figure 4 and 5).

Effect of Methanolic extract and Thioacetamide On primary hepatocytes

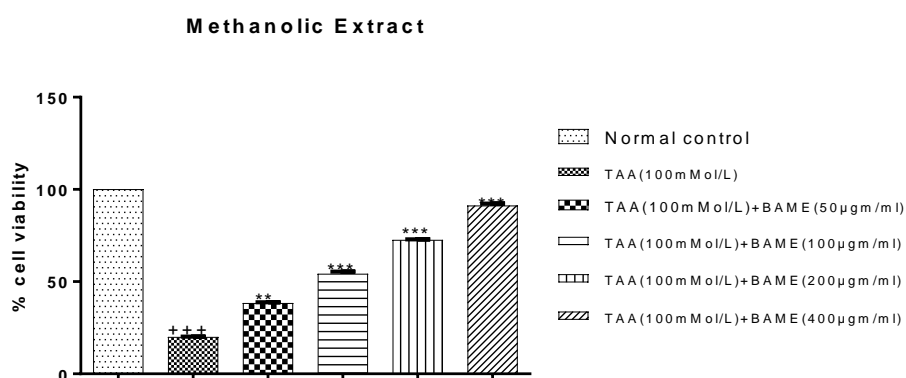
The effects of methanolic extract of *Bambusa arundinaceae* on freshly isolated rat hepatocytes intoxicated with thioacetamide are recorded in. Methanolic extract of *Bambusa arundinaceae* shows dose dependent increase in cell viability (P < 0.001), compared to Thioacetamide intoxicated group (Figure 6).



**Figure 4:** *In vitro* hepatoprotective activity of methanolic extract in thioacetamide induced cell injury in hep3b Cell lines. Data are expressed as mean ± SD. \*\*p< 0.001 &\*\*\*p< 0.0001 compared to TAA control group, and +++p<0.0001 compared to normal control group. BAME=*Bambusa arundinaceae* methanolic extract, TAA = Thioacetamide.



**Figure 5:** *In vitro* hepatoprotective activity of methanolic extract in thioacetamide induced cell injury in HepG2 Cell lines. Data are expressed as mean ± SD. \*\*p< 0.001 &\*\*\*p< 0.0001 compared to TAA control group, and +++p<0.0001 compared to normal control group. BAME=*Bambusa arundinaceae* methanolic extract, TAA = Thioacetamide.



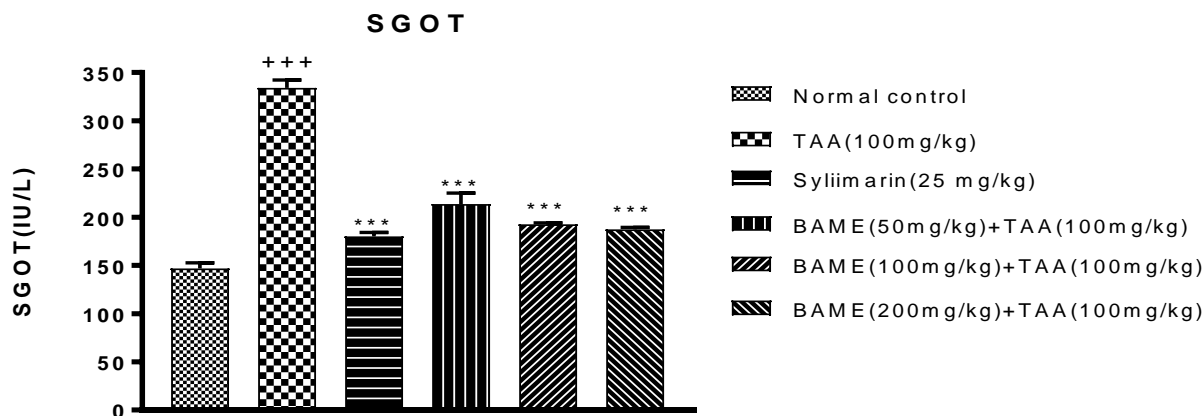
**Figure 6:** *In vitro* hepatoprotective activity of methanolic extract in thioacetamide induced cell injury in primary hepatocyte. Data are expressed as mean ± SD. \*\*p< 0.001 &\*\*\*p< 0.0001 compared to TAA control group, and +++p<0.0001 compared to normal control group. BAME=*Bambusa arundinaceae* methanolic extract, TAA = Thioacetamide.

***In vivo* hepatoprotective activity**

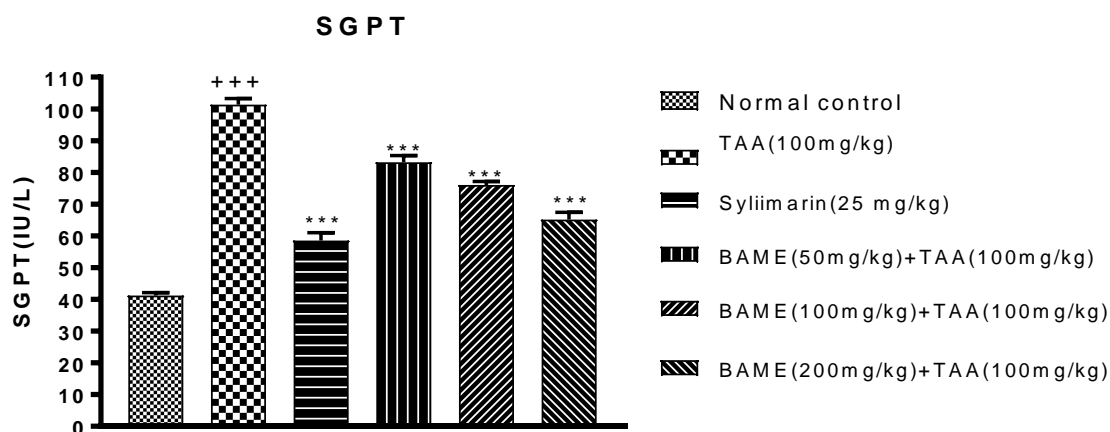
*Biochemical parameters*

Biochemical parameters like SGPT, SGOT, ALP and Total and direct bilirubin were significantly ( $P < 0.001$ ) increased in thioacetamide treated group when compared to control. The methanolic extract of

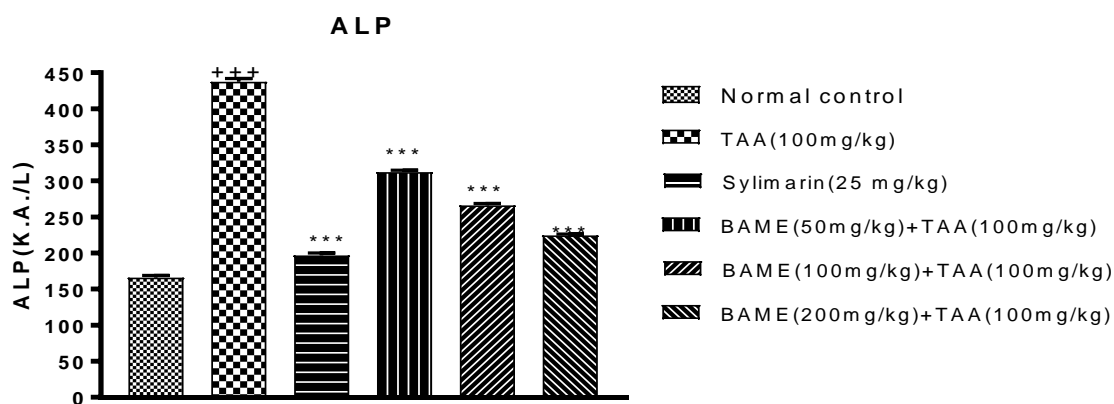
*Bambusa arundinaceae* at dose of 50, 100, 200 mg/kg significantly decrease the level ( $P < 0.001$ ) of these biochemical parameter in blood serum as compared to thioacetamide treated animals comparable with Silymarin ( $P < 0.001$ ). The dose of 200 mg/kg treated group was found to be more effective and similar to silymarin treated group (Figure 7,8,9, 10 and 11).



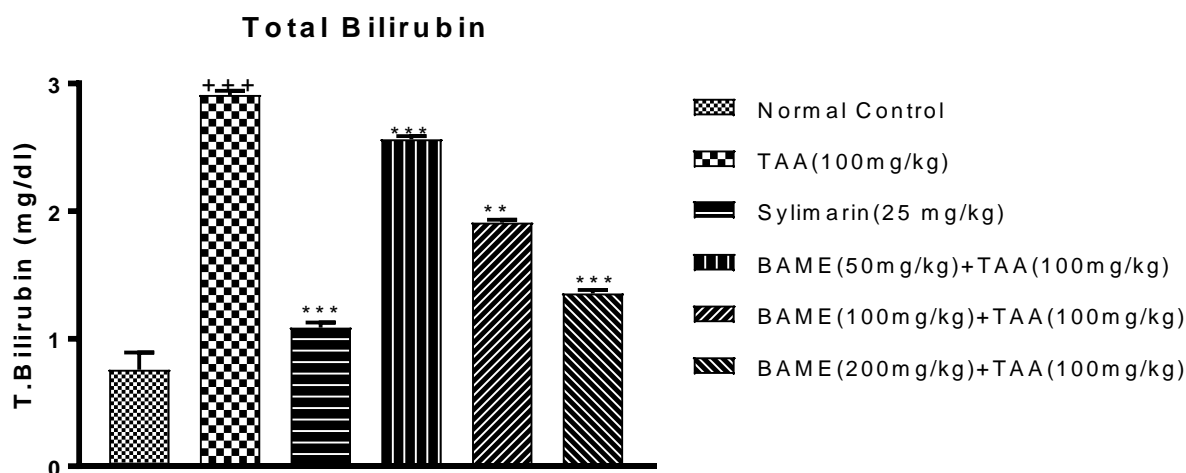
**Figure 7:** Effect of methanolic extract of shoots of *Bambusa arundinaceae* and Thioacetamide on SGOT in rats. Data are expressed as mean  $\pm$  SD. Means among groups (rate/group) show significant difference, \*\*\* $p < 0.0001$  compared to TAA control group, and +++ $p < 0.0001$  compared to normal control group. BAME= *Bambusa arundinaceae* methanolic extract, TAA = Thioacetamide



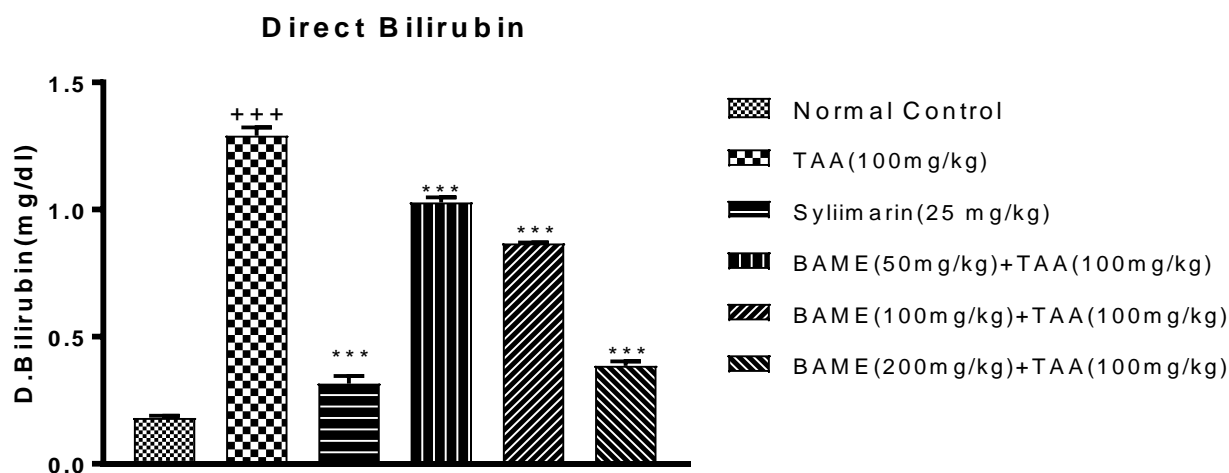
**Figure 8:** Effect of methanolic extract of shoots of *Bambusa arundinaceae* and Thioacetamide on SGPT in rats. Data are expressed as mean  $\pm$  SD. Means among groups (rate/group) show significant difference, \*\*\* $p < 0.0001$  compared to TAA control group, and +++ $p < 0.0001$  compared to normal control group. BAME= *Bambusa arundinaceae* methanolic extract, TAA = Thioacetamide



**Figure 9:** Effect of methanolic extract of shoots of *Bambusa arundinaceae* and Thioacetamide on ALP in rats. Data are expressed as mean  $\pm$  SD. Means among groups (rate/group) show significant difference, \*\*\* $p < 0.0001$  compared to TAA control group, and +++ $p < 0.0001$  compared to normal control group. BAME= *Bambusa arundinaceae* methanolic extract, TAA = Thioacetamide



**Figure 10:** Effect of methanolic extract of shoots of *Bambusa arundinaceae* and Thioacetamide on Total Bilirubin in rats. Data are expressed as mean  $\pm$  SD. Means among groups (rate/group) show significant difference, \*\* $p < 0.001$ , \*\*\* $p < 0.0001$  compared to TAA control group, and +++ $p < 0.0001$  compared to normal control group. BAME= *Bambusa arundinaceae* methanolic extract, TAA = Thioacetamide



**Figure 11:** Effect of methanolic extract of shoots of *Bambusa arundinaceae* and Thioacetamide on Direct Bilirubin in rats. Data are expressed as mean  $\pm$  SD. Means among groups (rate/group) show significant difference, \*\* $p < 0.001$ , \*\*\* $p < 0.0001$  compared to TAA control group, and +++ $p < 0.0001$  compared to normal control group. BAME= *Bambusa arundinaceae* methanolic extract, TAA = Thioacetamide

### Histopathology

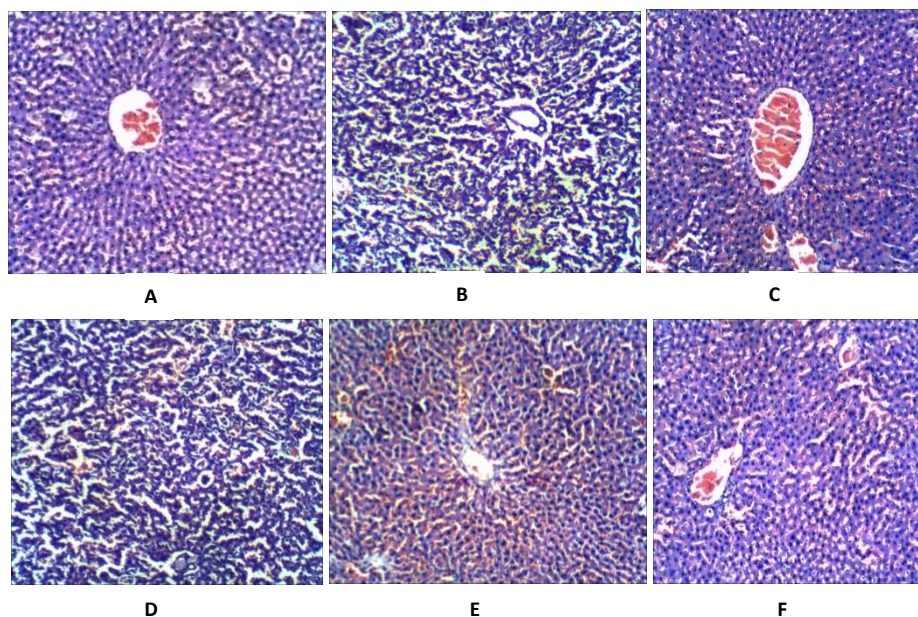
Livers from the normal, groups showed normal lobular architecture with distinct hepatic cells, sinusoidal spaces, and a central vein. On the other hand, the livers of the Thioacetamide treated animals exhibited loss of normal lobular architecture with eosinophilic infiltration. Liver of rat treated with methanolic extract of young shoots of *Bambusa arundinaceae* showed recovery of thioacetamide induced toxicity. Animals treated with the higher dose of the extract showed remarkable histological regeneration compared to those of the low dose group (Figure 12).

The present study was done to find out antioxidant and hepatoprotective activity of methanolic extract of young shoots of *Bambusa arundinaceae* in thioacetamide induced liver injury.

The total phenolic content of methanolic extract of young shoots of *Bambusa arundinaceae*, calculated from the calibration curve, was  $25.5 \pm 2.2$  gallic acid equivalents/gDW, and the total flavonoid content

was  $36 \pm 3.7$  Quercetin equivalents/gDW. Phenolic compounds have redox properties, which allow them to act as antioxidants. Their free radical scavenging ability is facilitated by their hydroxyl groups.<sup>[42]</sup> Flavonoids, have shown antioxidant activity *in vitro* and *in vivo* which depends on the presence of free OH groups, especially 3-OH.<sup>[43,44]</sup> The total phenolic and flavanoid concentration could be used as a basis for screening of antioxidant activity of the plant

Antioxidants possess the ability to protect the body from damage caused by free radical induced oxidative stress.<sup>[45]</sup> The electron donation ability of natural products can be measured by 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured solution bleaching<sup>[46]</sup>. This method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. In present study DPPH scavenging activity of methanolic extract was ( $IC_{50}=257.15$ ), while that of the standard, ascorbic acid, was ( $IC_{50}=170.03$ ).



**Figure 12:** Histopathology of methanolic extract of *Bambusa arundinaceae* treated rats shows recovery of thioacetamide induced liver toxicity in rats (10x). (A) Normal liver showing intact structure with and central vein, (B) Thioacetamide (100 mg/kg s.c.) produce necrosis and disrupted structure, (C) Syllimarin (25 mg/kg i.p.) treated liver showing less necrosis, less eosinophilic infiltration and more intact structure, (D) 50 mg/kg o.p, (E) 100 mg/kg o.p, (F) 200 mg/kg o.p treated rat liver showing dose dependent reduction in necrosis and more intact structure comparable with syllimarin treatment.

The process of lipid peroxidation has been suggested to proceed via a free radical chain reaction, which has been associated with cell damage in biomembranes. This damage leads to different diseases like cancer, liver disease, cardiovascular diseases and diabetes [47]. Methanolic extract showed inhibition of lipid peroxidation in liver homogenate with IC<sub>50</sub> of (IC<sub>50</sub>=197.91) comparable with standard (IC<sub>50</sub>=180.12).

In reducing power assay, the yellow colour of the test solution changes to green depending on the reducing power of the test specimen. The presence of the reductants in the solution causes the reduction of the Fe<sup>3+</sup>/ferricyanide complex to the ferrous form [48]. In reducing power assay the, methanolic extract shows significant changes in absorbance at 700 nm with increasing concentrations of extract (25-250, µg/mL).

Thioacetamide, a thiono-sulfur containing compound (CH<sub>3</sub>CSNH<sub>2</sub>), undergoes an extensive metabolism to produce acetamide and thioacetamide-S-oxid (TAA-S-oxide). TAA-S-oxide which is further metabolized, by cytochrome P-450 monooxygenases, resulting in formation of TAA-S-dioxide, which exerts hepatotoxicity by binding to hepatocyte macromolecules and causes centrilobular necrosis by generation of reactive oxygen species The cell viability is directly related to the structure of the cellular membrane [49,50]. In present study the methanolic extract of young shoots of *Bambusa arundinaceae* have shown increase in cell viability in dose dependent manner in HepG2, Hep3b cell lines and primary hepatocytes.

Thioacetamide administration in rats caused significant increase in SGPT, SGOT, ALP, Total and Direct Bilirubin. Methanolic extract of young shoots of *Bambusa arundinaceae* reduced those levels. Histopathological data also point toward a protective effect of Methanolic extract of young shoots of *Bambusa arundinaceae* against TAA-induced liver injury comparable with syllimarin.

## CONCLUSION

This study reveals that the methanolic extract of young shoots of *Bambusa arundinaceae* has significant antioxidant and *in vivo, in vitro* hepatoprotective activity may be due to presence of phenolic and flavonoid contents.

## Conflict of interest

None declared.

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