

Analysis of toxic, antidiabetic and antioxidant potential of *Bambusa balcooa* Roxb. leaf extracts in alloxan-induced diabetic rats

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Abstract *Bambusa balcooa* (Poaceae) is native to India and has been used traditionally by the tribes of Northeast India to treat diabetes. The present investigation was aimed to evaluate the toxicity, anti-diabetic activity along with in vitro antioxidant activity of the leaf of *B. balcooa* in alloxan-induced diabetic rats and also identify active compounds by using HPLC. The acute toxicity test of aqueous extract of *B. balcooa* leaf revealed that the median lethal dose (LD₅₀) of *B. balcooa* aqueous extract (BAQE) was 5.18 g/kg body weight in mice. Administration of BAQE at 100 and 200 mg/kg in alloxan-induced diabetic rats showed significant reduction in fasting blood glucose and glycated hemoglobin while plasma insulin level was elevated compared to diabetic control. Both the doses were effective when compared to diabetic glibenclamide rats. The BAQE treated diabetic rats showed significant increase in the endogenous antioxidant enzymes superoxide dismutase, glutathione peroxidase and decrease in malondialdehyde levels. HPLC analysis of BAQE showed the presence of rutin, gallic acid and β sitosterol. Thus, it can be inferred from this study that BAQE possess antidiabetic

and in vivo antioxidant activity. The overall activity might be possibly due to the presence of potential antioxidants.

Keywords *Bambusa balcooa* · Antioxidants · Diabetes · Toxicology · Flavonoids · Polyphenols

Introduction

Tropical medicines are the main stay of therapy for most of the patients in developing countries. Diabetes mellitus (DM) is prevalent among almost 200 million people worldwide, which is thought to increase exponentially to 300 million in the next two decades, type 2 being more common (Choi et al. 2008). Increased oxidative stress and exhausted antioxidant defences are mainly responsible for the chronic hyperglycemia, which further leads to dysfunction and failure of various organs like kidney, heart, eyes and nerves (Middha et al. 2011). The adverse effects of anti-diabetic drugs drifted scientific interest towards plant-based chemical moieties which, in turn, might reduce economic and clinical toll of DM (Singh et al. 2012; Palsamy and Subramanian 2008). It has also been proposed that decrease of lipid peroxidation and increased antioxidant would reduce diabetes disorders (Kiran et al. 2013; Prasad et al. 2012). The ethnobotanical lore of India is very rich. In India, numerous floras are used for the treatment of diabetes since ancient days and today scientific evidences also support their uses (Middha et al. 2009, 2016a).

Bambusa balcooa Roxb. (BB), (Poaceae) is an indigenous species native to India. The culms are tangled having a diameter of about 15 cm and attain a maximum height of about 18.3 m. The tender shoots are edible (Goyal et al. 2010a). The siliceous secretion of the culm is believed to be aphrodisiac and can also be used as tonic (Manandhar

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and Manandhar 2002). In Dibrugarh district, Assam, India crushed leaves of BB combined with *Cucumis sativus* is administered orally to treat epistaxis (Kalita and Phukan 2010). Apart from this, BB young leaves decoction is consumed orally to treat diabetes traditionally by the Moran folk of Tinsukia district of Assam, India (Kalita and Phukan 2009). In vitro antidiabetic studies of BB have shown promising results (Middha and Usha 2012). Survey of scientific literature revealed that the experimental evidence on the traditional use of this plant is not yet documented. To the best of our knowledge, no compounds have been detected in BB until now. Keeping this in view, the present study might be the first one to evaluate the toxicity, anti-diabetic activity, in vitro and in vivo antioxidant activity of the leaf of BB and also identify active compounds by using HPLC.

Materials and methods

Chemicals and reagents

Ascorbic acid, 2,2-diphenyl-1-picryl-hydrazyl (DPPH), ferric chloride (FeCl₃), gallic acid (GA), quercetin, sodium nitrite (NaNO₂), trichloroacetic acid (TCA) were obtained from Himedia Laboratories Pvt. Ltd, Mumbai, India. acetic acid (CH₃COOH), bromophenol blue, Di-potassium hydrogen phosphate (K₂HPO₄), disodium hydrogen phosphate (Na₂HPO₄), methanol, *n*-butanol, potassium di-hydrogen phosphate (KH₂PO₄), potassium ferricyanide (K₂Fe(CN)₆), sodium hydroxide (NaOH), sodium carbonate (Na₂CO₃), hydrogen peroxide (H₂O₂), ferrous sulfate, (FeSO₄·7H₂O), pyridine (C₅H₅N), ethanol (C₂H₅OH), and thiobarbituric acid were procured from Merck, Mumbai, India. Alloxan, 2,4-dinitro phenyl hydrazine, glutathione reductase, epinephrine, nicotinamide, pyridine, thiobarbituric acid, *t*-butyl hydrogen peroxide, reduced glutathione, adenine dinucleotide phosphate, 1,1,3,3-tetra methoxy propane, Sigma (St. Louis, MO, USA). Insulin was obtained from Biocon Pvt. Lmt., Bangalore, India. All organic solvents were of spectral grade and general chemicals were of analytical grade and were purchased from local companies.

Plant material and extraction

The BB leaf was collected from Sukna forests during July, 2012. A voucher specimen has been deposited at Bam-busetum, Kurseong Research Range, Sukna, Darjeeling, West Bengal (SUK/KRR/B005) (Goyal et al. 2012).

The material was shade dried and ground to fine powder. The pulverized leaves (10 g each) were extracted by Soxhlation using double-distilled water (BAQE), acetone

(BAE) and methanol (BME), respectively (Plant: solvent 1:15 w/v) (Middha et al. 2011) for 48 h. Prior to use, the freeze-dried extracts were dissolved in double-distilled water (DDW) in desired concentrations for further use.

Determination of plant extracts yield, total phenolic and flavonoid content

The yield of evaporated extracts (w/w), the total soluble phenolic (mg gallic acid equivalent (GAE)/g) and flavonoid content (quercetin equivalents (QE)/g) in the extracts were estimated according to standard protocol of Goyal et al. (2010b).

Determination of total flavonol content

The protocol developed by Kumaran and Karunakaran (2007) was used to estimate the total flavonols using quercetin as a standard. The absorbance at 440 nm was read after 2.5 h. The total amount of flavonol was calculated using previously formula used by Usha et al. 2014 and expressed as µg quercetin equivalents (QE)/g of extract.

Determination of total proanthocyanidin content

The total proanthocyanidin was determined as per protocol previously reported by Sun et al. (1998). Quercetin was used as standard. The absorbance was measured at 500 nm. Total proanthocyanidin contents were expressed as µg quercetin equivalents (QE)/g of extract, using the following equation of the curve: $Y = 0.024x - 0.034$, $R^2 = 0.995$, where x is the absorbance and Y is the quercetin equivalent.

In vitro antioxidant properties of the extract

The antioxidant activity of the extracts and standard were assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, ferric reducing power assay (FRAP) and scavenging of hydrogen peroxide determined as described by Goyal et al. (2010b).

Lipid peroxidation radical scavenging

Inhibition of lipid peroxidation induced in rat whole liver homogenate

Lipid peroxidation was induced by Fe²⁺ EDTA system in the rat liver homogenate in the presence and absence of BB extracts to form thiobarbituric acid-reacting substance (TBARS). The TBARS thus formed was determined by the method of Ohkawa et al. (1979).

HPLC analysis of the aqueous extract of BB leaf

Preparation of standard and sample solutions

Stock solutions of GA, Quercetin, β sitosterol and Rutin were prepared at a concentration of 1000 $\mu\text{g/ml}$ just prior to use and used as reference standards. The aqueous extract of BB leaf extract was dissolved in HPLC grade water to get the desired concentration of 4 mg/ml and used as sample. Prior to injection all the solutions were filtered through 0.22 μm Millipore filter.

Chromatographic conditions for HPLC

The liquid chromatographic (isocratic HPLC Shimadzu) system consisted of pump (LC-10ATVP, Shimadzu, Japan), UV-Vis detector (SPD-10A Shimadzu) equipped with chromtech N2000 software and a Rheodyne (7725i) sample injector fitted with a 20- μl sample loop. The chromatographic separation was carried out on Supelco C18 (250X4.6 mm i.d. 5 μm) column. The mobile phase was water: methanol (60:40 v/v). All the separations were performed isocratically at a flow rate of 1 ml/min at room temperature. The peak area was determined using a UV detector at a wave length of 254 nm. The chromatographic peaks of the analytes were confirmed by comparing their retention time (Rt) and UV spectra with those of the reference standards. Integrated peak areas of the samples using the corresponding standard were used to quantify the compounds (Middha et al. 2013).

In vivo hepatoprotective activity

Experimental animals

Male Wistar albino rats (150–200 g) were housed under standard laboratory conditions of 12-h light and dark cycles, temperature of 25 ± 2 °C and relative humidity of $60 \pm 1\%$. The animals were given standard rat pellet (Lipton India Ltd., Bangalore) and tap water ad libitum. The experimental procedures were conducted after the approval of Ethical Committee (1368/ac/10/CPCSEA) and were in strict accordance with the protocol approved by Maharani Lakshmi Ammanni College Ethical Committee, Bangalore for the care and use of laboratory animals.

Acute toxicity test

Swiss albino mice (25–30 g) of either sex were divided into five groups of ten each. Animals were fasted overnight but water was made available prior to experiment. Aqueous extract of *B. balcooa* leaf at different dose levels [5.0, 6.0, 7.0, 8.0, 9.0 g/kg body weight (BW)/ml] was administered

once to experimental groups. The mice were observed for 24 h, mortality was recorded and median lethal dose LD_{50} (median lethal dose) was determined using the arithmetic method of Karber modified by Aliyu and Nwude (Middha et al. 2011) as per OECD guideline. The acute toxicity (LD_{50}) was calculated using the formula:

$$\text{LD}_{50} = \text{LD}_y - \frac{1-r}{r} \Sigma(\text{Dd} \times \text{Md})/n,$$

where LD_y = highest dose and n = number of animals per group ($n = 10$), Dd = dose difference, Md = mean dead.

Alloxan-induced Wistar albino rat model for diabetes mellitus (DM)

DM was induced in overnight-fasted rats by administering alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) (150 mg/kg BW i.p) in 50 mM phosphate buffer (pH 7.0) in a volume of 1 ml/kg BW. Fasting blood glucose (FBG) with 220–260 dl/ml was considered diabetic and was used for further experimental purposes (Middha et al. 2011, 2012).

Experimental groups

Rats were randomly allotted into seven groups.

Group 1 served as normal control and received distilled water (NL).

Group 2 served as normal animals treated with BB 200 mg/kg BW (NL + BB).

Group 3 served as diabetic control animals treated with alloxan (DC).

Group 4 served as alloxan-induced diabetic rats treated with 100 mg/kg BW aqueous extract of BB leaf (DC + LB) for 45 days.

Group 5 served as alloxan-induced diabetic rats treated with 200 mg/kg BW aqueous extract of BB leaf (DC + HB) for 45 days.

Group 6 served as alloxan-induced diabetic rats treated with glibenclamide (DG) (600 $\mu\text{g/kg}$ BW) for 45 days.

Group 7 served as alloxan-induced Diabetic rats treated with standard drug insulin (DC + I) (2 units/kg BW) (Insugen, Biocon) daily intraperitoneally for 45 days.

The drug treatment was carried out every day morning with the help of 16 gauge ball-tipped feeding needle for a period of 6 weeks. FBG was determined after 1st and 6th week of drug treatment. However, BW was determined on everyday basis. After 6 weeks of drug treatment, rats were euthanized using 0.3 ml/100 g i/p Ketamine (300 mg/kg) + 0.15 ml/100 g Xylazine (30 mg/kg) as recommended by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and biochemical assays were performed with liver.

Estimation of blood glucose

Blood samples were withdrawn by end tail vein cutting method from overnight-fasted animals and blood glucose level was determined using one-touch electronic glucometer ACU check (Apollo Pharmacy, Bangalore, India) (Middha et al. 2012).

Preparation of tissue

Liver tissue was prepared by the method used by Middha et al. 2011. The tissue was homogenized in 50 mM phosphate buffer pH (7.0) containing 0.1 mM EDTA to obtain a 5% homogenate for the malondialdehyde (MDA) tests. Analytical procedures for superoxide dismutase (SOD) and glutathione peroxidase (GPx) were assayed in the supernatant obtained after centrifugation of the 5% homogenate at $600\times g$ for 10 min at 4 °C (RV/FM, super spin, Plastocraft, India).

Biochemical estimation

Markers of lipid per oxidation (LPO), malondialdehyde (MDA), glutathione peroxidase (GPx) and the activities of superoxide dismutase (SOD) in liver tissue were determined as described by Middha et al. (2011).

Statistical analysis

Data were expressed as mean \pm SE. Comparison between different groups was done using one-way ANOVA, followed by Tukey's Multiple comparison test's (Graph pad Prism windows 5). $p < 0.05$ was considered to be statistically significant. All determinations were carried out in triplicates.

Results and discussion

Plant yield

The plant yield of different fractions of *B. balcooa* leaf, i.e. BAQE, BME and BAE was found to be 7.56, 6.97 and 5.73%, respectively. The highest yield was obtained when extracted with water followed by methanol and acetone. Solvent selection was done based on the ethnopharmacological uses of the plants. Previous studies indicate methanol, water and acetone extraction as the most popular solvents used for the plant extraction research (Middha et al. 2013, 2014, 2016a, b; Goyal et al. 2011a, b, 2013).

Total phenolic, flavonoid, flavonol, proanthocyanidin contents

It was elucidated that the BME showed higher phenolic content than the BAQE and BAE in mg/g as GA equivalent, i.e. BME (378.55 ± 0.03) >BAQE (367.90 ± 0.016) >BAE (219.96 ± 0.026). BME also exhibited high content of flavonoid, flavonol and proanthocyanidin (Table 1). The higher polyphenolic content in BME might be due to the difference in the polarity of the solvent and the potential of methanol was to release the bound polyphenols present in the cell wall of the plants (Goyal et al. 2011a, b). Thus, methanol proved to be a suitable solvent for extraction of the plant as our studies are in accordance to the solvent used for various plants with previous reports (Middha et al. 2013, 2016a, b).

In vitro antioxidant assays

DPPH radical scavenging activity

The radical scavenging activity, using a DPPH-generated radical, was tested with different sample extracts (Fig. 1). It was observed that the BME exhibited the highest radical scavenging activity followed by BAQE. However, BAE extract showed the weakest activity.

Ferric reducing power assay (FRAP)

The reducing power of the extract was also found to be substantial. The reducing power in different leaf extracts of BB ranged from 0.006 to 0.217 at concentrations ranging from 20 to 200 $\mu\text{g/ml}$. The reducing capacity of extracts was as follows: BAQE > BME > BAE (Fig. 2). In the case of reducing power assay, the transformation of Fe^{3+} to Fe^{2+} in the presence of either the extract or the standard (ascorbic acid) is a measure of reducing capability. It is found that the reducing power of the extract increases with increase in concentration. The highest reducing ability was noted in BAQE (0.217) followed by BME (0.079) and BAE (0.027) compared to ascorbic acid (0.041) as standard at the concentration of 200 $\mu\text{g/ml}$. Similar trend shown by the BAE and BME was very similar at 20, 80 and 120 $\mu\text{g/ml}$. Results were in accordance to previous works on other bamboo species (Goyal et al. 2011a; Nagavani and Rao 2010). Mostly, the antioxidant activity in plants is due to the presence of polyphenols (Nakbi et al. 2010; Middha et al. 2013; Usha et al. 2014). Moreover, the higher the inhibitory action, the more powerful is the antioxidant activity (Ajith 2010).

Table 1 Polyphenol contents in different fractions of *B. balcooa* leaf ($n = 3, X \pm SEM$)

	BAQE	BME	BAE
TPC ^a	367.90 \pm 0.016	378.55 \pm 0.030	219.96 \pm 0.026
TFC ^b	355.20 \pm 0.014	365.20 \pm 0.034	227.20 \pm 0.007
TFLC ^c	3.62 \pm 0.002	12.30 \pm 0.006	1.80 \pm 0.001
TPrC ^d	38.44 \pm 0.010	298.24 \pm 0.010	20.39 \pm 0.009

^a Total phenol content analyzed as gallic acid equivalent (GAE) mg/g of extract

^b Total flavonoid content analyzed as quercetin equivalent (QE) mg/g of extract

^c Total flavonol content analyzed as quercetin equivalent (QE) μ g/g of extract

^d Total proanthocyanidin content analyzed as catechin equivalent μ g/g of extract

Fig. 1 DPPH scavenging activity of *B. balcooa* leaf extract compared to standard ascorbic acid

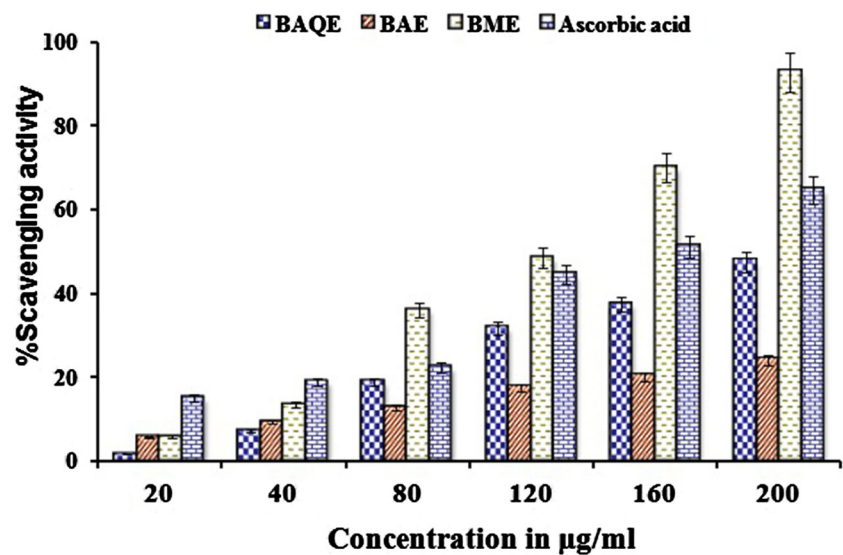


Fig. 2 Ferric reducing power assay of *B. balcooa* leaf extract compared to standard ascorbic acid

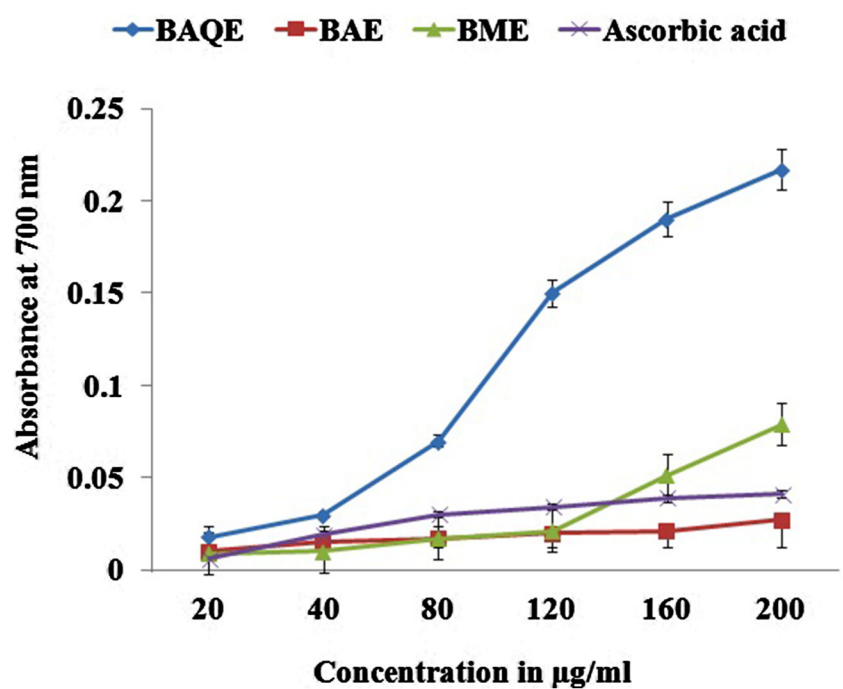
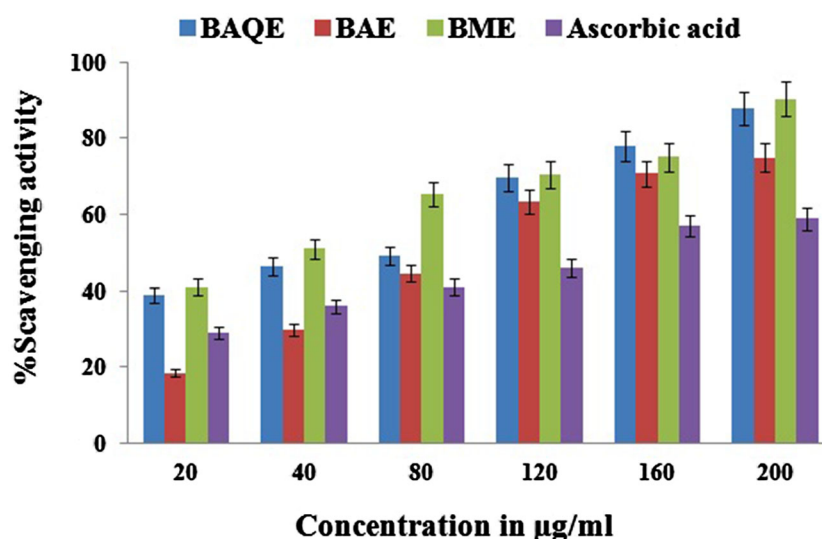


Fig. 3 Hydrogen peroxide scavenging activity of *B. balcooa* leaf extract compared to standard ascorbic acid



Hydrogen peroxide (H_2O_2) scavenging activity

Figure 3 represents the H_2O_2 scavenging activity of BME, BAQE and BAE. A high amount of H_2O_2 scavenging activity was observed. The conversion of H_2O_2 into water may occur according to the antioxidant compounds as the antioxidant component present in the extract are good electron donors, they may accelerate the conversion of H_2O_2 to H_2O . H_2O_2 although being a weak oxidizing agent has the potential to turn off a few enzymes directly (Middha et al. 2013). In the presence of redox active transition metals Fe^{2+} and Cu^{2+} , H_2O_2 is transformed to hydroxyl radical which might be the key to its toxic effect (Goyal et al. 2011a). Thus, the amount of H_2O_2 accrued in the cells should be monitored. Figure 3 illustrates the leaf extract is a good scavenger of H_2O_2 .

Lipid peroxidation assay

Lipid peroxidation assay was used to access the antioxidant properties of the extracts in this study. The effects of various concentrations of BAQE, BME and BAE (10–80 mg/ml) on lipid peroxidation are depicted in Table 2. As indicated in the study, BME could inhibit lipid

Table 2 Lipid peroxidation profile of *B. balcooa* different leaf extract ($n = 3$, $X \pm SEM$)

Lipid peroxidation (I%)			
Concentration (mg/ml)	BAQE	BME	BAE
Control	00.00 \pm 0.00	00.00 \pm 0.00	00.00 \pm 0.00
10	7.72 \pm 0.70	28.38 \pm 1.21	22.60 \pm 0.34
20	10.27 \pm 0.91	36.06 \pm 2.12	37.41 \pm 1.44
30	27.21 \pm 1.45	39.17 \pm 3.34	44.96 \pm 1.07
40	28.21 \pm 2.41	65.31 \pm 5.15	55.08 \pm 1.22
80	36.26 \pm 1.32	71.10 \pm 4.53	68.74 \pm 3.11

peroxidation effectively than that of control followed by BAE and BAQE. The lipid peroxidation inhibition increased with increasing concentrations of all the three extracts. BME effectively and dose dependently protected the lipid peroxidation in the liver homogenate. So, it can be inferred that due to high contents of antioxidants in BME, it showed high antioxidant activity on lipid peroxidation.

HPLC analysis of BAQE leaf extract

In the present study, only the aqueous extract of BB leaf was considered for HPLC analysis primarily due to two reasons. One being the presence of moderate amount of antioxidants and the other being its traditional use to treat diabetes as aqueous extract by the Moran folk in Tinsukia district of Assam (India) (Kalita and Phukan 2010) in the same form. The chromatogram tested at 254 nm is depicted in Fig. 4. The HPLC estimation of BAQE leaf showed the presence of different phytoconstituents. However, the only three phytoconstituents or biomarkers were identified viz. rutin (1.03 mg), gallic acid (0.007 mg) and β sitosterol (0.065 mg) (Fig. 4). Rutin is the glycosidic form of quercetin. The pharmacological effect of rutin is well documented for antidiabetic conditions (Middha et al. 2013; Sharma et al. 2013). The presence of rutin in the bamboo extract can induce the β cells to produce insulin and/or protect the β cells from deterioration and, thus, help in reducing diabetes. Similarly, the antioxidant property of gallic acid is also reported (Ow and Stupens 2003) and the property of gallic acid to enhance the insulin receptor sensitivity might be the reason for its anti-hyperglycaemic activity (Huang et al. 2005). Moreover, rutin was higher in content compared to gallic acid and hence rutin may be responsible for the antidiabetic activity and the gallic acid, in turn, may act as adjuvant with rutin and enhance the pharmacological action of BB leaf extract. The third compound

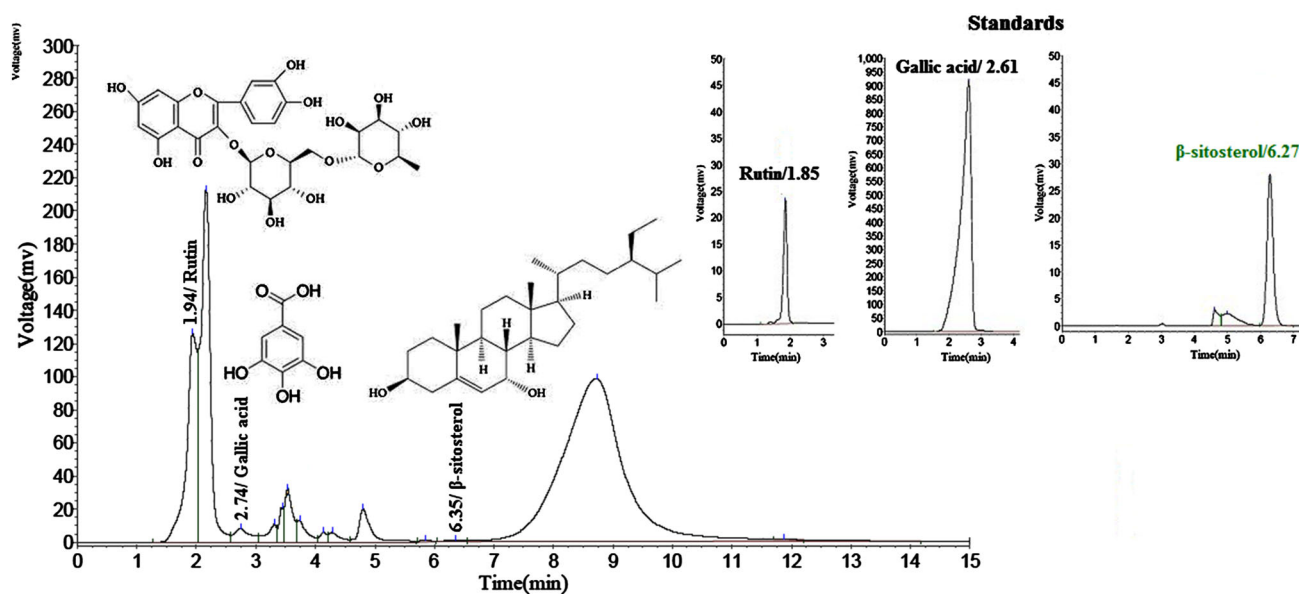


Fig. 4 HPLC chromatogram of *B. balcooa* aqueous leaf extract

detected is the β sitosterol, which is already established as an active phytosterol having multiple pharmacological properties (Mutai et al. 2009). The presence of β sitosterol in the plant extract might also complement for the antidiabetic activity of BB leaf extract. The possible reason might be that it elevates insulin secretion either through its antioxidant activity (Vivancos and Juan 2005) or regeneration of β cells of Islets of Langerhans (Gupta et al. 2012).

In vivo anti-hyperglycemic activity

Here, an attempt was made to study the anti-hyperglycemic effect of the aqueous leaf extract of *Bambusa balcooa* in alloxan-induced diabetic rats.

Acute toxicity test

The primary sign of toxicity was noticed only after 10–12 h of the administration of extract with decrease in locomotor activity and the sense of touch. This was followed by reduced feed intake and prostration 5.18 g/kg body after about 18 h. LD₅₀ of the aqueous extract of BB was found to be weight in mice and thus can be considered to be relatively safe as per Loomis and Hayes LD₅₀ Scale (1996). In general, the lesser the LD₅₀ value, the more lethal the compound is.

Effect on fasting blood glucose, plasma insulin and glycated hemoglobin

Table 3 infers the fasting blood glucose (FBG), plasma insulin and glycated hemoglobin (glycated Hb) level in alloxan-

induced diabetic rats treated with 100 and 200 mg/kg of BAQE extract, insulin, glibenclamide and control groups. FBG increased up to three folds after alloxan induction compared to normal control rats. The higher dose (200 mg/kg BW) of BAQE showed more significant reduction (50.86%) in FBG than lower dose (100 mg/kg BW) (35.64%) of extract and glibenclamide-treated rats (44.98%) as compared to the diabetic control rats. The possible mechanism by which BAQE brings about its hypoglycemic action might be that it possess insulin-like effect either by promoting glucose uptake or inhibiting hepatic gluconeogenesis (Tanko et al. 2008) also it might act by eliciting either the pancreatic secretion of insulin from the β cells of Islets of Langerhans or its release from the bound form (Middha et al. 2012). Similar to FBG level, the administration of extract at 100 and 200 mg/kg BW and glibenclamide significantly reduced the glycated Hb level by 34.81, 54.27 and 40.78% as compared to diabetic control rats. This decrease in the glycated Hb level might be due to increase in insulin secretion and reactivation of glycogen synthase enzyme system (Pandhare et al. 2011). The infusion of BAQE extract at both the doses significantly increased the plasma insulin level from 2.09 ± 0.27 μ U/ml in diabetic control to 7.08 ± 0.41 and 11.08 ± 0.54 μ U/ml at 100 and 200 mg/kg BW, respectively. These observations suggest that the hypoglycaemic activity of this bamboo may be mediated through enhancement of peripheral metabolism of glucose and an increase in insulin release.

Effect of BAQE leaf extract on SOD, GPx and MDA

In the current study, the activity of endogenous enzymatic antioxidants such as SOD and GPx has been evaluated.

Table 3 Effect of *B. balcooa* aqueous leaf extract on fasting blood glucose, plasma insulin and glycated Hb levels in control and alloxan-induced diabetic rats

Experimental groups	Fasting blood glucose (mg/dL)	Plasma insulin (μ U/ml)	Glycated Hb (% total Hb)
NL	89.54 \pm 6.74	13.15 \pm 0.65	1.32 \pm 0.07
NL + BB	90.12 \pm 8.87	13.09 \pm 1.71	1.28 \pm 0.21
DC	289 \pm 3.87 ^a	2.09 \pm 0.27 ^a	5.86 \pm 0.74 ^a
LB	186.61 \pm 8.7 ^b	7.08 \pm 0.41 ^b	3.82 \pm 0.27 ^b
HB	142.86 \pm 7.17 ^b	11.08 \pm 0.54 ^b	2.68 \pm 0.42 ^b
DG	159.0 \pm 9.47 ^b	9.83 \pm 0.47 ^b	3.47 \pm 0.54 ^b
DI	108.60 \pm 1.43 ^b	12.08 \pm 0.46 ^b	1.98 \pm 0.42 ^b

NL normal, NL + BB normal control rats treated with *Bambusa balcooa* leaf, DC diabetic control, LB diabetic control rats treated with *B. balcooa* leaf (100 mg/kg BW), HB diabetic control rats treated with *B. balcooa* leaf (200 mg/kg BW), DG diabetic control treated with glibenclamide, DI diabetic insulin. Values are mean \pm SE ($n = 6$ animals/group) in experimental animals

^a Significant ($p < 0.05$) as compared to normal control rats

^b Significant ($p < 0.05$) as compared to alloxan induced diabetic rats

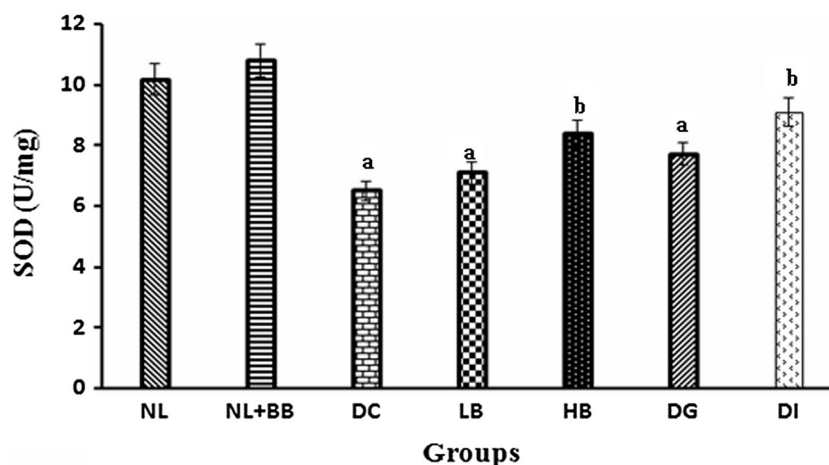


Fig. 5 Effect of *B. balcooa* leaf extracts (BB) supplementation on superoxide dismutase in liver of experimental rats. NL normal, NL + BB normal + BB (200 mg/kg b.w), DC diabetic control, DG diabetic glibenclamide, DI diabetic insulin, LB low BB (100 mg/kg

b.w.), HB high BB (200 mg/kg b.w). Values are means + SE ($n = 6$ animals/groups) in liver of experimental animals. Results were considered significant between the groups at $p < 0.05$ in comparative studies with DC, those are not sharing the same letter (a, b)

SOD is an important antioxidant defence enzyme which protects tissue against oxygen free radicals by catalyzing the dismutation of superoxide radicals converting it into hydrogen peroxide and molecular oxygen (Chis et al. 2009). The reactive oxygen species of SOD is more effective when its activity is followed by GPx. GPx is involved in detoxifying the hydrogen peroxide (H_2O_2) generated by SOD (Halliwell 2012) and other organic hydroperoxides (ROOH) and thus protects the membrane from lipid peroxidation.

In diabetic rats, the activity of SOD was significantly decreased in the liver as depicted in Fig. 5. Oral supplementation of *B. balcooa* leaf extract exhibited an increase in the SOD activity in the liver by 9.49 and 28.98% at a dose of 100 and 200 mg/kg BW. A similar trend was noticed in glibenclamide treated rats by 18.71%. Similarly,

GPx activity was reduced in diabetic rats with respect to the control rats (Fig. 6). Treatment of diabetic rats with both low and high doses of extract up regulated the GPx activity by 9.96 and 16.06%, respectively. Glibenclamide-treated rats also attained an elevation of 12.51% in GPx activity. In insulin treated rats, the activity was restored to normal. These observations emphasizes that the two enzymes are interconnected and lowering of their enzymatic activity leads to deposition of lipid peroxides which in turn increases the oxidative stress in the diabetic rats. Treatment with BAQE leaf extract increased the enzyme activity significantly and thus may help in eliminating the free radicals generated during diabetes mellitus.

The concentrations of MDA in liver of normal and experimental rats are depicted in Fig. 7. MDA is one of the final products of polyunsaturated fatty acid peroxidation

Fig. 6 Effect of *B. balcooa* leaf extract (BB) supplementation on glutathione peroxidase in liver of experimental rats. *NL* normal, *NL + BB* normal + BB (200 mg/kg b.w.), *DC* diabetic control, *DG* diabetic glibenclamide, *DI* diabetic insulin, *LB* low BB (100 mg/kg b.w.), *HB* high BB (200 mg/kg b.w.). Values are means + SE ($n = 6$ animals/groups) in liver of experimental animals. Results were considered significant between the groups at $p < 0.05$ in comparative studies with DC, those are not sharing the same letter (a–c)

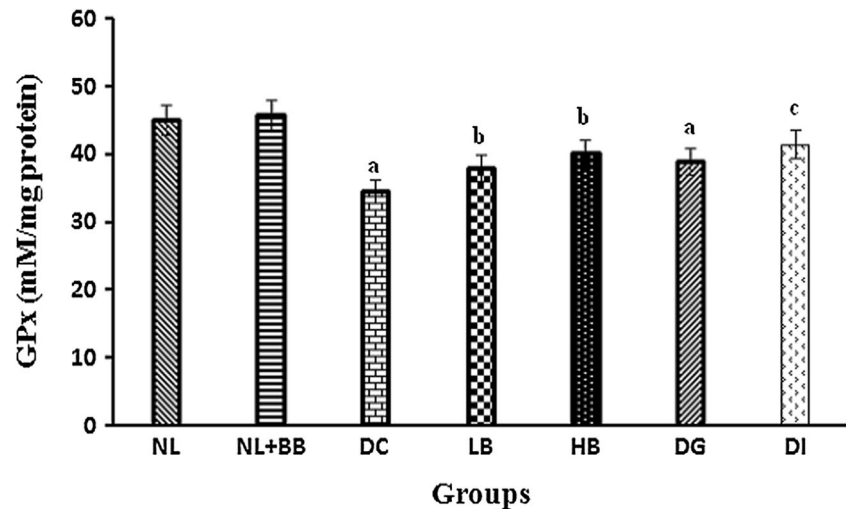
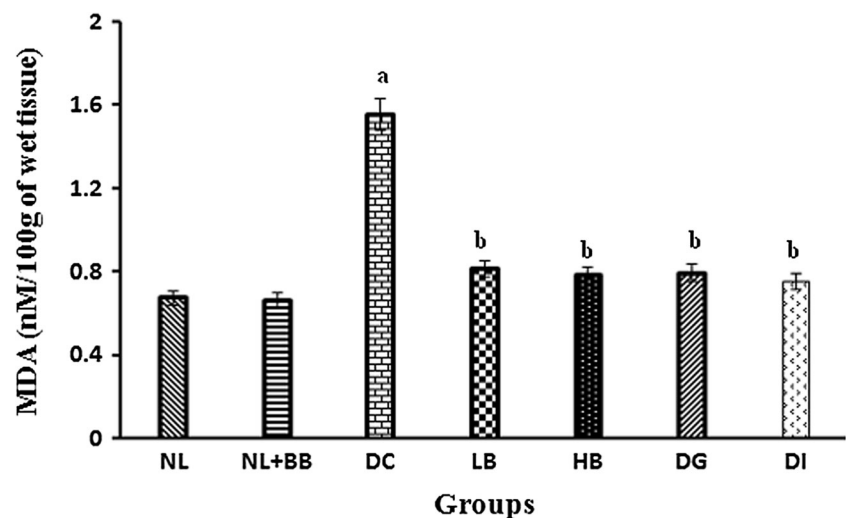


Fig. 7 Effect of *B. balcooa* leaf extract (BB) supplementation on lipid peroxidation in liver of experimental rats. *NL* normal, *NL + BB* normal + BB (200 mg/kg b.w.), *DC* diabetic control, *DG* diabetic glibenclamide, *DI* diabetic insulin, *LB* low BB (100 mg/kg b.w.), *HB* high BB (200 mg/kg b.w.). Values are means + SE ($n = 6$ animals/groups) in liver of experimental animals. Results were considered significant between the groups at $p < 0.05$ in comparative studies with DC, those are not sharing the same letter (a, b)



whose production increases with the increase in free radicals in the cells. MDA level is popularly designated as marker of oxidative stress (Gawel et al. 2004). In the present study, diabetic rats showed a significant increase in MDA compared to normal rats. Supplementation of LB extract lowered the lipid peroxidation by 47.44% whereas 49.36% reduction was observed in the rats fed with HB extract. The effect of glibenclamide treatment on MDA was in between the low and high dose and reduced MDA by 48.72%. Thus, it can be inferred that this bamboo extract could be effective in reducing lipid peroxidation.

These results are in accordance with the other reports on the bamboo's activity. Nzreen et al. (2011) reported the hypoglycaemic activity of *Bambusa arundinacea* in streptozotocin induced diabetic rats. In their experiment, they found a significant decrease in the blood glucose level in treated rats along with reduction in the glutathione and lipid peroxidation level and thus elevation in the enzymes activity. In another experiment on *Dendrocalamopsis oldhamii* leaf extract, Lv

et al. (2012) also observed significant decrease in the SOD and GPx activity in the liver when the animals were fed with the same. Their study suggested that the diabetic animals are exposed to oxidative stress and the leaf extract of *B. balcooa* can partially reduce the imbalances between the events of generation of reactive oxygen species and the scavenging enzyme activity. Thus, it can be inferred from the above study that BB could be a supplement as an antioxidant therapy due to its resemblance in activity with insulin and may prove to be beneficial in eliminating the hyperglycemia and preventing diabetic complications occurring due to lipid peroxidation and free radicals.

Conclusion

Thus, from the above analysis, it can be concluded that all the three compounds detected have the potential to treat diabetes individually and in this case they might

complement with each other and enhance the activity of *B. balcooa* to treat diabetes and thus justifies its traditional value by the ethnic communities in India to conserve good health. However, further studies on *B. balcooa* and its isolated compounds are essential, for better understanding the mechanism of action by which it modulates the oxidative stress in liver due to diabetes and thus develops a potential antidiabetic drug.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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