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Scientific validation of toxicological and anti-hyperglycemic effect of *Bambusa tulda* leaf

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Bambusa tulda (Poaceae) is one of the most valuable bamboo species in terms of health-promoting effects. The goal of this research work was to carry out the *in-vivo* acute toxicity, anti-diabetic and anti-oxidative activities of hydro-methanolic extract of *B. tulda* leaves. The median lethal dose (LD50) of the hydro-methanolic extract of *B. tulda* leaves was found to be 6088.13 mg/kg body weight in mice. Supplementing the low (100 mg/kg) and high dose (200 mg/kg) of *B. tulda* leaf extract showed significant elevation in the endogenous enzymes level of superoxide dismutase (24.81%) and glutathione peroxidase (31.60%) with a decline in malondialdehyde levels (21.90%) when compared to untreated alloxan-induced diabetic control rats. The histopathological assessment of pancreas also showed an increase in β-cells, though not at a significant level. Hence the presence of phyto-constituents substantiates the pharmacological activities of hydro-methanolic extract of *B. tulda* leaf particularly as a potential candidate for anti-diabetic activity. However, detailed studies are needed to elucidate its exact mechanism of action against diabetes.

Keywords: Anti-diabetic, Anti-hyperglycaemia, *Bambusa tulda*, Glutathione peroxidase, Superoxide dismutase, Toxicity

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Plants are used in Ayurveda, an ancient Indian traditional knowledge, to cure and manage various ailments since the days of yore and bamboo is no exception[1-3]. As resourceful natural antioxidants and their bioactive compounds, bamboo species have been well documented in the recent years[4]. The major documented species are *Bambusa vulgaris* 'Vittata'[5,6], *Dendrocalamus strictus*[7], *Dendrocalamus hamiltonii*[8], *Phyllostachys edulis*[9], *Sasa borealis*[10], *Bambusa balcooa*[11,12]. There are only a few reports of scientific evidences to name the compounds present in bamboo[8,11] though, the authors have not come across any document on the mechanism of action with regards to disease prevention. However, only a few scientific documentations are available to validate the anti-hyperglycemic activity of "Giant Grass" bamboo species[11,13-16]. Few bamboo species such as *B. vulgaris*, *B. pallida*, *B. balcooa*, *D. hamiltonii* and *D. sikkimensis* had shown prominent anti-diabetic activity *in vitro* using glucose oxidase method[17].

Bambusa tulda Roxb. (family: Poaceae) is dark green in colour, attaining a height of about 17 m and a diameter of about 16.51 cm[4]. Popular among the local tribe as *Owa gubwai* (*Bodo*), its tender shoots are widely consumed by the *Bodos* in various culinary preparations[18]. Though common in the wild, *Bambusa tulda* is also very frequent in the homestead plantation. It is closely associated with the livelihood of the rustic tribes in various ways[19-23].

Bambusa tulda forms an ingredient of the traditional medicine used by the people of Northeast India[24,25]. The shoot exudates (juice) are effective in treating nail injury when used fresh[26]. The fermented shoot exudates of *Bambusa tulda* forms a component of the traditional

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formulation used in tumor management[26]. Dey *et al.*[25] evaluated antioxidant activity, preliminary phytochemical analysis, total phenolic content and total flavonoids content of *Bambusa tulda* leaves. Though as per our knowledge, there is no substantial scientific evidence found on the *in vivo* anti-diabetic activity. Therefore, with this understanding, the current investigation could be the one to analyze the antihyperglycemic and antioxidative properties of *Bambusa tulda* leaf extract in alloxan-induced diabetic rats.

Materials and Methods

Plant material collection and extraction

The leaves of *Bambusa tulda* (BT) were amassed from the forest of Kokrajhar District, BTAD, Assam, (Latitude: 26°23'59.99"N, Longitude: 90° 16' 7.20" E) India during July, 2016. After an authentication by a plant taxonomist a voucher specimen (Voucher No. DBT/BU/Bamboo/007) was placed at Bodoland University herbarium, Kokrajhar, BTAD, Assam, India.

The air-dried bamboo leaves were finely ground using an indigenous mechanical grinder. Ten-gram of powdered sample was extracted using a Soxhlet apparatus with 70% aqueous methanol (*v/v*) (the ratio of plant matter to solvent was 1:15 *m/v*)[27] for 6 h in three cycles. The extract obtained was dried under pressure at 50°C to get a steady weight and stored at 4°C until required. Before use, the extract was suspended in double-distilled water (DDW) in the desired concentrations[25].

In vivo activity

Experimental animals

Male *Wistar albino* rats (200-250 g) and Swiss mice (25-30 g) were adapted at 26±3°C, relative humidity of 62±5% and 12:12-h light–dark cycle. The animals were kept on a regular rat chow-chow diet (Lipton India Ltd., Bengaluru) and tap water *ad libitum*. The standard procedural routines were followed, once the consent was provided by Maharani Lakshmi Ammanni College Internal Animal Ethical Committee, Bengaluru (1368/ac/10/CPCSEA/SKM/ BT/17) and were in compliance with the guidelines approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for the care and utilization of laboratory animals.

Acute toxicity test

The randomly segregated Swiss mice (25-30 g) of any sex were fasted (n=10 animals per group) overnight and made accessible prior to the toxicological trial. The hydro-methanolic extract of *BT* leaf extract having diverse dose levels (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 g/kg BW/mL) were orally supplemented once per day to experimental groups as indicated previously by our group[28]. The experimental mice were kept under observation for next 48 h, any casualties were recorded and the median lethal dose LD_{50} was studied using OECD guidelines and as our previous study[8]. The following formula was used for calculating LD_{50}: LD_{50} = Highest dose (LDy) – $^{l\text{-}r}\Sigma$ [Dose difference (Dd) X Mean dead (Md)]/ number of animals per group (n)

Alloxan-induced Wistar albino rat model for Diabetes Mellitus (DM)

Experimental groups

After a week of acclimation, rats were prepared/ induced diabetic using alloxan (2,4,5,6(1H,3H)-Pyrimidinetetrone) (150 mg/kg BW/mL i.p.) in 50 mM phosphate buffer Saline (pH 7.2) in overnight fasted animals excluding normal animal group. To avoid initial alloxan-induced lethal hypoglycemia, rats were administered glucose (5 mL of 10% solution) using 16 gavage (16 g) ball point needle and were permitted 5% glucose in drinking water subsequently for 24 h. Rats which had fasting blood sugar (FBS) less than 220-260 dL/mL were excluded from the experiment. Others were included as diabetic animals and used further for experimental purposes and were randomly grouped into 5 groups29. The groups include DC (Diabetic control), LBT (100 mg/kg BW/mL, lower dosage of B. tulda), HBT (200 mg/kg BW/mL, higher dosage of B. tulda), DG (5 mg/kg BW/mL; Diabetic glibenclamide) and DI (Diabetic insulin) groups, each having 6 rats per group. Rats (n=6) neither treated with alloxan nor with extract were considered as a normal (NL) group. NL and DC groups received distilled water until the experiment ended. LBT and HBT groups were orally fed with 100 mg/kg BW and 200 mg/kg BW hydro-methanolic extract of *B. tulda* leaf, respectively. DG and DI were included as Positive control groups and treated with standard drug glibenclamide (600 µg/kg BW) and insulin (2 units/ kg of BW) (Insugen, Biocon) daily intraperitoneally (*i.p.*), respectively. All the groups were treated in a similar pattern for six continuous weeks. FBS was found out after the 7th day and last week before sacrifice. Body weight (BW) was determined on a daily basis. After 6 weeks, testing animals were euthanized using standard protocol (0.3 mL/100 g *i/p* Ketamine (300 mg/kg) + 0.15 mL/100 g Xylazine (30 mg/kg)) as recommended by IAEC (CPCSEA) and liver tissue was used for further biochemical assays[11].

Estimation of blood glucose

Blood withdrawn from overnight fasted trail animals, were used to check FBS by using one touch automated ACCU-CHEK® blood glucose meter check[29].

Preparation of tissue

The 5% crude liver homogenate was obtained for malondialdehyde (MDA) assay, a marker of lipid peroxidation. The 5% homogenate centrifuged (Eppendorf 5810R, Germany) and collected, at 600 xg for 10 min, were used for antioxidant enzyme estimation such as superoxide dismutase (SOD) and glutathione peroxidase (GPx)[28].

Statistical analysis

All analysis followed in methodology was performed in triplicates and articulated as mean ± Standard Error (SE). Relationships amid diverse experimental groups were established using one-way ANOVA using MS Excel at $p<0.05$.

Results and Discussion

Plants have been postulated and fondly acting as conventional medicine since the time immemorial. They are understood to be the major components of several formulations (herbal) that could help in reducing a range of health ailments. Therefore, a comprehensive learning on medicinal plants is in spotlight for the progression and quality management of traditional formulations.

Ying *et al.*[30], previously reported that bamboo leaf extract might exert anti-diabetic activity because of its effect on the improvement of insulin sensitivity although without a significant change in HbA1C values in streptozotocin (STZ)-induced animal model. There was nothing reported about the dosage toxicity and pancreatic anatomy in that study. In this context, the current research may offer a promising strategy to understand the acute toxicity, *in vivo* anti-hyperglycemic and antioxidative activity of hydro-methanolic extract of *B. tulda* leaf native to Bodoland, India.

***In vivo* studies**

Acute toxicity test

LD_{50} of BT leaf extract was 6088.13 mg/kg BW in mice**.** There were not many reports with regards to toxicity studies of BT. Earlier, Goyal *et al.*[11] have reported the LD_{50} value of *B. balcooa* (5.18 g/kg BW). The loss of locomotors activity noticed after 12 h of the drug administration indicated the toxicity of the extract. However, no mortality was seen in experimental animals for the given dosages. Loomis and Hayes scale[31] indicated a chemical entity ranging within 5–15 g/kg BW, is regarded as practically non-toxic and relatively safe[11,32].

Effect of hydro-methanolic extract of BT leaves on glucose level

A significant anti-hyperglycemic bustle was noted from the third day onwards (Table 1). The decrease was prominent up to the 6th week (42nd day) in experimental animals receiving the high dose of BT, though the results were not found significant. A significant ($p<0.05$) increase in FBS was recorded after alloxan administration when compared to NL group. The BT supplementation significantly ($p<0.001$) trimmed down the blood glucose levels in hyperglycemic animals when compared to the DC group. The HBT was noted to be more effective than the LBT, indicating that HBT has strong anti-diabetic activity and acted as an efficient dose. Goyal *et al.*[11] also have evaluated and found similar pattern in *B. balcooa* leaves extract. Earlier, the anti-hyperglycemic activity of *Bambusa arundinacea* leaves was evaluated by the Nazreen and coworkers[33] in streptozotocin (STZ) induced diabetic animals. Dey *et al.*[34] reported the preliminary anti-hyperglycemic activity of *B. tulda* in alloxan induced animals.

Biochemical assays

The SOD and GPx concentration of distilled water (normal) and extract-treated animals were studied in hepatic (liver) tissue (Fig. 1 and 2). In diabetic rats, the SOD activities were significantly lowered than the NL control group (Fig. 1). Oral supplementation of BT extract revealed an increase in SOD activity. A similar trend was observed in DG and DI groups

Table 1 — Blood glucose level (mg/dL) in experimental diabetic rats

Group	0th Day	3rd Day	42nd Day
NL	99±2.91	93±2.11	99±3.52
DC	101±1.31	280±6.91a	391±5.43a
DG	102±2.51	276±7.81a	219±6.48b
DI	103±1.89	285±4.53a	124±4.32c
LBT (100 mg/kg)	101±2.61	286±8.96a	224±8.61b
HBT (200 mg/kg)	100±2.11	278±5.73a	204±5.27b

Abbreviations indicates NL, Normal; DC, Diabetic Control; DG, Diabetic glibenclamide, DI, Diabetic insulin; LBT (100 mg/kg), Lower dosage of *B. tulda*; HBT(200 mg/kg), Higher dosage of *B. tulda*. The values are means + S.E. (n=6 animals/groups) in the liver of experimental animals. Results (columns-wise) were considered significant between the groups at $p<0.05$ in a comparative study with DC. Those are not sharing the common letters (a & b) are significantly different.

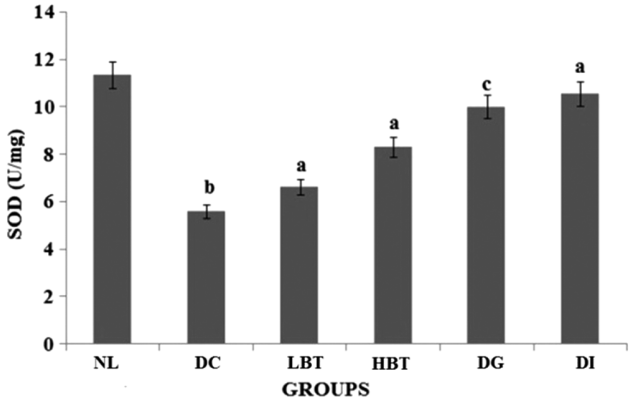

Fig. 1 — Effect of *B. tulda* leaf extract supplementation on superoxide dismutase in liver of experimental rats. Values are means + S.E. (n=6 animals/groups) in the liver of experimental animals. Results were considered significant between the groups at $p<0.05$ in a comparative study with DC. Those are not sharing the common letters (a, b & c) are significantly different.

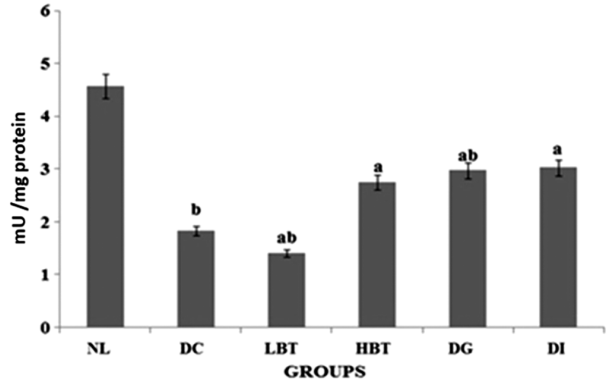

Fig. 2 — Effect of *B. tulda* leaf extract supplementation on glutathione peroxidase in liver of experimental rats. Values are means + S.E. (n=6 animals/groups) in the liver of experimental animals. Results were considered significant between the groups at $p<0.05$ in a comparative study with DC. Those are not sharing the common letters (a & b) are significantly different.

with significant effects compared to DC group. Similarly, GPx activity was moderated in DC group (Fig. 2). A significant Increase in GPx activity was seen in rats treated with HBT extract (but not with LBT). HBT had increased the levels of SOD (24.81%) and GPx (31.60%) antioxidant enzymes in the liver. This study underlines that these enzymes are inter-related and lowering their enzymatic activity can directly amplify oxidative stress in the alloxan-treated diabetic animals. The BT extract treatment (at least at high dose) can improve the activity of these enzymes radically and might assist in getting rid of the free radicals spawned during diabetes mellitus. Lv *et al.*[35] reported a boost in antioxidant enzymes such as SOD

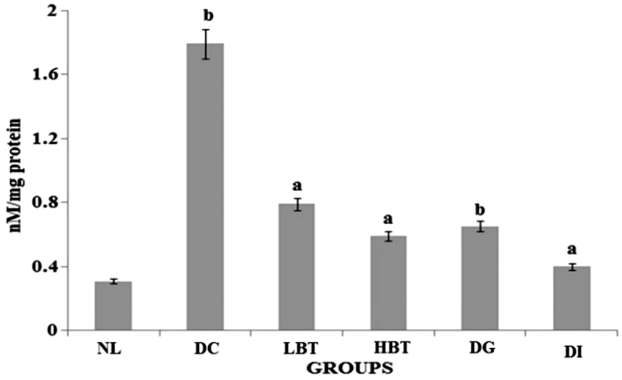

Fig. 3 — Effect of *B. tulda* leaf extract supplementation on lipid peroxidation in the liver of experimental rats. Values are means + S.E. (n=6 animals/groups) in the liver of experimental animals. Results were considered significant between the groups at $p<0.05$ in a comparative study with DC. Those are not sharing the common letters (a & b) are significantly different.

and GPx activity in hepatic tissue (liver), when the STZ-induced diabetic rats were supplemented with *Dendrocalamopsis oldhamii* extract. Ying *et al.*[30] proved that supplementing bamboo could reduce weight and glucose level in hyperglycemic rats. The decreased glycosylated hemoglobin A1c (HbAlc) and increasing SOD activity were also observed in their study following the supplementation.

A significant decrease of MDA in the liver with the higher dose of BT was recorded when compared with DC group animals (Fig. 3). MDA is known to be the end product of poly-unsaturated fatty acid peroxidation, whose fabrication augments with the amplification in free radicals. In this study, the DC group demonstrated a noteworthy increase in MDA as compared to the NL group. Supplementation of BT extract lowered the lipid peroxidation (LPx). The effect of the standard drug (DG group) was found to be more significant (34.1%) than the rats treated with BT (21.9%). The results reported in this study are in agreement with the previous report on the giant grass[11]. Lipid peroxidation level (MDA) was also seen decreasing in diabetic animals after bamboo leaf extract supplementation. The group also indicated that bamboo leaf supplementation could improve diabetic nephropathy conditions by triggering the protein kinase B (AKT) pathway in investigational animals.

Nazreen and her co-workers[33] indicated the anti-hyperglycaemic properties of *Bambusa* sp. in STZ-induced diabetic models. Their study confirmed a reduction in FBS in experimentally treated animals, followed by the reduction in the GPx level and thus elevating the enzyme activity. Another report by Lv

and his team[35] on *Dendrocalamopsis oldhamii* extract, observed a significant reduction in the antioxidative enzymes like SOD and GPx, in the liver after the animals were treated with the same. The same study also proposed that the animals in the DC group (diabetic) are in depiction to oxidative stress. They also indicated that *D. oldhamii* extract could moderately decrease the inequities between the generation of reactive oxygen species (ROS) and the foraging enzyme activity. Goyal and his co-workers[11] also substantiated the anti-hyperglycaemic and antioxidant potential of *Bambusa balcooa* in alloxan-induced diabetic rats.

Escalating confirmation ties the pathological transformations of pancreatic tissue with high glucose-stimulated, even though the fundamental mechanism remains unclear with regards to complementary and alternative medicines treatment[36-39]. To more profoundly understand the beneficial effect of the bamboo extract on diabetic rats, authors utilized histo-pathological evidence to prove their claim (Fig. 4).

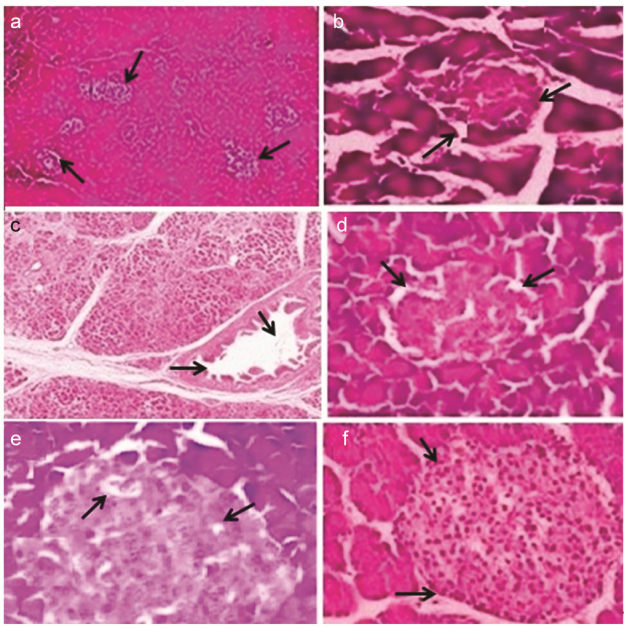

Fig. 4 — Histopathology of pancreas stained by haematoxylin (H) and eosin (E) stain of (a) Normal control: Normal control group display the normal islet (arrow) (b) Diabetic control: Diabetic control rat histopathology display the islet enlargement and necrosis (arrow) (c) LBT (100 mg/kg BW): BT tested drug histopathology display increasing islets and necrosis (arrow) (d) HBT (200 mg/kg BW) (e) Glibenclamide (400 µg/kg BW): Glibenclamide treated group rat histopathology showing normal islet close to the normal control group (f) Insulin (1 U/kg BW): The histopathology images of insulin-treated group rat pancreas showing normal islet close to normal control animal group. For each group 6 animals were examined and 30 photos were captured. (Original magnification, 40 X)

Result evidenced that diabetic rats have deteriorated changes in the β-cells of pancreases after alloxan treatment. Conversely, the animals treated with bamboo leaf extract could reverse β-cells of pancreases with little side-effect such as hair fall noticed. The data recorded here clearly demonstrated that bamboo leaf extract moderately shields pancreatic tissue against diabetes and its side effects.

Conclusions

To conclude, it could be understood from the above learning that *Bambusa tulda* leaf could be complemented as an antioxidant remedy due to its similar insulin-like activity and may provide evidence in eliminating the hyperglycemia and averting side effects due to diabetes including free radicals. This could possibly be attributed to the synergistic effect of the various bioactive phyto-constituents recorded in *Bambusa tulda* leaf like *p*-hydroxy benzoic acid, salicylic acid, *o*-coumaric acid, vanillic acid, ferulic acid and 2,4-dihydroxybenzoic acid as reported by Dey and his team in their previous study[23]. However, further studies at molecular level have to be carried out to prove its anti-diabetic efficiency and to bring out a drug molecule from the extract and to understand the extract's molecular mechanism of function.

Limitations/Future directions

Here, the readers must understand the limitations of this study, hence can be taken as future directions. In this work, the authors have only indicated the probable pathway using histopathological studies indicating partially growing pancreatic β -cells. Thus, other signalling pathways, such as Akt, or linked pathways that improve diabetes conditions, may also be involved. The bioactive constituents of the leaf extract of BT should be elucidated to correlate with the observed anti-diabetic activity which can help us to study the activity guided fractionation of the extract for further confirmation. Using gene knock-out animals and inhibitors, researchers may able to clearly confirm the efficacy of bamboo extract in alleviating diabetic symptoms. In-vitro trials (cell lines) will be continued further to convolute specific mechanisms whereby bamboo extract reduces the injuries persuaded by elevated sugar levels. This work was conducted with male animals and thus effect of the extracts on the female rats should also be studies in the future. It needs another trail in either sex (female) rats also. Furthermore, data of this research were all obtained from preclinical study and the prospective

testing at the clinical level need to be worked out in future.

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Ethical statement

All institutional and national guidelines for the care and use of laboratory animals were followed.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

AKG conceptualized the study, AKG, SKM and TU designed the study, SKD, EH, AK, RS carried out the research work, acquired the data. AKG, SKM, AIF and HSY analyzed the data and wrote the first draft of the manuscript. All the authors edited the manuscript and approved the final version for submission.

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