HYPOGLYCEMIC ACTIVITY OF BAMBUSA ARUNDINACEA LEAF ETHANOLIC EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Summary

The hypoglycaemic activity of the ethanolic extract and different fractions of leaves of *Bambusa arundinacea* were carried out in streptozotocin induced diabetic rats. The effect of the extract and different fractions on biochemical parameters were also assessed to evaluate their activity in controlling diabetes related metabolic conditions. The biochemical parameters include lipid peroxidation, reduced glutathione levels and activity of antioxidant enzymes. The results indicate that the ethyl acetate fraction at 150 mg/kg b.w. significantly lowers blood glucose level (105±15.58 mg/dL) comparable to standard drug glibenclamide (95 ± 10.64 mg/dL) (3 mg/kg b.w.) with insignificant ulceration compared to the standard. Also, there was reduction in lipid peroxidation level and glutathione levels and elevation in the activity of antioxidant enzymes. From the phytochemical investigation, *β*-sitosterol glucoside and stigmasterol have been isolated in pure form from the ethyl acetate and chloroform fractions. This study suggests that leaf extract of *Bambusa arundinacea* could be potentially useful for treatment of hyperglycaemia.

Keywords: *Bambusa arundinacea;* diabetes; *β*-sitosterol glucoside; stigmasterol.

Introduction

Diabetes mellitus (DM) is a common chronic metabolic disease characterized by hyperglycaemia and various metabolic imbalances. Its prevalence is about 6% worldwide and the number of cases, presently estimated at more than 150 million, is predicted to double by 2025 [1-3].

Currently available therapies for diabetes include insulin and various oral anti-diabetic agents which have a number of serious adverse effects; thus, managing diabetes without any side effects is still a challenge [4]. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of investigation. In this context, plant based antidiabetic therapeutics are the current days interest, and the hypoglycemic effect of several plants used as antidiabetic remedies has been confirmed, and the mechanisms of hypoglycemic activity of these plants are being studied [5-7].

Bambusa arundinacea Retz. (Poaceae) is commonly known as bans. The leaves of Bambusa are emmenogogue [8] and are used for the treatment of inflammatory diseases, wound healing, ulcers and paralytic complaints [9-11]. Further *in vitro* antioxidant activity of aqueous, methanol and butanolic extracts and hypoglycemic activity of the aqueous extract has also been reported [12-13]. However, to the best of our knowledge, no detailed phytochemical investigation has been attempted to find out the active ingredients responsible for its antidiabetic activity.

Keeping the biological importance of *Bambusa* as an antidiabetic plant, we envisaged our programme on the investigation of active components responsible for its antidiabetic property as a part of our ongoing project on the investigation of new antidiabetic plants. In the present study, *in vivo* antidiabetic effect of the ethanolic leaf extract and its different fractions in normal and streptozotocin induced diabetic rats and effectiveness on various biochemical parameters, namely lipid peroxidation, reduced glutathione levels and activity of antioxidant enzymes (superoxide dismutase and Catalase) has been investigated and finally isolated the pure compounds from the active fractions.

Materials and methods

Collection of plant Material

The leaves of *B.arundinacea* (Retz.) were collected from Saket Nursery, New Delhi in March 2009 and authenticated by Dr. H. B. Singh, Taxonomist, National Institute of Science Communication and Information resources, New Delhi. A voucher specimen (No.1386/188) has been deposited in the author's laboratory.

Preparation of crude plant extracts and its fractions

The leaves of *B.arundinacea* (Retz.) were shade dried for 2-3 days and coarsely powdered. The grounded leaves (10 kg) were extracted with 95% ethanol in a Soxhlet apparatus. The ethanolic extract was concentrated under reduced pressure to yield a brown viscous mass (650 g). The ethanolic extract was fractionated with petroleum ether (3 x 1.0 L), $CHCl_3$ (3 x 1.0 L), EtOAc (3 x 1.0 L) and MeOH (3 x 1.0 L) to furnish petroleum ether fraction (200 g), $CHCl_3$ fraction (150 g), EtOAc fraction (100 g) and MeOH fraction (200 g). The extract and its different fractions were kept under refrigeration until used for its biological and phytochemical testing.

Experimental animals

Albino wistar rats of either sex (150 to 200 g) were obtained from Central Animal House, Jamia Hamdard University, New Delhi. The animals were kept in cages at the room temperature and fed with food and water ad libitum. Fourteen hours before the start of the experiment the animals were sent to lab and fed only with water ad libitum. The experiments were performed in accordance with the rules of Institutional Animals Ethics Committee (registration number 173-CPCSEA).

Preparation of diabetic rats

The rats were fasted overnight and diabetes was induced by injecting streptozotocin (STZ) (60 mg/kg body weight) intraperitoneally. STZ was prepared freshly in 0.1 M citrate buffer (pH 4.5). The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the 3rd day after STZ injection. The treatment was started on the 4th day after STZ injection and this was considered as 1st day of treatment. The treatment was continued for 30 days.
The rats were divided into nine groups comprising of six animals in each group as follows: Group I: Control rats receiving 0.1 M citrate buffer (pH 4.5),Group II: Diabetic controls (STZ induced),Group III: Diabetic rats given bamboo extract (150 mg/kg bd wt) in aqueous suspension orally for 30 d, Group IV: Diabetic rats given bamboo extract (350 mg/kg bd wt) in aqueous suspension orally for 30 d, Group V: Diabetic rats given bamboo extract chloroform fraction (80 mg/kg bd wt) in aqueous suspension orally for 30 d, Group VI: Diabetic rats given bamboo extract chloroform fraction (150 mg/kg bd wt) in aqueous suspension orally for 30 d, Group VII: Diabetic rats given bamboo extract ethyl acetate fraction (80 mg/kg bd wt) in aqueous suspension orally for 30 d, Group VIII: Diabetic rats given bamboo extract ethyl acetate fraction (150 mg/kg bd wt) in aqueous suspension orally for 30 d , Group IX: Diabetic rats given glibenclamide (3 mg/kg bd wt) in aqueous solution orally for 30 d.

Biochemical Parameters

At the end of the experimental period, the rats were anaesthetized and sacrificed by cervical dislocation. Blood was collected in tubes containing EDTA. Organs (pancreas, kidney and liver) were removed for histopathological evaluation and biochemical parameters. Lipid peroxidation (LPO) was assayed by the method of Wright *et al.* [14], reduced glutathione (GSH) was assayed by the method of Jollow *et al.* [15] Superoxide dismutase and Catalase activity was assayed by the method of Yen and Chen [16] and Claiborne [17] respectively.

Histopathological studies

Animals were sacrificed by decapitation and tissues (liver, kidney and pancreas) were removed immediately for histopathological studies. The tissues was fixed with 10% phosphate-buffered neutral formalin, dehydrated in graded (50-100%) alcohol and embedded in paraffin. Thin sections (4-5µM) were cut and stained with routine hematoxylin and eosin (H & E) stain for photomicroscopic assessment.

Statistical analysis

Data was analyzed by one way ANOVA followed by Dunnett's ´t´ test (n=6), *$p<0.05$, **$p<0.01$ significant from control; ***$P<0.001$ extremely significant from control; ns- not significant.

Results

Phytochemical screening: Phytochemical screening of the ethanolic extract revealed the presence of flavanoids, tannins, steroids and phenolic glycosides.

Effect of *B. arundinacea* ethanolic extract, chloroform and ethyl acetate fractions on blood glucose levels of diabetic rats

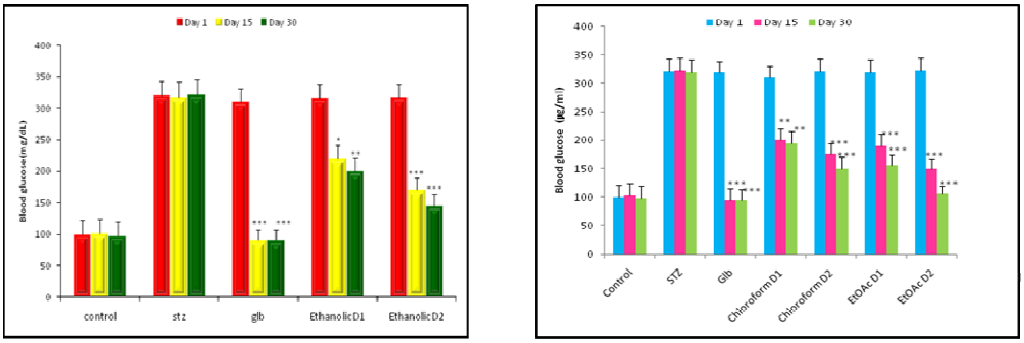
The ethanolic extract of *B. arundinacea* when administered to diabetic rats at 150 mg/kg b.w and 350 mg/kg b.w, caused a significant hypoglycaemic effect throughout the studies by reducing blood glucose level to 178±16.28 and 124±15.23 mg/dL respectively comparable to the standard glibenclamide which caused 95 ± 10.64 reduction in blood glucose after 30 days of study (Figure I). Of the two fractions, chloroform and ethyl acetate, the ethyl acetate fraction was found to be more effective in reducing blood glucose level (105±15.58) than the chloroform fraction (140±13. 26).

Effect of *B. arundinacea* ethanolic extract, chloroform and ethyl acetate fractions on lipid peroxidation and reduced glutathione (GSH) level in pancreas of diabetic rats

It was observed that the ethanolic extract, chloroform and ethyl acetate fractions decrease the levels of lipid peroxidation in pancreas of diabetic rats (Figure II A). Also, the ethanolic extract, chloroform and ethyl acetate fractions and glibenclamide when administered to diabetic rats brought GSH levels to near normal level (Figure II B).

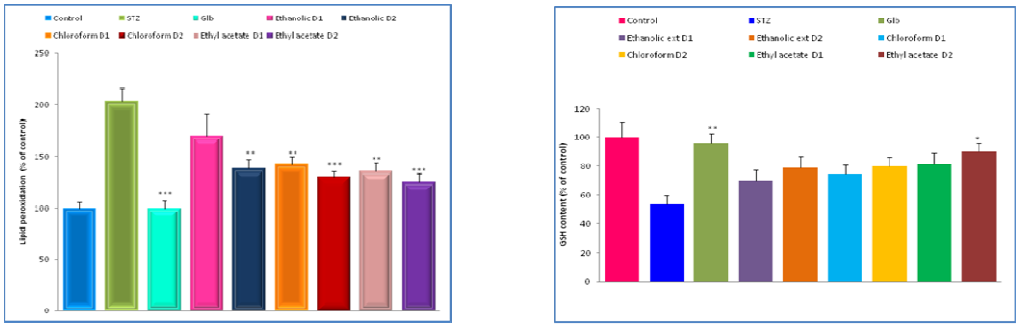
Effect of *B. arundinacea* ethanolic extract, chloroform and ethyl acetate fractions on superoxide dismutase (SOD) and catalase (CAT) enzymes in pancreas of diabetic rats

B. arundinacea ethanolic extract, chloroform and ethyl acetate fractions and the standard drug, glibenclamide recovered the activities of SOD and catalase enzymes to close to control values in rats in which diabetes had been induced (Figure III).



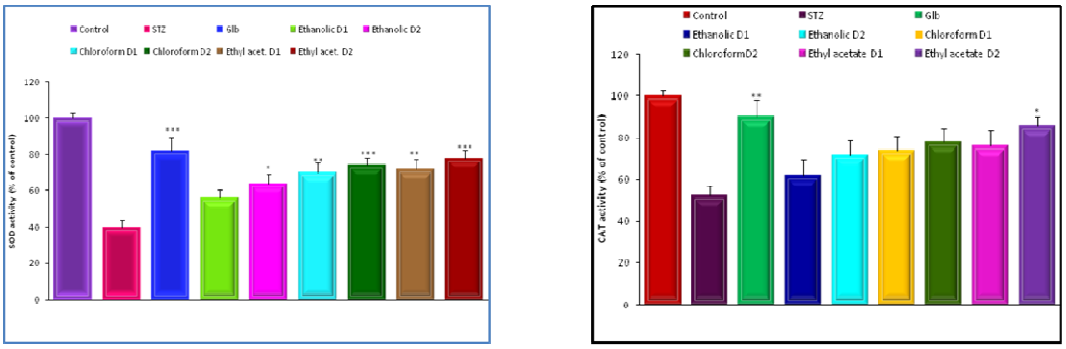
A **B**

Figure I. Effect of (**A**) ethanolic extract and (**B**) chloroform fractions and ethyl acetate fraction of Bambusa on blood glucose levels of diabetic rats. Blood glucose was measured on days 1, 15 and 30 of induction of diabetes



A **B**

Figure II. Effect of ethanolic extract and chloroform fractions and ethyl acetate fraction of Bambusa on **(A)** lipid peroxidation and (B) reduced glutathione (GSH) level in pancreas of diabetic rats. Results are expressed as % of control. Each value is mean ± S.E. (*n=5*)



A **B**

Figure III. Effect of ethanolic extract and chloroform fractions and ethyl acetate fraction of Bambusa on (A) SOD and (B) CAT activity in pancreas of diabetic rats. Results are expressed as % of control. Each value is mean ± S.E. (*n=5)*

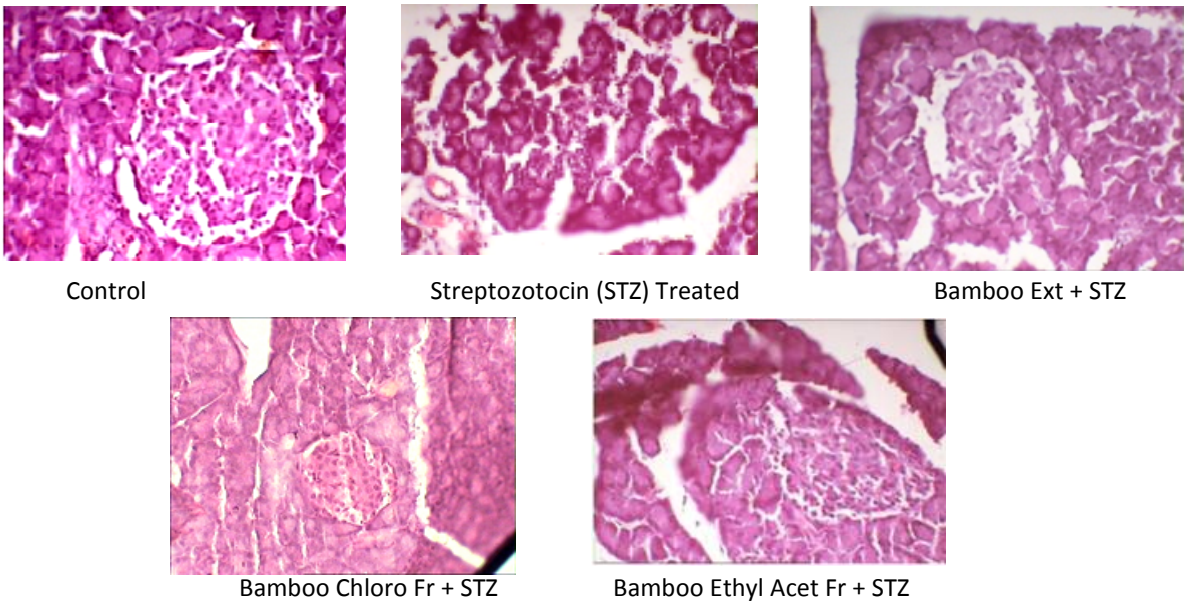


Figure IV. Effect of Bamboo extract and its fractions on histopathological changes in pancreas of rats in which diabetes had been induced by STZ.

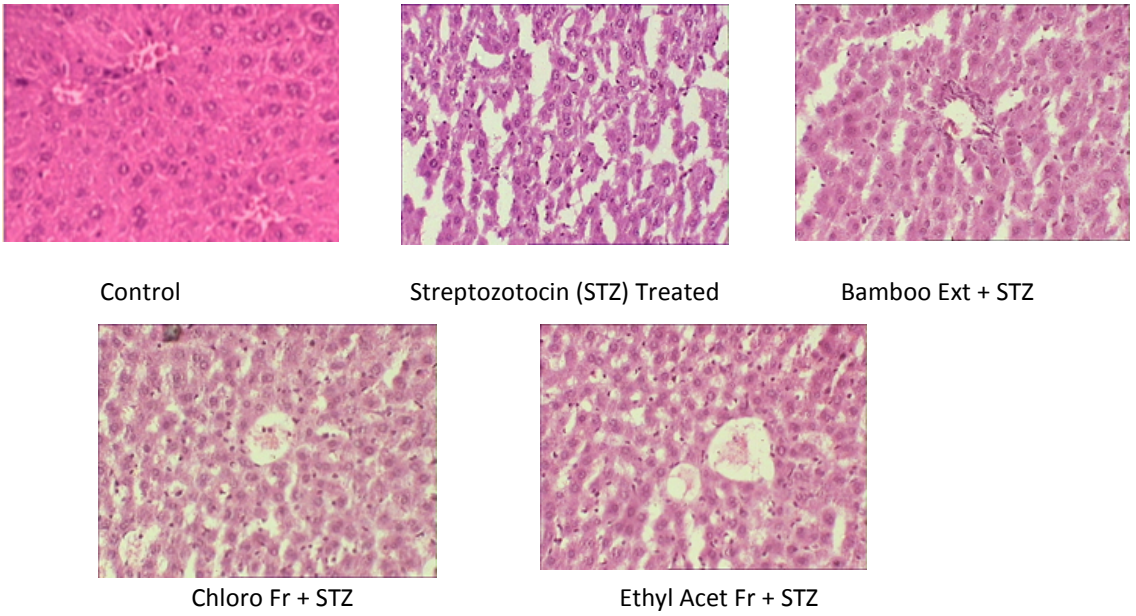
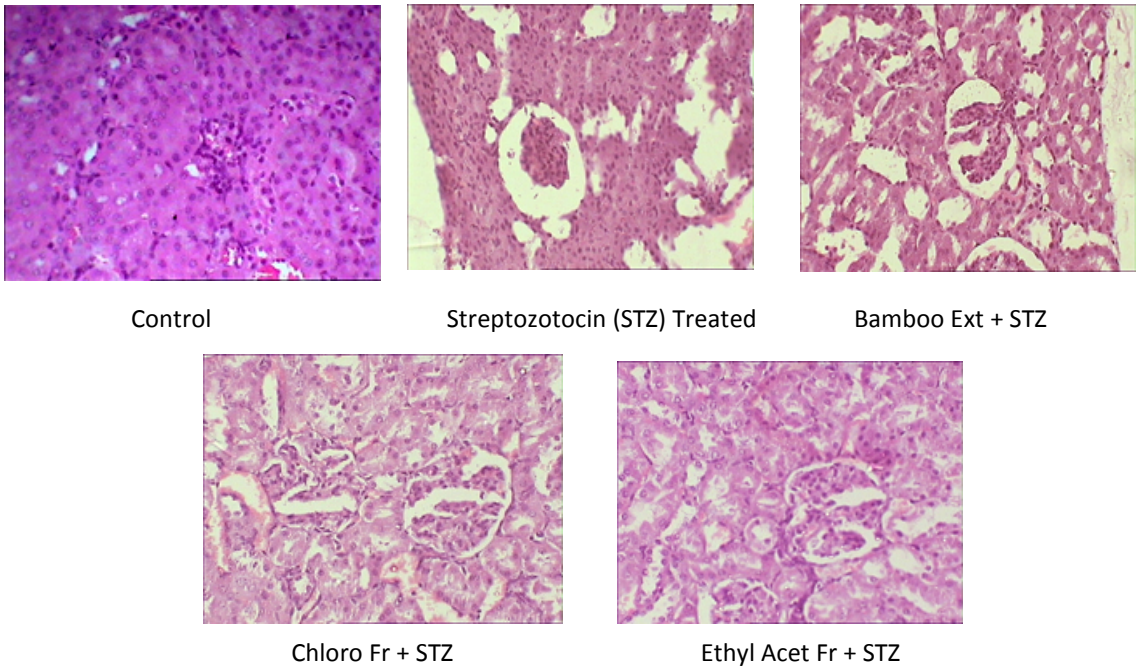


Figure V. Effect of Bamboo extract and its fractions on histopathological changes in liver of rats in which diabetes had been induced by STZ.

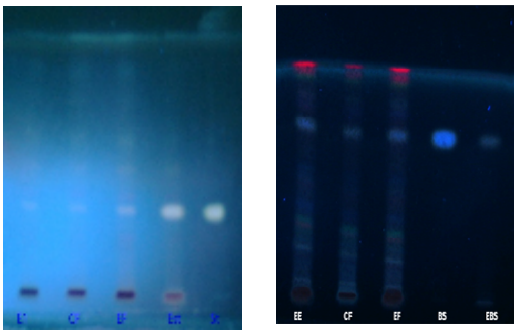


Figyre VI. Effect of Bamboo extract and its fractions on histopathological changes in kidney of rats in which diabetes had been induced by STZ.

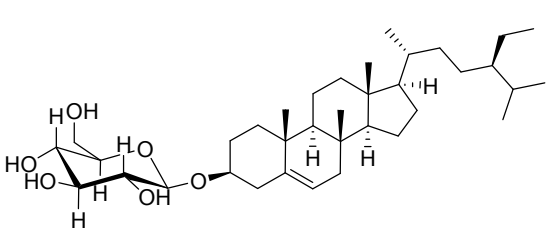
Discussion

It was observed that there was a significant elevation in glucose levels 48 h after administration of STZ, which persisted until the end of the study, i.e., after 1 month indicating persistent hyperglycemia. Administration of Bamboo extract, chloroform fraction, ethyl acetate fraction and standard glibenclamide (3 mg/kg b.w) to diabetic rats significantly decreased the level of blood glucose as compared with that of diabetic mellitus model (Fig. 3). In diabetic control the blood glucose level remained high and the standard glibenclamide caused a reduction in blood glucose level (95 ± 10.64mg/dL) after 30 days of study (Figure I). The ethanolic extract of *Bambusa arundinacea* when administered to diabetic rats at 150 mg/kg b.w and 350 mg/kg b.w, caused a significant hypoglycaemic effect throughout the studies by reducing blood glucose level to 178±16.28 and 124±15.23 mg/dL respectively after one month. The chloroform and ethyl acetate fractions when administered at two different doses, 80 mg/kg b.w and 150 mg/kg b.w to diabetic rats, both the fractions at a dose of 150 mg/kg b.w lowers blood glucose level to 140±13.26 and 105±15.58 respectively suggesting that the ethyl acetate fraction was more potent in lowering the blood glucose level.

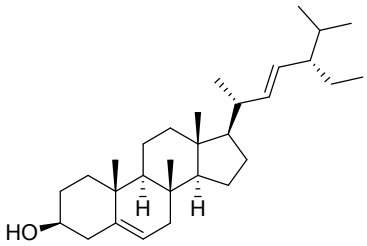
The phytochemical analysis has revealed the presence of glycosides, flavanoids, sterols, triterpenes in chloroform and ethyl acetate fractions. It was reported that the stigmaoidal sitosterol glycoside called 'Charantin' isolated from *Momordica charantia* exhibited a significant fall in blood glucose levels when administered orally or intravenously. [18] Further hypoglycaemic effect of *Parkia speciosa* seeds due to the synergistic action of *β*-sitosterol glucoside and stigmasterol has been explained [19]. S-9-4, a mixture isolated from chloroform extracts of *Parkia speciosa* seeds containing two related sterols *β*–sitosterol glucoside and stigmasterol (66:34 mg/kg BW) shown the synergistic antidiabetic effect, whereas its pure components did not produce any significant decrease in blood sugar levels. Nevertheless the mixture of the above authentic compounds in the ratio 66:34 did show similar hypoglycaemic activity to S-9.4, with a similar percentage activity (84%). From the phytochemical investigation of chloroform and ethyl acetate fractions from ethanolic extract of *Bambusa*, *β*-sitosterol glucoside and stigmasterol were isolated in pure form for the first time from this plant. The HPTLC pattern of pure compounds against its extract and its fractions are given below.



HPTLC of the isolated pure components: E1, EE-ethanolic fraction, CF-chloroform extract, EF-ethylacetate fraction, ESt- Co-spot of ethylacetate fraction and stigmasterol, St-pure stigmasterol, EBS-co spot of ethylacetate fraction and *β*-sitosterol glucoside, BS-pure *β*-sitosterol glucoside. Mobile phase: (A) n-hexane: EtOAc (8:2), (B) $CHCl_3$:MeOH (9:1), Sprayed with Liebermann Burchard reagent.



β-Sitosterol glucoside



Stigmasterol

The antidiabetic activity shown by these extracts is mainly due to the synergistic effect of these two compounds. Analytical data of these two compounds has been compared with the literature data and were found identical.

Lipid peroxidation is one of the cellular features of chronic diabetes. Oxidative stress that leads to an increased production of reactive oxygen species (ROS) and finally cellular lipid peroxidation has been found to play an important role in the development of diabetes mellitus. A reduction in lipid peroxidation was observed in streptozotocin induced diabetic rats when treated with Bamboo ethanolic extract and its fractions. The ethyl acetate fraction at a dose of 150 mg/kg brings down the levelof lipid peroxidation in pancreas more effectively than chloroform fraction at the same dose. Similarly, it was found that there was a significant decrease in the concentration of GSH in streptozotocin induced diabetic rats when compared to control group of rats. Treatment with bamboo extract and its fractions brought GSH levels to near normal level (Figure II).

The concentration of SOD and CAT enzymes in the diabetic rats was significantly lowered than in the normal rats Feeding of bamboo extract and its chloroform and ethyl acetate fractions recovered the activities of SOD and CAT enzymes in pancreas of diabetic rats (Figure III).

Histopathology studies also supported our findings. STZ was found to induce significant damage to islets of langerhans of pancreas. Diabetic rats showed markedly reduced islet cells, which were restored to near normal upon treatment with the extract, chloroform fraction, ethyl acetate fraction (Figure IV).The liver of control rat showed normal architecture. The liver of diabetic rat showed perivenular inflammatory infiltration filling over the sinusoidal vacuolation of the hepatocyte nuclei. The pathomorphological changes observed in STZ-induced diabetes became apparently normal after treatment with bamboo extract, its chloroform and ethyl acetate fractions and glibenclamide (Figure V). The kidney of control rat showed normal glomeruli and tubules. The kidney of diabetic rat showed thickening of vesicles, glomeruli showed some cellular proliferation with fibrosis. Bamboo extract or its chloroform/ethyl acetate fractions or glibenclamide treated diabetic rat showed reversion to near normal glomeruli (Figure VI).

In conclusion the present study shows the potential of *Bambusa arundinacea* as an antidiabetic agent and the activity of ethanolic extract may be due to the synergistic action of *β*-sitosterol and stigmasterol with insignificant side effects. Admixtures of *β*-sitosterol glucoside and stigmasterol may therefore be used as a new orally effective hypoglycaemic agent, besides their hypocholesterolaemic property in animals and humans. Further phytochemical studies are under progress to identify the other active components that are responsible for its antidiabetic activity.

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