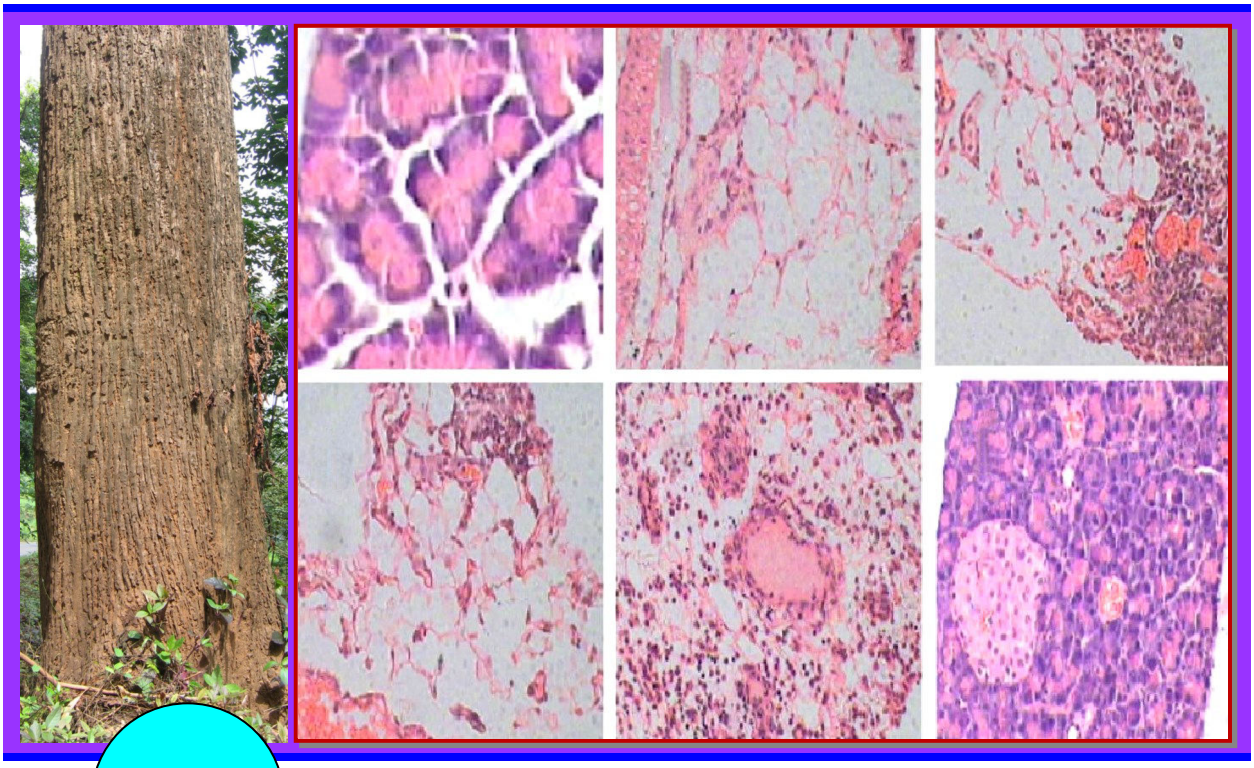

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Potential anti-diabetic activity of *Bombax ceiba*

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Abstract

Bombax ceiba bark extract was evaluated for its hypoglycemic and hypolipidemic potential through normal and streptozotocin-induced diabetic rats administered with graded oral doses (200, 400, 600 mg/kg/day) for 21 days. The results showed that a dose of 600 mg/kg of *B. ceiba* extract is the most effective to cause significant ($p<0.001$) hypoglycemic and/or hypolipidemic effects on streptozotocin-induced diabetic rats. This dose also significantly ($p<0.001$) lowered the total cholesterol and triglyceride level in severely diabetic rats. Phytochemical and GC-MS studies confirmed the presence of the triterpenoid compounds in the extract, which may account for its significant hypoglycemic activity. The present study thus provides a scientific rationale for the traditional use of this plant in the management diabetes.

Introduction

Kantesavar botanical name *Bombax ceiba* L bark family Malvaceae is commonly known as silk cotton tree, found in dry, moist and mixed deciduous forest, throughout in India. The tender bark is used as famine food, demulcent, emetic and tonic, and its aqueous extract mixed with curd to check blood-dysentery. Externally it is used as styptic and also for fomenting wounds (The wealth of India, 2005). The bark reported to contain Shamiminol: A new aromatic glycoside from the stem bark of *B. ceiba* (Faizi et al., 2011). The bark is also reported to have anti-oxidant activity (Gandhare et al., 2012). Cardioprotective effect of *B. ceiba* flowers has been reported by (Patel et al., 2011). Pharmacognostical, phytochemical and biological evaluations of the stem bark of *B. ceiba* were carried out (Ansari et al., 2007).

The tribal's of toranmal use and prescribe the extract in the form of powder for the treatment of diabetes, however there is no scientific proof regarding its use as an anti-diabetic agent in modern literature. Thus the present study was undertaken to find out the anti-diabetic activity of the extract in streptozotocin-induced hyperglycemic rat using glibenclamide as reference standard.

Material and Method

The bark was collected from Toranmal, Maharashtra, India in December 2007 and authenticated from Botany department of Dr. P. R. Ghogre Science College Dhule, Maharashtra. A voucher specimen (HRPIPER-02) has been conserved for future reference. The bark was washed, cleaned, shade-dried, pulverized and passed through a 40-mesh sieve, and kept in a well-closed container for further extraction.

Chemicals

Diagnostic kits for estimation of triglyceride and cholesterol were purchased from Pointe Scientific Inc., USA, for measurement of glucose from RFCL Limited, India

Preparation of bark extract

The bark powder (1 kg) was successively extracted with petroleum ether (60-80°), ethyl acetate and ethanol with hot continuous percolation. All the extracts were screened for preliminary phytochemical test, out of the three extracts screened; only ethyl acetate extract showed Libermann-Burchards test positive for the presence of triterpenoids. Accurately weighed 3 g of ethyl acetate

extract was dissolved in ethyl acetate and shaken well, this solution then treated with 5%hydrochloric acid for several times, resulted in formation of two layers, which then separated by separating funnel. Aqueous layer was discarded and ethyl acetate layer was treated with 5% potassium hydroxide and shaken for 3 days. Formed two layers were then separated by separating funnel. Ethyl acetate layer was collected, evaporated, dried and used for further study.

Animals

Animal experimentation part was performed by strictly adhering to Indian regulations and approved by the Institutional Research Committee (Approval letter no. RCPIPER/2008-9/12). Wister rats weighing 250–300 g were used for the study. Animals were maintained under controlled environmental conditions: 12 hours light/dark cycles with *ad libitum* access to standard rodent chow and water. Food was removed 4 hours before experiments were conducted.

Acute toxicity study

Healthy adult Wistar rats (male) were subjected to acute toxicity studies as per guideline (AOT no. 425) suggested by Organization for Economic Co-operation and Development (OECD) (2001). The rats were observed by housing them individually in the polypropylene metabolic cages continuously for 2 hours for behavioral, neurological and autonomic profiles; and for any lethality during next 48 hours.

Experimental design

Group I: Normal control (vehicle treated); Group II: Diabetic control (streptozotocin 45 mg/kg); Group III: Diabetic rats orally administered glibenclamide (10 mg/kg) in 0.5 mL of 5% alcohol; Group IV: Diabetic rats orally administered *B. ceiba* extract (200 mg/kg) in 0.5 mL of 0.1%methylcellulose suspension; Group V: Diabetic rats orally administered *B. ceiba* extract (400 mg/kg) in 0.5 mL of 0.1% methylcellulose suspension; Group VI: Diabetic rats orally administered *B. ceiba* extract (600 mg/kg) in 0.5 mL of 0.1% methylcellulose suspension.

In vivo hypoglycemic activity

A freshly prepared solution of streptozotocin (45 mg/kg) in 0.1M citrate buffer, pH 4.5 was injected intraperitoneally to overnight fasted rats. After 3 days, blood glucose level was analysed and those rats above blood glucose 250 mg/dL were considered as diabetic. Treatment began 3 days after streptozotocin injection with various concentrations of *B. ceiba* extract once a day for 3 weeks/21 days. Fasting blood glucose level in all the rats were determined on 21th day by glucose oxidase method (Trinder, 1969). Blood samples withdrawn from retro orbital sinus on 22nd day and serum was separated from blood by centrifugation at 4,000 rpm for 10 min to obtain biochemical parameters.

Biochemical parameters

Total cholesterol and triglycerides in blood were determined by cholesterol esterase method (Allain et al., 1974) and lipoprotein lipase method (Muller et al., 1977) respectively. High density lipoprotein was determined by Burstein method (Burstein et al., 1970). Low density lipoprotein was analyzed by the method described elsewhere (Friedewald et al., 1972).

Histopathological studies

On 22nd day of study, rats were sacrificed by cervical dislocation. Pancrea samples were isolated from the control and treated groups of animals, washed separately with normal saline, and fixed in 10% neutral buffered formalin solution for 48 hours. The formalin-fixed Pancrea samples were stained with hematoxylin–eosin for photomicroscopic observations.

GC-MS analysis of ethyl acetate fraction

Ethyl acetate fraction of *B. ceiba* was analyzed by GC-MS, gas chromatograph- Make: Perkin Elmer, USA, and Model: auto system XL, silica based capillary column was used for separation of component. mass spectrometer- make; Turbomass spectrometer, Perkin Elmer, Detector: Flame Ionization Detector, Temperature range was 50 to 450°C, used for determination of molecular weight of separated component.

Statistical analysis

Results were expressed as mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. A value of $p<0.05$ was considered to be statistically significant.

Results

Body weight was significantly lowered (23% on day 21) in the diabetic group throughout the research signifying the appropriateness of the animal model. Animal treated with the extract improved body weight (0.98%) on 14 days and after 21-day supplementation, 14.12% increase was observed which was significant. (data not shown).

Before the treatment, fasting blood glucose of all animals was within the normal range. Blood glucose was elevated significantly after 48 hours of streptozotocin injection and was monitored for 7 days. Animals having 250 mg/dL blood glucose were selected for the study. Oral administration of ethyl acetate extract for 21 days resulted in significant reduction of fasting blood glucose at a dose of 600 mg/kg body weight when compared with diabetic control (Table I). At a dose of 200 mg/kg body weight no significant reduction in blood glucose level was observed.

In *B. ceiba* ethyl acetate extract treated rats, a significant

Table I

Effect of 21 daily dose of various concentration of *Bombax ceiba* bark ethyl acetate extract on fasting plasma glucose, cholesterol, triglycerides, HDL and LDL concentrations of STZ-treated rats

Groups	Blood glucose (mg/dL)	Total cholesterol (mmol/L)	Triglyrceride (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Normal control	161 ± 21^{c}	1.7 ± 01^{c}	0.69 ± 0.19^{c}	1.1 ± 0.44^{b}	1.1 ± 0.44^{c}
Diabetic control	433 ± 11	2.3 ± 07	2.2 ± 0.10	0.59 ± 0.07	1.5 ± 0.17
Glibenclamide 10 mg/kg	159 ± 14^{c}	1.8 ± 0.11^{c}	2.1 ± 0.18	0.60 ± 0.08	1.4 ± 0.14
Bombax ceiba 200 mg/kg	424 ± 44	2.1 ± 0.14^{b}	2.1 ± 56	0.81 ± 0.22	1.1 ± 0.17
Bombax ceiba 400 mg/kg	327 ± 80^{a}	1.8 ± 0.06^{c}	1.6 ± 0.24^{c}	0.92 ± 0.96^{a}	1.3 ± 0.26^{b}
Bombax ceiba 600 mg/kg	156 ± 8.6^{c}	1.8 ± 0.12^{c}	0.9 ± 1.33^{c}	0.99 ± 0.1^{a}	0.8 ± 0.33^{c}

Data are expressed as mean ± SD; (n = 6); $^{a}p<0.05$ significant from diabetic control; $^{b}p<0.01$ significant from diabetic control; $^{c}p<0.001$ significant from diabetic control

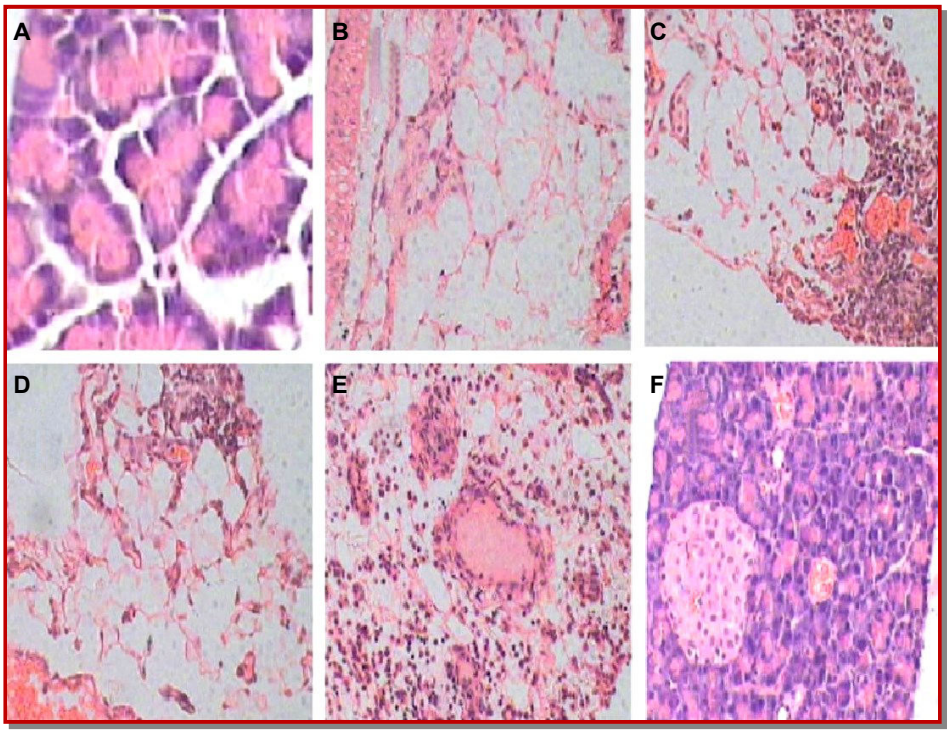

Figure 1: (A) Normal pancreas showing no pathological changes with normal islets of Langerhans; (B) Pancreas (diabetic control) showing diffused necrotic changes of moderate to marked degree as a result of which they were significantly reduced in size and number; (C) Pancreas treated with glibenclamide 10 mg/kg), after 22 days: Showing improvement and minimal degenerative changes; (D) Pancreas treated with *Bombax ceiba* ethyl acetate extract 200 mg/kg), after 22 days: Not showing improvement or restoration of normal cellular size of islets; (E) Pancreas treated with *Bombax ceiba* ethyl acetate extract 400 mg/kg), after 22 days: showing less improvement with nearly normal islets of Langerhans; (F) Pancreas treated with *Bombax ceiba* ethyl acetate extract 600 mg/kg), after 22 days: Showing marked improvement with nearly normal islets of Langerhan

decrease ($p<0.001$) in serum cholesterol was observed at dose of 600 mg/kg body weight after the treatment period, when compared to the diabetic untreated rats. Treatment with the ethyl acetate extract and glibenclamide caused a significant decline in total cholesterol by 24 and 19% correspondingly.

Significant increased level of HDL was observed in diabetic group of rats on treatment with the ethyl acetate extract, as compared with diabetic control. The LDL level fell to 54.2% at dose of 600 mg/kg body weight of *B. ceiba* ethyl acetate extract in diabetic group of animal.

Histological changes were shown in Figure 1. In streptozotocin-diabetic rats the islet is considerably reduced and shrunken. There is damage of some β-cells with central hyalinization; a few cells showed pyknotic nuclei and the number of cells were decreased (Figure 1A). After 22 days of oral administration with the extract, when blood glucose came down to normal level, islets of Langerhans also showed improvement in the β-cells granulation which is comparable to standard

(Figure 1F).

Compounds in the ethyl acetate fractions were separated by Gas chromatogram with different retention time of which compound 1 (RT 40.54) and Compound 2 (RT 41.60) were found to have mass fragmentations pattern same as that of triterpenoids.

Discussion

Anti-hyperglycemic activity of medicinal plants or plant derived products needs extensive research as the number of diabetic patients is continuously on the rise and according to WHO projections; it will be the single largest non-communicable disease worldwide by the year 2025 with the largest diabetic population in India (Wild et al., 2004). Hypoglycemic effect of various triterpenoids and steroids from natural resources had been investigated and documented by various researchers (Sugihara et al., 2000; Tan et al., 2008; Hai-Xue et al., 2011). The extract is widely used and prescribed by tribal of India as an alternative or complementary diabetes treatment (Verma et al., 2011). The extract reported to have triterpenoids but its anti-diabetic activity is not investigated till date. In light of these facts, the present study was undertaken to evaluate the anti-diabetic status of the extract.

In the preliminary phytochemical investigation of all extracts, ethyl acetate extract shows the presence of triterpenoids and hence selected for anti-diabetic activity.

Among the various doses of ethyl acetate fraction of the bark extract, a dose of 400 mg/kg body weight and 600 mg/kg body weight showed significant anti-hyperglycemic effect in the streptozotocin-treated group. The administration of extract in the streptozotocin-treated group prevented increase in cholesterol and triglyceride concentrations. In diabetes, hyperglycemia is accompanied with dyslipidemia i.e. characterized by increase in LDL and fall in HDL (Bierman et al., 1966). This altered serum lipid profile was reversed towards normal after treatment with the extract. Streptozotocin is a highly cytotoxic agent inducing diabetes by damaging the pancreatic β-cells that causes reduction in insulin release (Elsner et al., 2000). The insulinogenic effect of various medicinal plant extracts resulting in activation of β-cells has been reported (Kedar and Chakrabarti, 1982). The possible mechanism through which the exerts its anti-hyperglycemic effect might have been due to the increased release of insulin from β-cells and/or regenerated β-cells. In this circumstance, a number of other plants have been reported to have anti-hyperglycemic activity with a stimulatory effect on insulin release (Esmaeili and Yazdanparas, 2004). Since the extract produced highly significant anti-hyperglycemic effect even in diabetic rats in which most of the β-cells are damaged, it is likely that the extract might have extra-pancreatic mechanism of action.

GC-MS is valuable tool for the separation and identification of natural constituents in extract (Jacques et al., 2007). Hence the ethyl acetate fractions subjected to GC-MS analysis. The total ion chromatogram of ethyl acetate extract two triterpenoids detected and identified by comparing the mass spectra of separated fraction with the Wiley library of the GC/MS). A compound was only considered positively identified when the percent of similarity was superior to 90%.

Conclusion

B. ceiba was found to lower blood glucose in streptozotocin-induced diabetic rats, the extract also has hypocholesterolemic and hypotriglyceridemic effects.

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