EFFECT OF METHANOL AND ACETONIC EXTRACTS OF *Bombax ceiba* LEAVES ON ALLOXAN INDUCED DIABETIC RATS

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Abstract: The present study aims to determine the effect of methanol and acetonic extracts of *Bombax ceiba* leaves on blood glucose levels of alloxan-induced diabetic rats. Diabetes was induced in Wistar rats by intraperitoneal injection of alloxan (150 mg/kg) and afterwards, the normal rats and diabetic rats were randomly allocated into 5 groups; normal control rats, diabetic control rats, diabetic rats administered metformin (120 mg/kg), diabetic rats received methanol extract of *Bombax ceiba* (400 mg/kg) and diabetic rats received acetonic extract of *Bombax ceiba* (400 mg/kg). The extracts and metformin were administered orally for 7 days. Significant ($p \leq 0.05$) reduction in blood glucose of diabetic rats in comparison with diabetic control group 'was observed'. The methanol extract of *Bombax ceiba* was found to be more effective as hypoglycemic agent than both metformin and acetone which are almost at same level.

Keywords: *Bombax ceiba;* diabetes mellitus; plant extracts; blood glucose level

Introduction

Diabetes mellitus (DM), commonly known as diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a prolonged period (WHO, 2014), higher than blood glucose level of 90-140 mg/dl. Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications (WHO, 2013). Complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death (Kitabchi *et al.*, 2009) and others like cardiovascular disease, stroke, chronic kidney disease (WHO, 2013). The disease states underlying the diagnosis of diabetes mellitus are now classified into four categories: type 1, *insulin-dependent diabetes;* type 2, *noninsulin-dependent diabetes*; type 3 and 4, gestational diabetes mellitus (Nolte and Karam, 2007).

Bombax ceiba is commonly known as silk cotton tree and semal which belongs to family Bombacaceae. It is one of the important medicinal plants found in tropical and subtropical India and also occurs in Sri Lanka, Pakistan, Bangladesh, Myanmar, Malaysia, Java, Sumatra and Northern Australia. It has number of traditional uses and its medicinal usage has been reported in the Indian traditional systems of medicine such as Ayurveda, Siddha and Unani (Anandarajagopal, 2013). The various parts of *Bombax ceiba* have been reported for hypotensive and hypoglycaemic, antiangiogenic, analgesic, antiulcer, antioxidant, hepatoprotective and antimicrobial activities. Also it was used for the treatment of sexual debility, bleeding wounds and vaginal infections (Anandarajagopal, 2013).

Materials and Method

Sample collection and preparation

The leaves of *Bombax ceiba* (Bombacaceae) were collected from Gombe Jewel Suite, Gombe, Gombe State on 1st July, 2019. The leaf was identified and authenticated by a taxonomist at the herbarium of the Department of Biological Sciences, Faculty of Sciences, Gombe State University, Gombe, to be *Bombax ceiba* where a specimen with a voucher No. 128 has been deposited. The plant leaves material was removed from the rest of the plants part and shade dried for 2 weeks and manually size reduced using mortar and pestle.

Extraction of plant material

The crushed powder leaves of *Bombax ceiba* was weighed (128 g) and transferred into the clean thimble extracted with 100% methanol 1.2 liter using Soxhlet apparatus. Extract obtained was condensed using condenser and allowed to evaporate to dryness under vacuum to remove solvent completely. The same procedure was done but with 100% acetone now in place of methanol. Extracts were kept in desiccators.

Phytochemical screening

These involves the screening and identification of the chemical constituents in plants which includes sugars, fats, amino acids, alkaloids, glycosides, tannins, Saponins, steroids, resins etc based on their different reactions to organic chemical.

Test for carbohydrate (Benedict's test)

To 5 ml of Benedict's reagent, 1 ml of Leaves extract of *Bombax ceiba* solution was added and boiled for 2 min and cooled. Formation of a red precipitate shows the presence of carbohydrate (Khurshid Alam *et al.*, 2018).

Test for saponins (foam test)

Ten (10) mg of Leaves extract of *Bombax ceiba* was taken in a test tube and shaken vigorously with 5 ml of water. Production of persistent foam indicates the presence of saponins (Khurshid Alam *et al.*, 2018).

Test for flavonoid, phenol and tannin (ferric chloride test)

Five (5) mg of Leaves extract of *Bombax ceiba* was dissolved in 2 ml of water. Then 1 ml of neutral 5% ferric chloride solution was added. A dark green color indicates the presence of flavonoid, phenols and tannin (Khurshid Alam *et al.*, 2018).

Test for proteins (Biuret test)

Three to five (3-5) mg of Leaves extract of *Bombax ceiba* was added in 4% NaOH and few drops of 1% $CuSO_4$ solution was added in this solution. A violet or pink color indicates the presence of protein (Khurshid Alam *et al.*, 2018).

Test for steroids (Liebermann-Burchard test)

The Leaves extract of *Bombax ceiba* 5 mg was dissolved in 1 ml of chloroform. 2 ml acetic anhydride and 1 ml concentrated sulfuric acid were added. Formation of greenish color solution indicates the presence of steroids (Khurshid Alam *et al.*, 2018).

Test for phytosterol (Salkowski's reaction)

To 2 mg of Leaves extract of *Bombax ceiba*, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 were added and shaken vigorously. Chloroform layer shows greenish yellow fluorescence, which indicates the presence of phytosterol (Khurshid Alam *et al.*, 2018).

Test for amino acids (Ninhydrin test)

To an aliquot of diluted extract, 2 ml of ninhydrin solution was added. A violet color formation indicates the presence of amino acid (Khurshid Alam *et al.*, 2018).

Test for glycosides (Keller- Killani Test)

About 2 mg of extracts was taken in a test tube followed by 1 ml of glacial acetic acid containing trace amount of $FeCl_3$ and 1 ml of concentrated H_2SO_4 were added to the extract carefully. A reddish-brown colour is formed at the junction of two layer and the upper layer turns bluish green in presence of glycosides (Khurshid Alam *et al.,* 2018).

Test for alkaloids (Wagner's Test)

The leaves extract of *Bombax ceiba* 3 mg was taken in a test tube. 5 ml of 1% HCl was added to the test tube and stirred on a stream bath. After filtering the solution, few drops of Wagner's reagent was added. Formation of a reddish brown precipitate indicates the presence of alkaloids (Khurshid Alam *et al.,* 2018).

Drug preparation

Standard metformin (120 mg/kg) body weight is prepared by using sterilized water. Leaves extract of *Bombax ceiba* was dissolved in sterilized water to prepare dose at a concentration of 400 mg/kg body weight (Khurshid Alam *et al.,* 2018).

Induction of diabetes

For the development of diabetic model rats, the rats were grouped into 5 (groups I-V). The group I rats were kept as normal control group. Oral glucose solution (10%) was administered 1 h after alloxan administration to overcome initial hypoglycemia. After 48 hours of alloxan induction, blood samples were collected by tail puncture and measure by a glucometer (SD Code Free blood Glucose meter, SD Biosensor, Inc., Korea). All the rats in group II-V were kept overnight fasting and a freshly prepared solution of Alloxan (150 mg/kg body weight in 0.9% normal saline) was administered intraperitoneally. Rats with significant elevated blood glucose levels above 11.1 mmol/L were selected for the study. Baseline blood glucose level of group II-V rats was also measured just prior to the administration of alloxan (Khurshid Alam *et al.,* 2018).

Grouping of experimental rats

Confirmed hypoglycemic rats were randomly assigned into five groups (n=5). (normal control) rats received 0.9% normal saline (1 mL/ kg, bw), (diabetic control) rats received alloxan (150 mg/kg, bw) in 0.9% normal saline, (positive control/standard) rats received metformin hydrochloride (120 mg/kg, bw) in 0.9% normal saline, rats received methanol leaf extract of *Bombax ceiba* (400 mg/ kg, bw) in 0.9% normal saline, rats received acetonic leaf extract of *Bombax ceiba* (400 mg/ kg, bw) in 0.9 % normal saline.

Method of acute toxicity studies

Acute toxicity was carried out on Wistar rats according to the method proposed by Lorke (1983); this method has two phases which are Phase I and Phase II.

Phase I: This phase requires twelve rats; the twelve rats were divided in to three groups, group one received 10 mg/kg/p.o of extract of *Bombax ceiba*. Group two was administered 100 mg/kg/p.o extract of *B. ceiba* and group three received 1000 mg/kg/p.o of extract. The rats were placed under observation for 24 h to monitor their behavior such as increased motor activity, salivation, convulsion, coma, as well as mortality.

Phase II: This phase involved the use of three Wistar rats, which were distributed into three groups of one rat each. The rats were administered higher doses 1600, 2900, and 5000 mg/kg/p.o of test extracts, respectively. The rats were then observed for 24 h for behavior as increased monitor their behavior such as increase motor activity, salivation, convulsion, coma, as well as mortality.

Then LD_{50} (lethal dose) is calculated by the

$$LD50 = \sqrt{(DO \times D100)}$$

Where: D0 = highest dose that gave no mortality; D100 = lowest dose that produced mortality (Lorke, 1983).

Statistical analysis

The results were expressed as mean ± standard errors of the mean (SEM) for all values. The data for blood glucose analysis was analyzed using one-way ANOVA followed by Dunnetts comparison test. Results were considered to be significantly when $P \leq 0.05$ (Mali *et al.,* 2017).

Results and Discussion

Yield obtained

Extraction with acetone yield 8.2% while with methanol, 10.7% yield was obtained.

Phytochemical quantitative screening

The phytochemical screening revealed the presence of phenolic compounds flavonoids, tannins and alkaloids in both methanol and acetonic extracts of *Bombax ceiba* in larger amounts. Acetonic extract was found to contain steroids and phytosterols in large amounts which were absent in methanol extract, whereas cardiac glycosides, saponins, amino acids and proteins were found in methanol extract in moderate level. Table 1 shows the differential distribution of phytoconstituents in both methanol and acetonic extracts.

Table 1: Phytochemical constituents of methanol and acetonic extracts of *Bombax ceiba* leaves

S/N	Name of Test	Name of Phytochemicals	Methanol Extract of *B. ceiba* Leave	Ethanolic Extract of *B. ceiba* Leaves
1	Benedict's test	Carbohydrate	-	-
2	Foam test	Saponin	++	-
3	Ferric chloride test	Flavonoids	+++	+++
4	Ferric chloride test	Phenolic compound & Tannin	+++	+++
5	Biuret test	Proteins	++	-
6	Lieberman-Burchard test	Steroid	-	+++
7	Salkowski's test	Phytosterol	-	+++
8	Ninhydrin test	Amino acids	++	-
9	Keller-killani test	Cardiac glycosides	++	-
10	Wagner's test	Alkaloid	++	++

– = absent; ++ = present in moderate amount; +++ = large amount

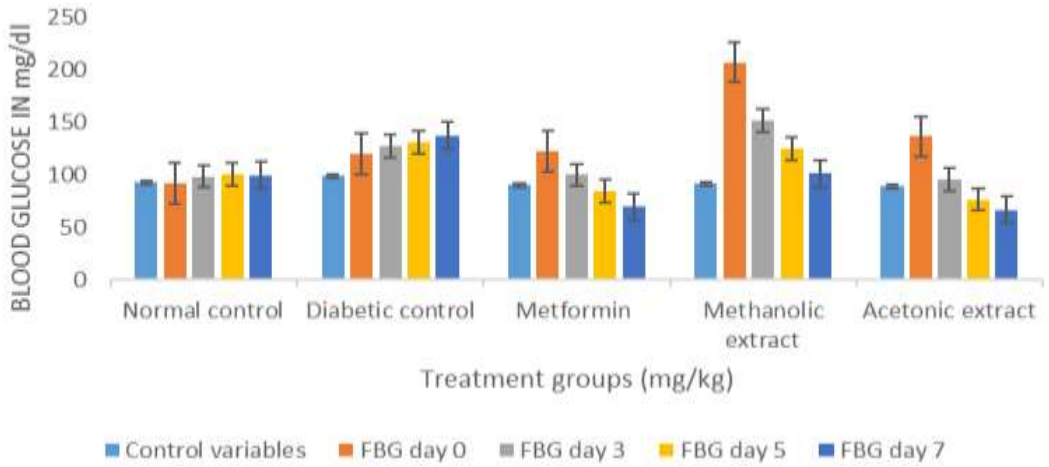

Fig. 1: Effect of methanol and acetonic extract of *Bombax ceiba* on blood glucose level in alloxan-induced diabetic rats

Effect of methanol and acetonic extract on blood glucose level in alloxan-induced diabetic rats

The methanol leaf extract of *Bombax ceiba* (400 mg/kg, bw) and acetonic leaf extract of *Bombax ceiba* (400 mg/kg, bw), and the standard metformin (150 mg/kg bw) were administered i.p. in alloxan-induced diabetic rats and the blood samples were collected by cutting the tail tip of the rats at day 0, 3, 5 and 7 of treatment. The methanol extract significantly reduced the blood glucose levels at different 'Duration' (0, 3, 5 and 7 days) when compared with the untreated alloxan-induced diabetic rats. Interestingly, the reduction activity of glucose level in blood was the maximum of day 0 and found to be 82.8%, whereas the acetonic extract and standard metformin reduced the blood glucose level to 54.53 and 48.8%, respectively by day 0 suggest that methanol extract has higher hypoglycemic activity than that of acetonic extract and standard metformin (Fig. 1).

Table 2: Results for acute toxicity studies of *Bombax ceiba* in rats

Treatments	Dose (mg/kg)	No. of Animals used	Mortality	% Mortality
Phase I				
Methanol extract of leaf of *Bombax ceiba*	10	3	0	0
	100	3	0	0
	1000	3	0	0
Acetonic extract of leaf of *Bombax ceiba*	10	3	0	0
	100	3	0	0
	1000	3	0	0
Phase II				
Methanol extract of leaf of *Bombax ceiba*	1600	1	0	0
	2900	1	0	0
	5000	1	1	100
Acetonic extract of leaf of *Bombax ceiba*	1600	1	0	0
	2900	1	0	0
	5000	1	1	100

The median lethal (LD_{50}) of the extracts (methanol and acetonic) of *Bombax ceiba* is 3807.89 mg/kg

This shows that the yield of *Bombax ceiba* can vary with the nature of the solvent used for the extraction of the active constituents, and in this case methanol is a better solvent for its extraction. This might be due to the presence of high content of secondary metabolites which may be soluble in high polar solvent (Anandarajagopal *et al.*, 2013).

The acute oral toxicity study of *Bombax ceiba* showed no mortality up to 3807.89 mg/kg but shown mortality at the dose of 5000 mg/kg. There was no obvious sign of convulsion, salivation, increase motor activity with all the doses administered in both Phases I and II (Table 2). Reported acute toxicity studies on leaves extract of *Bombax ceiba* showed no mortality rate at 2000 mg/kg body weight (Bhargava and Shah, 2016).

Alloxan monohydrate destroys the β-cells of islets of Langerhans of the pancreas and inhibits the production of insulin, which affect it ability to move glucose into the body tissues, resulting to high level of glucose in the blood (Khurshid Alam *et al.*, 2018). The administration of Alloxan injection caused a sharp increase in blood glucose levels of diabetic control and treated rats compared with those of normal controls. However, treatment with standard drug (metformin) and *Bombax ceiba* extracts resulted in marked decrease in blood glucose throughout the period of the study.

From Fig. 1, it can be observed that treatment with methanol *extract* of *Bombax ceiba* reduces the blood glucose level of the treated rats (as expected) better than methformin and acetonic extract and at the same time *Bombax ceiba* extracts shown significant hypoglycemic activity. The hypoglycemic activity of *Bombax ceiba* is due to possession of a chemical constituent called Shamimin, a C-flavonol glycoside from the leaves (Saleem and Syed, 1999).

Conclusion

From antidiabetic studies, it can be concluded that the leaves of *Bombax ceiba* possess hypoglycemic activity and methanol extract contain more hypoglycemic constituent than acetonic extract, and more hypoglycemic activity than that of both metformin and acetonic extract of the leaves. The phytochemicals present in this plant might account for the observed pharmacological actions. Further study is necessary to potential compound (s) and their role in the control and management of diabetes.

Conflict of Interests

The authors declare that there is no conflict of interest related to this work.

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