Asian Journal of Chemistry; Vol. 23, No. 6 (2011), 2716-2718

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

# Antihyperglycemic Effect of Isolated Fractions of *Ceiba pentandra* in Alloxan Induced Diabetic Rats

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(*Received*: 7 September 2010; *Accepted*: 24 February 2011) AJC-9646

The present study investigated antihyperglycemic effect of different fractions of methanol extract of *Ceiba pentandra* leaves. Petroleum ether, chloroform, ethyl acetate and methanol fractions of *Ceiba pentandra* leaves were prepared and administered individually at different doses to different batches of rats (both normal and alloxan diabetic rats) after an overnight fast. The blood glucose levels were measured at 0, 2, 4 h, 7 and 14 days after the treatment. The methanol fraction of *Ceiba pentandra* at a dosage of 200 mg/kg body weight is showing maximal blood glucose lowering effect in diabetic rats. The antihyperglycemic activity of *Ceiba pentandra* leaves was compared with the treatment of glibenclamide, an oral hypoglycemic agent.

**Key Words: Acute toxicity, *Ceiba pentandra*, Antihyperglycemic activity, Alloxan.**

## INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hypoglycemia, altered metabolism of lipids, carbohydrates and proteins. It is an increased risk of complications from vascular diseases[1,2]. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications effecting eyes, kidneys, nerves and arteries[3,4]. These may be delayed, decreased or prevented by maintaining blood glucose values close to normal. The increasing number of aging, population, consumption of calories rich diet, obesity and secondary life style have lead to tremendous increase in the number of diabetes worldwide. It is apparent that due to the side effects of the currently used drugs, there is a need for safe agents with minimal adverse effects, which can be taken for long duration[5]. Recently, the search for appropriate hypoglycemic agents has been focused on plants used in traditional medicine partly because of leads provided by traditional medicine to natural products that may be better treatments than currently used drugs[6].

*Ceiba pentandra* (L) Gaertner known as silk cotton tree and locally as "dum" belongs to the Bombacaceae family. Various parts of this plant are widely reputed in African traditional medicine. The plant has been reported to be a useful diuretic and effective remedy against diabetes, hypertension, headache, dizziness, constipation, mental trouble, fever, peptic ulcer, rheumatism, leprosy[7].

## EXPERIMENTAL

The leaves of *Ceiba pentandra* were collected from Melghat, India. This plant was identified and authenticated by Dr. Minoo Parabia at the VNSGU, Surat. A voucher specimen (No. 13) has been deposited at the Department of Pharmceutical Sciences, Dibrugarh.

Alloxan monohydrate were purchased from Sigma Chemical Co. All other chemical reagents used in this study were of analytical grade.

**Preparation of *Ceiba pentandra* leaf extracts:** The air dried and powdered leaves of *Ceiba pentandra* (1 Kg) were macerated with methanol for 7 days. Methanolic extract fractionated by petroleum ether, chloroform, ethyl acetate and methanol.

**Acute oral toxicity:** Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar rats (n = 3) of either sex selected by random sampling technique were used for the study. The animals were acclimatized for 5 days. The animals were kept fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 2000 mg/kg body weight by intragastric tube and observed for 14 days[8,9].

**Induction of diabetes:** Diabetes was induced in male wistar albino rats, aged 10-12 weeks (body weight 180-220 g) by intraperitoneal administration of ice-cold aqueous alloxan monohydrate[1,2] (150 mg/kg body weight). After 14 days, rats

with marked hyperglycemia (fasting blood glucose > 200 mg/dl) were selected and used for the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in plastic cages.

**Experimental design:** The rats were divided into 7 groups and each group consisted of six rats.

Group I: Normal untreated group; Group II: Diabetic untreated group; Group III: Diabetic rats treated with 0.4 mg/kg b.w. of glibenclamide; Group IV: Diabetic rats treated with 200 mg/kg b.w. of petroleum ether fraction; Group V: Diabetic rats treated with 200 mg/kg b.w. of chloroform fraction; Group VI: Diabetic rats treated with 200 mg/kg b.w. of ethyl acetate fraction; Group VII: Diabetic rats treated with 200 mg/kg b.w. of methanol fraction.

After an overnight fast, the plant fractions suspended in 1 % sodium CMC was fed to the experimental rats by gastric intubation, using a force feeding needle.

**Effect of different fractions of methanolic extract of *Ceiba pentandra* on alloxan-induced hyperglycemia**

**Acute treatment:** Hyperglycemia was induced by a single I.P. injection of 150 mg/kg of alloxan monohydrate in sterile solution. After 5 days of alloxan injection, the diabetic rats (glucose level > 200 mg/dl) were separated and divided into seven groups of six animals each. Group I served as a control and was given 1 % sodium CMC, Group II treated as diabetic control, Group III treated with glibenclamide (0.4 mg/kg), groups IV-VII were treated orally with petroleum ether, chloroform, ethyl acetate, methanol fractions of methanolic extract of *Ceiba pentandra* respectively. Blood samples were collected from the tail vein just prior to and 2 and 4 h after extracts administration (Table-1).

**Sub-acute treatment:** The diabetic rats (glucose level > 200 mg/dl) were divided into seven groups of six rats each. Group I served as a control and was given distilled water, Group II treated as diabetic control, Group III treated with glibenclamide (0.4 mg/kg), groups IV-VII were treated orally with petroleum ether, chloroform, ethyl acetate, methanol fractions of methanolic extract of *Ceiba pentandra*, respectively. The administration of extracts was continued for 14 days, once daily. Blood samples were collected through retro orbital plexus just prior to and on days 2, 7 and 14 of extract administration. The blood glucose levels were determined for all the samples by glucose-oxidase method (Table-2).

**Statistical analysis:** The experimental results were expressed as mean ± SD. Statistical comparison was done using one-way ANOVA followed by Duncan's multiple range test (DMRT) when more than two groups were involved.

## RESULTS AND DISCUSSION

**Acute oral toxicity studies:** In the acute toxicity *Ceiba pentandra* extract up to the dose level of 2000 mg/kg of body weight did not exhibit any lethality or toxic symptoms. Further dosing to estimate the $LD_{50}$ of the extract was not performed. According to organization for economic cooperation and development (OECD) guidelines, an $LD_{50}$ dose of 2000 mg/kg and above category is classified as unclassified and hence the drug is found to be safe.

**Effect of *Ceiba pentandra* extracts on alloxan induced hypoglycemia:** The antihyperglycemic effect of the fractions of methanolic extract on the blood sugar levels of diabetic rats is shown in Tables 1 and 2. Administration of alloxan (150 mg/kg) led to over 2.5 fold elevation of blood glucose level ($p < 0.001$) which was maintained over 2 weeks out of the four different fractions tested chloroform, ethyl acetate and methanol fractions showed fall in blood glucose level to 4.83, 10.11 and 20.02 %, respectively. The petroleum ether fraction

TABLE-1
EFFECT OF ACUTE TREATMENT WITH DIFFERENT FRACTIONS OF METHANOL EXTRACT OF *Ceiba pentandra* ON ALLOXAN INDUCED DIABETIC RATS

| Group | Treatment | Blood glucose (mg/dl) | | |
|---|---|---|---|---|
| | | 0 h | 2 h | 4 h |
| I | Normal | 73.46 ± 1.758 | 73.98 ± 1.651 | 73.18 ± 1.716 |
| II | Diabetic control | 232.29 ± 6.786 | 234.58 ± 6.558 | 235.39 ± 5.987 |
| III | Glibenclamide (0.4 mg/kg) | 239.52 ± 9.862 | 208.21 ± 7.896** | 179.33 ± 6.587** |
| IV | Petroleum ether fraction (200 mg/kg) | 247.19 ± 12.323 | 245.72 ± 12.592 | 241.38 ± 11.993 |
| V | Chloroform fraction (200 mg/kg) | 243.44 ± 11.596 | 238.27 ± 9.893 | 231.66 ± 10.527* |
| VI | Ethyl acetate fraction (200 mg/kg) | 251.96 ± 7.881 | 239.18 ± 7.224 | 226.48 ± 6.925* |
| VII | Methanol fraction (200 mg/kg) | 248.29 ± 6.192 | 229.36 ± 5.924** | 198.58 ± 6.887** |

Values are expressed as mean ± SD (n = 6). Significance $p < 0.05$ (*), $p < 0.01$ (**) as compared to diabetic control.

TABLE-2
EFFECT OF SUB-ACUTE TREATMENT WITH *Ceiba pentandra* FRACTIONS ON ALLOXAN INDUCED DIABETIC RATS

| Group | Treatment | Blood sugar level (mg/dl) | | | Body weight | |
|---|---|---|---|---|---|---|
| | | 0 day | 7 day | 14 day | Before treatment | After treatment |
| I | Normal | 74.17 ± 3.27 | 74.04 ± 2.05 | 74.13 ± 2.61 | 247 ± 10.78 | 275 ± 6.65 |
| II | Diabetic control | 236.2 ± 22.87 | 238.05 ± 22.76 | 237.24 ± 23.14 | 239 ± 15.69 | 198 ± 10.53 |
| III | Glibenclamide (4 mg/kg) | 237.38 ± 13.83 | 118.94 ± 7.60 | 101.26 ± 10.23 | 263 ± 3.6 | 283 ± 5.5 |
| IV | Petroleum ether fraction (200 mg/kg) | 240.16 ± 12.34 | 212.05 ± 3.07 | 208.82 ± 3.46 | 233 ± 12.28 | 247 ± 7.76 |
| V | Chloroform fraction (200 mg/kg) | 243.27 ± 15.88 | 176.72 ± 2.5 | 157.87 ± 3.04 | 244 ± 6.8 | 265 ± 1 |
| VI | Ethyl acetate fraction (200 mg/kg) | 231.04 ± 11.45 | 138.21 ± 12.34 | 122.65 ± 6.19 | 242 ± 6.5 | 249 ± 12 |
| VII | Methanol fraction (200 mg/kg) | 234.35 ± 7.71 | 121.03 ± 3.90 | 107.55 ± 2.62 | 242 ± 12.09 | 255 ± 18.33 |

Values are expressed as mean ± SD (n = 6). Significance $p < 0.05$ (*), $p < 0.01$ (**) as compared to diabetic control.

showed only 2.35 % reduction in blood glucose level. After 2 weeks of daily treatment with 200 mg/kg body weight of fraction of petroleum ether, chloroform and ethyl acetate led to fall in blood glucose level to 13.04, 35.10 and 46.91 %. While methanolic fraction shown maximum fall in blood glucose level 54.15 %. Treatment with glibenclamide at a dosage of 0.4 mg/kg body weight resulted in 57.38 % fall in the blood glucose level of diabetic rats.

The results indicate that *C. pentandra* possess significant antihyperglycemic activity. All fractions except petroleum ether fraction even if with a different degree, have shown significant reduction in blood glucose levels in alloxan induced diabetic rats. The methanol fraction has shown maximum effect in all the two tests compared to the other extracts.

It is generally accepted that alloxan treatment causes permanent destruction of β-cells. It is therefore, conceivable that the hypoglycemic principles in the methanol fraction of *Ceiba pentandra* exert their effect by an extrapancreatic mechanism in diabetic rats[10].

## ACKNOWLEDGEMENTS

The authors are grateful to the Director, Jai Research Foundation, Valvada, for the facilities and animal ethics permission and to Dr. Dharmesh Patel, for his kind help.

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