ABSTRACT

Clerodendrum colebrookianum is commonly distributed in North eastern region of India. Clerodendrum colebrookianum leaves are reported to be used in the herbal antidiabetic medicines. The aim of this study was to investigate the antidiabetic property of Clerodendrum colebrookianum leaves in diabetic mice. Diabetes mellitus was induced in experimental mice by intraperitoneal administration of alloxan monohydrate. Diabetic mice showed increase in blood glucose levels after 72 hrs of alloxan administration. Effects of aqueous extracts of Clerodendrum colebrookianum leaves on normal and alloxan-induced diabetic mice were investigated in four groups of mice (six mice per group). The levels of blood glucose of mice were examined in all four experimental groups. Phytochemical screening of the leaf extracts of Clerodendrum colebrookianum indicated the presence of carbohydrates, flavonoids, tannins, phenolic compounds, glycosides, and terpenoids. The mice were administered orally with the aqueous extracts of Clerodendrum colebrookianum leaves with a single dose of 400 mg/kg body weight per day. The leaf of Clerodendrum colebrookianum was found to have blood sugar lowering potential in alloxan induced diabetic mice. The present study has established the anti-diabetic effect of Clerodendrum colebrookianum leaves.

Keywords: Diabetes mellitus, Alloxan monohydrate, antidiabetic, hypoglycemic, aqueous extract.

INTRODUCTION

Diabetes is one of the most serious metabolic syndromes. Diabetes mellitus is mainly chronic complication of carbohydrate metabolism due to insufficient insulin secretion or insulin resistance action. Various conventional therapies are available for the treatment of diabetes mellitus including different oral antidiabetic drugs and insulin. A large number of drugs are available for the treatment of diabetes such as biguanides, thiazolidinediones, α-glucoside inhibitors, and sulfonylureas. These currently available therapies have various limitations and different adverse side effects. A large number of medicinal plants are reported to be source of alternative and safe medicines for the treatment of diabetes mellitus. Different traditional herbal folk medicinal systems are using various crude plant extracts for the management of diabetes. Several phytochemicals are reported to have strong antidiabetic effects. Secondary metabolite compounds are biosynthesized from primary plant metabolites. A large number of secondary metabolite compounds are reported to possess therapeutic activity. Various indigenous medicinal plant species are found to be very much effective to control diabetes. Recently herbal drugs are getting significant importance in the management of Diabetes. Nowadays a large number of herbal formulations are available in the market for the treatment of diabetes. There is an increasing demand for herbal antidiabetic products. Therefore, medicinal plants are continuously scrutinized to evaluate their antidiabetic activity.

Medicinal plants are playing an important role in improving the quality of life. Clerodendrum colebrookianum is one kind of perennial shrub. It is widely distributed in tropical as well as subtropical regions of the world. Clerodendrum colebrookianum is commonly distributed in Bangladesh, China, India, Myanmar, Nepal, and Vietnam. It is found to be a common medicinal plant in all the states of North East India including Assam. The plant is reported to be used for the control and management of hypertension and diabetes in North East India. Clerodendrum colebrookianum is traditionally used as therapy for the treatment of various diseases like diabetes, cough, fever, asthma, dysentery, malaria, blood pressure, stomach upset, and dizziness. A large section of people of North Eastern region of India are using Clerodendrum colebrookianum for the treatment of diabetes.

MATERIALS AND METHODS

Chemicals

All the reagents used in the experiments were of analytical grade. Alloxan monohydrate was obtained from Sigma-Aldrich.

Plant material

Clerodendrum colebrookianum leaves were collected from the village area of Nalbari District, Assam. The collected...
Plant parts were properly identified as well as authenticated in the Botany Department of Royal Global University.

**Preparation of water extract of Clerodendrum colebrookianum leaves**

The matured *Clerodendrum colebrookianum* leaves were cut into small pieces and were dried under shade. Dried leaves were then powdered. Powdered leaves were mixed with distilled water and stirred in a round bottom flask for 24 hours at room temperature. In the next phase the resultant mixture was heated at 80 °C for eight hours with regular stirring. The extract was then filtered. The filtrate obtained was concentrated in a rotary flash evaporator. The concentrated water extracts were lyophilized in a lyophilizer. The lyophilized aqueous extract was stored in airtight container for further uses. The extract yield was found to be 11%.

**Animal used**

Male albino adult mice weighing about mice 25-30g were used for the present study. The experimental mice were housed in polypropylene cages, maintained under normal environmental conditions. The mice were allowed free access to clean water as well as standard pellet diet. All the mice were kept maintaining standard experimental protocols and as per internationally accepted guidelines for laboratory animal use throughout the study. This experimental study was done after getting proper approval of institutional animal ethics committee of Gauhati University, Guwahati, Assam, India (IAEC approval registration number :902/ac/05/CPCSEA).

**Diabetes induction**

The induction of diabetes was done by intra peritoneal injection of Alloxan monohydrate. Alloxan monohydrate was freshly dissolved in distilled water and was injected at a single dose of 180 mg/ kg of body weight. The mice were provided 10% of glucose solution in water bottles for the next 24hrs to prevent hypoglycemia. After 72 hrs of injection, fasting blood glucose levels were measured. Fasting blood glucose levels of mice were measured after 7 days of injection to check the hyperglycemia. Diabetic mice with hyperglycemia were considered for experimental studies.

**Experimental designs**

The mice were divided into four groups of six animals each and the treatment was done as follows:

- **Group I:** Normal non-diabetic control mice received only normal regular diet and water.
- **Group II:** Normal mice received water extract of *Clerodendrum colebrookianum* leaves at a single dose of 400 mg/kg body weight daily one time.
- **Group III:** Alloxan-induced diabetic control mice served as diabetic controls without any therapy.
- **Group IV:** Diabetic mice were treated with water extract of *Clerodendrum colebrookianum* leaves at a single dose of 400 mg/kg body weight daily one time.

**Statistical analysis**

The results of the experiment were expressed as mean ± standard error of mean (SEM). The significant difference in the means was determined by using ANOVA. The level of significant was considered at p ≤ 0.05.

**Oral administration to mice**

The plant extracts were administered orally by dissolving minimum volume of water by using syringe of a long needle.

**Estimation of Blood Glucose**

The estimation of blood glucose levels was estimated by one touch commercial glucometer. GLUCOCARDTM 01 SENSOR glucometer was used in the experiment.

**RESULTS**

**Preliminary phytochemical screening**

The crude plant extract was subjected for qualitative phytochemical screening using standard procedures. After the phytochemical investigation it has been found that the plant extract contained different phytochemical constituents like carbohydrates, flavonoids, tannins, phenolic compounds, glycosides, and terpenoids.

**Table 1:** Phytochemical present in aqueous extract of *Clerodendrum colebrookianum*

| Phytochemical       | Aqueous extract |
|---------------------|-----------------|
| Steroids            | -               |
| Glycosides          | +               |
| Alkaloids           | -               |
| Flavonoids          | +               |
| Terpenoids          | +               |
| Carbohydrates       | +               |
| Saponins            | -               |
| Tannins             | +               |
| Phenolic compounds  | +               |

Key: + = Present; - = Absent

**Toxicity studies**

Toxicity studies were conducted for water extract of *Clerodendrum colebrookianum* leaves. The mice were orally treated with the water extract at doses of 200, 300, 400, 500, 1000 and 2000 mg/kg body weight. No mortality was observed even at a dose of 2000 mg/ kg body weight in the mice. Even at very high dose also, no gross behavioural change was observed in the mice. This toxicity study has indicated a high margin of safety.
Effect on body weight

Weight loss is very commonly observed among the diabetic patients. Unexpected weight loss is one of the common symptoms of diabetes. In diabetes, people can’t utilize sugar they consume for energy. Body utilizes fat and muscle for energy production resulting in weight loss. In our study also weight loss has been observed in experimental diabetic mice. Normal control mice were found to maintain stable body weight during the study period. Administration of alloxan monohydrate leads to a loss in body weight in the diabetic mice after the treatment. All the experimental data on body weight are summarized in the Table-2. A loss in body weight was observed in the diabetic control group of mice (from 29.37 g to 22.43 g). The average body weights in the mice of group IV were slightly increased (from 23.11 g to 24.11 g) by the treatment of Clerodendrum colebrookianum leave extract after 14 days of treatment.

Table 2: Effect of aqueous extract of Clerodendrum colebrookianum leave on body weight (g) in normal and alloxan-induced diabetic mice.

| Groups                             | Treatment                  | Mean body weight in gram |
|------------------------------------|----------------------------|--------------------------|
| Group I (Untreated normal control mice) | Without any therapy       | Initial: 28.96 ± 0.84, 0th day: 28.24 ± 0.85, 7th day: 27.79 ± 0.69, 14th day: 27.82 ± 0.45 |
| Group II (Plant extract treated normal mice) | plant extract             | Initial: 29.07 ± 0.62, 0th day: 28.95 ± 0.78, 7th day: 29.11 ± 0.27, 14th day: 29.81 ± 0.22* |
| Group III (Diabetic control mice)   | Alloxan only               | Initial: 29.37 ± 0.41, 0th day: 24.38 ± 0.51, 7th day: 23.19 ± 0.22, 14th day: 22.43 ± 0.71 |
| Group IV (Plant extract treated diabetic mice) | Alloxan + plant extract  | Initial: 29.04 ± 0.23, 0th day: 23.11 ± 0.76*, 7th day: 23.66 ± 0.18, 14th day: 24.11 ± 0.73* |

Results were expressed as mean values ± standard error of mean (Mean ± SEM) for 6 mice in each group (n=6); *p < 0.05 Vs Control.

Figure 1: Effect of aqueous extract of Clerodendrum colebrookianum leave on body weight (g) in normal and alloxan-induced diabetic mice.

Effect on blood glucose level

The results of the present investigation have shown that treatment of alloxan monohydrate at a dose of 180 mg/kg body weight after 7 days caused significant increases in blood glucose levels of mice in group III (276 mg/dl), group IV (280 mg/dl), when compared with the untreated normal control mice, group I (110 mg/dl) and plant extract treated normal mice, group II (109 mg/dl). The blood glucose levels of non-diabetic mice in group II were reduced after the treatment of aqueous extract of Clerodendrum colebrookianum leaves (by 5.50 % reduction). In the study, Clerodendrum colebrookianum leaves extract given at the dose of 400 mg/kg body weight showed significant lowering of blood glucose level (239 mg /dl) on day 14 as compared to control group (294 mg/dl, p <0.05) (by 18.70 % reduction). So, the Clerodendrum colebrookianum leaves extract showed significant hypoglycemic activity in alloxan induced diabetic mice.
Table 3: Effect of aqueous extract of *Clerodendrum colebrookianum* leave on blood glucose level of normal and alloxan-induced diabetic mice.

| Groups                               | Treatment                  | Blood glucose level (mg/dL) |
|--------------------------------------|----------------------------|-----------------------------|
|                                      |                            | 0<sup>th</sup> day | 7<sup>th</sup> day | 14<sup>th</sup> day |
|                                      |                            |                          |                  |                   |
| Group I (Untreated Normal Control mice) | Without any therapy | 110 ± 2.30               | 104 ± 2.44       | 107 ± 1.88       |
| Group II (plant Extract treated Normal mice) | plant extract       | 109 ± 1.22               | 101 ± 2.66*      | 103 ± 2.55       |
| Group III (Diabetic control mice)     | Alloxan only              | 276 ± 2.33               | 280 ± 3.17       | 294 ± 2.19       |
| Group IV (plant Extract treated Diabetic mice) | Alloxan + plant extract | 280 ± 2.45*              | 257 ± 2.12       | 239 ± 1.88*      |

Results were expressed as mean values ± standard error of mean (Mean ± SEM) for 6 mice in each group (n=6); *p < 0.05 Vs Control.

Figure 2: Effect of aqueous extract of *Clerodendrum colebrookianum* leave on blood glucose level in normal and alloxan-induced diabetic mice.

**DISCUSSION**

Diabetes is a chronic disease of carbohydrate metabolism. Alloxan can induce diabetes. Animal models with chemical induction by alloxan could provide lots of valuable information in the investigation. Alloxan is one kind of cyclic-urea derivative. It is commonly used as an effective tool to induce experimental diabetes in laboratory animals. Alloxan selectively destroys the beta-cells of pancreas. Alloxan has the structural similarities with glucose and it can be equally transported by the GLUT2 glucose transporter. The diabetogenic activity of alloxan monohydrate was used to induce diabetes in the present investigation. The present study has revealed that the induction of alloxan monohydrate produced diabetes in the experimental mice by destroying β-cells at a single dose of 180 mg/kg of body weight.

Medicinal plants may be considered as possible source of anti-diabetic agents. A large number of plant origin natural biomolecules have been tested for their antidiabetic activities using both in vivo as well as in vitro approaches. Phytochemicals are generally found to be free of side effects. Different natural compounds isolated from medicinal plants are reported to be active against diabetes. Natural biomolecules can be considered as potential alternatives for the treatment and management of diabetes. Natural antidiabetic compounds present in medicinal plants can be considered as an attractive alternative to synthetic drugs. Several research studies have shown that flavonoids, tannins, saponins, alkaloids etc. are potential source of antidiabetic principles. Aqueous extract of *Clerodendrum colebrookianum* leaves were subjected to preliminary phytochemical screening for chemical constituents using various common standard chemical laboratory tests for qualitative phytochemical screening. Natural antioxidants are gaining importance because of their various health benefits. Alkaloids are reported to have different pharmacological effects and are...
widely used as medicine. Flavonoids are reported to have significant glucose lowering effect. Several alkaloids are reported to have strong antidiabetic activity. Alkaloids have the ability to inhibit α-glucosidase. Alkaloids play an important role against hyperglycemia. Alkaloids use a complicated mechanism by changing the activities of enzymes involved in the carbohydrates metabolism.

Exact mechanism of herbal antidiabetic drugs is not clear. Some medicinal herbs may exert antidiabetic action by stimulating the activities of pancreatic β cells and thus increasing secretion of insulin. In some cases herbal extracts can reduce the activities of some selected enzymes like glucose-6-phosphatase, fructose 1, 6-bisphosphatase, etc. Some plants show antidiabetic activities by decreasing the carbohydrate absorption as well as inhibiting glucose transport. Some herbal extracts bring hypoglycemic effects by the stimulation of peripheral glucose absorption. Many medicinal plants are reported to stimulate the release of insulin into the bloodstream. Additional investigations are required for isolation and purification of bioactive antidiabetic compounds from the leaf of Clerodendrum colebrookianum. Further studies are required to identify the exact mechanism of hypoglycemic activities of the active principles in Clerodendrum colebrookianum. Different parts of Clerodendrum colebrookianum are used as a therapy for various medical conditions in the traditional ayurvedic medicinal system. β-sitosterol and sterol compounds were found to be present in the leaves of Clerodendrum colebrookianum. Clerodendrum colebrookianum leaf extract have strong antihypertensive property. Herbal preparation was reported to be used in various forms like decoction, fresh juice, raw leaf, infusion, water extract, etc. Oral administration of the aqueous leaf extract caused a significant reduction in blood glucose level in diabetic mice. Clerodendrum colebrookianum leaf extract may have some chemical compounds that exert regenerative effects on pancreatic β cells. The hypoglycemic effects of this leaf extract were probably due to enhanced secretion of insulin. The plant extract may help in the restoration of the β cells in alloxan treated diabetic mice.

CONCLUSION

In spite of large influence of modern medicine there is an increasing demand of herbal medicines. In the present study the assessment of the antidiabetic activity of aqueous extract of Clerodendrum colebrookianum leaf was investigated. The phytochemical analysis of Clerodendrum colebrookianum leaves showed the presence of carbohydrates, flavonoids, tannins, phenolic compounds, glycosides, and terpenoids. This study has shown that Clerodendrum colebrookianum leaves can be seen as a potential source of antidiabetic drug. The exact mechanism by which Clerodendrum colebrookianum leaf extracts stimulate the antidiabetic effects are still not clear. However, there is requirement of further study on this plant to find out the mechanism of its antidiabetic activity and to explore possibilities of developing a new effective antidiabetic drug.

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