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# EFFECT OF *CUSCUTA REFLEXA* STEM AND *CALOTROPIS PROCERA* LEAF EXTRACTS ON GLUCOSE TOLERANCE IN GLUCOSE-INDUCED HYPERGLYCEMIC RATS AND MICE

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## Abstract

*Cuscuta reflexa* (whole plant) and *Calotropis procera* (leaves) are used in folk medicine of Bangladesh to control blood sugar in patients suffering from diabetes mellitus. The hypoglycemic effects of methanol and chloroform extracts of whole plants of *Cuscuta reflexa*, and methanol extract of leaves of *Calotropis procera* were investigated in oral glucose tolerance tests in Long Evans rats and Swiss albino mice, respectively. Both methanol and chloroform extracts of *Cuscuta reflexa* whole plant demonstrated significant oral hypoglycemic activity in glucose-loaded rats at doses of 50, 100 and 200 mg/kg body weight. The methanol extract of leaves of *Calotropis procera*, when tested at doses of 100 and 250 mg/kg body weight did not demonstrate any oral hypoglycemic effect when tested in glucose-loaded mice.

**Key words:** *Cuscuta reflexa*, *Calotropis procera*, hypoglycemic activity, oral glucose tolerance test.

## Introduction

*Cuscuta reflexa* Roxb. (family: Cuscutaceae alternate Covolvulaceae, nature: a parasitic vine) is prevalent in various regions of Bangladesh. *Cuscuta reflexa* is known to contain a number of α-glucosidase inhibitory compounds (Anis et al., 2002). A new flavanone- reflexin (Tripathi et al., 2005), tetrahydrofuran derivatives and a coumarin (Uddin et al., 2007) have been isolated from stems of the plant. Methanol extracts of the stem reportedly demonstrated anti-steroidogenic (Gupta et al., 2003), and antibacterial activities (Pal et al., 2006).

The various pharmacological activities of whole plant or plant parts including latex of *Calotropis procera* (Ait.) R. Brown (family: Asclepiadaceae, nature: shrub, synonym: *Calotropis gigantea*) have been documented. Reported activities included analgesic and anthelmintic activities in the flowers (Pathak and Argal, 2007; Iqbal et al., 2005), antipyretic activity in the roots (Chitme et al., 2005), antinociceptive activity in latex (Soares et al., 2005), anti-diarrheal activity in aerial parts (Chitme et al., 2004), anti-fertility activity in the roots (Kamath and Rana, 2002), neuromuscular blocking activity in aerial parts (Mossa et al., 1991), and hepatoprotective activity in stems (Lodhi et al., 2009). The latex of the plant, in addition, reportedly demonstrated antioxidant and protective effect against alloxan-induced diabetes in rats (Roy et al., 2005).

Diabetes mellitus (especially Type 2 diabetes) is a disease which affects a considerable section (more than 4%) of the rural population of Bangladesh (Sayeed et al., 2003). Traditional or folk medicinal practitioners, who form the primary healthcare providers to most of the rural population, use a variety of medicinal plants or plant parts to treat this disease. Two of the widely used plants for treatment of this disease are the stems (vines) of *Cuscuta*

*reflexa* and leaves of *Calotropis procera*. It was the objective of the present study to evaluate the hypoglycemic potential of methanol and chloroform extracts of stems of *Cuscuta reflexa* and methanol extract of leaves of *Calotropis procera*.

## Materials and Methods

Preparation of extracts

Five hundred g of air-dried powdered stems (*Cuscuta reflexa*) or leaves (*Calotropis procera*) were extracted by maceration with 4 volumes (w/v) of methanol (MeOH) or chloroform until exhaustion. The extracts were freed from solvent using a vacuum rotary evaporator at 50$^{o}$C. The residues were stored at 4$^{o}$C in a refrigerator till use. Immediately prior to using, extracts were suspended thoroughly in distilled water containing 1% Tween-80. Following collection and prior to extraction, specimens of both plants were authenticated at the Bangladesh National Herbarium.

### Animals

Long Evans rats (weighing 90-120 g) and Swiss albino mice (weighing 15-25 g) were obtained from the animal house, International Cenre for Diarrheal Disease Research, Bangladesh (ICDDR, B). Rats and mice were housed in proper cages with adequate lighting and ventilation with an ambient temperature of 23-26$^{o}$C. All experimental studies were conducted following the ethical guidelines of the National Institute of Health, USA and appropriate permissions obtained from the relevant authorities of the University of Development Alternative prior to commencing the experiments.

### Studied activity

Oral glucose tolerance tests were conducted with slight modifications of method as described earlier (Joy and Kuttan, 1999). Two separate experiments were conducted, one for the testing of hypoglycemic activity present in methanol and chloroform extracts of *Cuscuta reflexa* stems, and the second for testing any hypoglycemic activity present in methanol extract of *Calotropis procera* leaves. For testing of *Cuscuta reflexa* stem methanol and chloroform extracts, 16 hr-fasted rats were divided into eight groups of five rats each. Group 1 served as control and received distilled water (containing 1% Tween-80). Group 2 received a standard drug glibenclamide at a dose of 10 mg/kg body weight (bw). Groups 3-5 received *Cuscuta reflexa* stem methanol extract at doses of 50, 100 and 200 mg/kg bw, respectively. Groups 6-8 received *Cuscuta reflexa* stem chloroform extract at doses of 50, 100 and 200 mg/kg bw, respectively. For testing of *Calotropis procera* leaf methanol extract, 16 hr-fasted mice were divided into four groups of six mice each. Group 1 received distilled water and group 2 received glibenclamide at a dose of 10 mg/kg bw. Groups 3 and 4 received methanol extract at doses of 100 and 250 mg/kg bw, respectively. All administrations were done by gavage. After 60 mins of extract administration, rats or mice of all groups were orally treated with 2 g/kg bw of glucose. Following another 120 mins after glucose loading, animals were sacrificed and serum collected. Serum glucose levels were measured immediately by glucose oxidase method (Trinder, 1969).

### Statistical analysis

The results were expressed as means ± S.E.M, and analyzed for statistical significance using Student's t-test. P values$< 0.05$ were considered significant.

## Results

### Cuscuta reflexa stem methanol and chloroform extracts

The methanol extract of *Cuscuta reflexa* stems caused a statistically significant and dose-dependent reduction in serum glucose levels in oral glucose-challenged rats, when compared to control group (Group 1). However, even at the highest dose tested (500 mg extract/kg bw), serum glucose levels were higher than that obtained with the standard drug glibenclamide (Table 1). Nevertheless, the results are promising enough to conduct further studies on isolation of active principle(s) from the extract that is responsible for the hypoglycemic effect. On, the other hand, the chloroform extract, although it demonstrated statistically significant reductions in serum glucose

levels, did not show any dose-dependency. However, even at the lowest dose administered (100 mg extract/kg bw), serum glucose levels were lower in glucose-challenged rats than obtained with a comparable dose of methanol extract. Thus both extracts showed promise in their hypoglycemic potential.

**Calotropis procera leaf methanol extract**

In contrast to the results obtained with *Cuscuta reflexa*, methanol extract of *Calotropis procera* did not demonstrate any significant lowering of serum glucose levels at both experimental doses than the control group (Group 1) in mice (Table 1). However, serum glucose levels fell from 132.9 ± 13.3 mg/dL (at a dose of 100 mg extract/kg bw) to 95.0 ± 17.1 mg/dL (at a dose of 250 mg/kg bw). It is to be noted that at the latter dose, the serum glucose levels were lesser than the control group (114.2 ± 11.7 mg/dL), even though this result was not statistically significant from the control. Any higher doses were not tried, as all experimental doses were administered based on amounts administered to human patients by traditional medicinal practitioners.

**Table 1:** Effect of oral administration of *Cuscuta reflexa* stem methanol and chloroform extracts or *Calotropis procera* leaf methanol extract on oral glucose tolerance in rats and mice[a]

| Group | Serum glucose (mg/dL) | |
|---|---|---|
| | *Cuscuta reflexa* | *Calotropis procera* |
| 1 | 87.0 ± 3.8 | 114.2 ± 11.7 |
| 2 | 37.8 ± 4.1* | 57.5 ± 7.3** |
| 3 | 74.6 ± 3.3*** | 132.9 ± 13.3 |
| 4 | 71.5 ± 4.0** | 95.0 ± 17.1 |
| 5 | 69.1 ± 3.9** | - |
| 6 | 54.1 ± 2.8* | - |
| 7 | 60.0 ± 6.8** | - |
| 8 | 58.9 ± 4.0* | - |

[a]Values are means ± S.E.M.; *P<0.001, **P<0.01, ***P<0.05 vs. Group 1.

## Discussion

*Cuscuta reflexa* contains a number of compounds like flavonoids (kaempferol, quercitin), coumarins, and flavonoid glycosides (Ghani, 2003). Earlier studies have shown that both kaempferol and quercetin could significantly improve insulin-stimulated glucose uptake in mature 3T3-L1 adipocytes. It was further reported that these two compounds act at multiple targets to ameliorate hyperglycemia (Fang et al., 2008). Aqueous and butanolic extracts of aerial parts of *Equisetum myriochaetum* containing kaempferol glucosides demonstrated hypoglycemic effects in streptozotocin-induced diabetic rats (Andrade Cetto et al., 2000). Quercetin 3-*O*-β-galacturonopyranoside and quercetin 7-*O*-β-glucopyranoside, isolated from aerial parts of *Cynanchum acutum* reportedly exhibited significant antioxidant and antidiabetic activities (Fawzy et al., 2008).

It has been reported earlier that the latex of *Calotropis procera* demonstrated antioxidant and anti-hyperglycemic effects against alloxan-induced diabetes in rats (Roy et al., 2005). Our experimental results with the methanol extract of leaves of this plant did not show any significant hypoglycemic activity. This can stem from two factors. First, leaves, instead of latex was used in the present experiment. Second, in the earlier reported results, latex was fed for days to observe the anti-hyperglycemic effect. In the present study, leaf extract was administered only 60 mins prior to glucose loading. An additional factor may be that the doses used in the present study were not large enough to observe significant hypoglycemic effects. It may be noted that a dose-dependent reduction of serum glucose was observed and at the highest dose tested, serum glucose in experimental mice was less than that in the control group.

Taken together, our results in combination with previously published reports justify the use of *Cuscuta reflexa* by traditional medicinal practitioners of Bangladesh to treat diabetes, and suggest that administration of leaves of *Calotropis procera* might also serve as an effective way to bring blood sugar in diabetic patients under control, when given in high doses.

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