Research Article

Antidiabetic activity of *Cuscuta reflexa*

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ABSTRACT

More than 100 million people worldwide are affected by a complex and heterogeneous disorder of Type 2 diabetes causing serious socio-ecologic problems. The different extracts of the bark of *Cuscuta reflexa* (Cuscutaceae – Dodder family) were tested for anti-diabetic activity, by alloxan induced diabetic rats and glucose tolerance test in normal rats. The blood glucose level of normal and experimental animals after oral administration of glucose (2 g/kg) the extract as well as standard drug treated animals showed more significant decrease in peak blood glucose level after 1 h. After 2 h, the extract treated animals tended to bring the values near normal. The extract produced significant decrease in the blood glucose level when compared with the controls in alloxan induced hyperglycemic rats in the single dose experiment at the tested dose level and are comparable with the standard drug glibenclamide. The ethanolic extract reversed the weight loss of the diabetic rats and they returned to near normal. This study gave a clear view that the ethanolic extract prevented significant elevation of glycosylated hemoglobin in vitro, with IC50 value being 11.25 µg/ml that is comparable with the reference drug α- tocopherol, since the non-enzymatic glycosylation of hemoglobin is an oxidative reaction.

Keywords: *Cuscuta reflexa*, hypoglycemic activity, alloxan, glibenclamide, α- tocopherol.

INTRODUCTION

Cuscuta reflexa is tropical and subtropical herbs found as parasite weed on host plants. The bilious disorders are treated by stems. The motion of bowl is affected by whole plant. Internally in treating protective fevers and externally in itchy skin and body pains. The leafless, twinning is used in Ayurveda to treat difficulty in urinating, jaundice, muscle pain and cough. Its decoction made with aromatics is given in flatulence, induration in liver, diarrhea.1 It contains coumarin, flavonoids, α- amarin, β-amarin, oleonolic acid, stigmasterol and β-sitisterol which were detected from roots of the plant.2,3 The major glycoside, Isohamentin 3-O-neohesperidoside was isolated along with flavonol glycoside.4 and have antiviral activity against HIV.5,6

Diabetes mellitus (especially Type 2 diabetes) affects a considerable section, Traditional or folk medicinal practitioners, use a variety of medicinal plant parts to treat this disease, The widely used plants for treatment of Diabetes are the stems (vines) of *Cuscuta reflexa*.

MATERIALS AND METHODS

Drugs and chemicals used

Bovine serum albumin (Sigma chemical St. Louis, MO, USA), thiobarbituric acid, nitro blue tetrazolium chloride (NBT), hemoglobin (Loba Chemie, Mumbai, India), trichloro acetic acid (Merck Ltd, Mumbai, India), 5,5'-dithio bis-2-nitrobenzoic acid (DTNB) were used. All the solvents were of analytical grade and purchased from local market.

Animals

Wistar albino rats of either sex, weighing 180-250 g. The selected animals were housed in acrylic cages in standard environmental conditions (25-30 °C). They were allowed free access to standard dry pellet diet and water ad libitum. All experiments were carried out as per the guidelines of the Institutional Animal Ethical Committee of Scientific and Applied Research Center, East marredpally, secunderabad, India (Approval No: SIP/CPCSEA/IAEC/2013/02).

Collection and authentication of plant material: The plant material was collected from wild sources around Babhnan, Gonda, authenticated by National Botanical Research Institute (Council of Scientific and Industrial Research)

Rana Pratap Marg Lucknow (Ref No: NBRI/CIF/413/2013) and the voucher specimens were deposited in department herbarium for future reference. The weed was shade dried at room temperature; the dried weeds were subjected to size reduction to coarse powder by using dry grinder (Philips, India) and passed through the sieve before stored in a closed vessel for further use.

Preparation of extract

The powdered plant material (400 g) was defatted with petroleum ether (60-80 °C) and then extracted with 1.5 litre of ethanol (95%) in a soxhlet apparatus. The solvent was removed under reduced pressure by rotary vacuum evaporator, which obtained a greenish-black sticky residue (yield: 11.6% w/w with respect to dried plant material). Aqueous extracts were prepared by using distilled water as solvent for the experiment. The dried extract was stored in a desiccator till further study.

Preliminary phyto-chemical screening: The weeds extracts of Cuscuta reflexa were subjected to qualitative tests for the identification of various active constituents viz. carbohydrate, glycoside, alkaloid, amino acids, flavonoids, fixed oil, tannins, gum and mucilage and phyto-sterols using standard test procedure. 7,8

Acute toxicity study: the acute toxicity studies were conducted using Wistar albino rats of either sexes taking the weed extract at various dose levels (5, 50, 300, 2000 mg/Kg), by adopting fixed dose method as per the OECD guidelines. 9 The animal were observed continuously for 2 hours and then occasionally for further 4 hours and finally overnight mortality/survival was recorded and LD_{50} was extapoated graphically.10

Screening for antidiabetic activity

The method of Joy and Kuttan was followed (11). The acclimatized animals were kept fasting for 24 h with water ad libitum and injected intraperitoneally a dose of 150 mg/kg of alloxan monohydrate in normal saline. After one hour, the animals were provided feed ad libitum. The blood glucose level was checked before and 72 h after alloxan injection. The animals were considered diabetic when the blood glucose level was raised beyond 300 mg/dl of blood. This condition was observed at the end of 72 h after alloxan injection.

Effect on oral glucose tolerance in rats

After overnight fasting, a 0-min blood sample was taken from the tip of the tail of each rat of different groups under mild ether anesthesia. Without delay a glucose solution (2 g/kg) was administered by a gavage. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration (12). All blood samples were taken for the estimation of the blood glucose. Estimation of blood glucose was carried out with the haemoglucostrips supplied by M/s Lifescan, Inc. USA with the help of a Johnson & Johnson ONE TOUCH blood glucometer.

Single dose study

The animals were segregated into five groups of six rats in each. Group I and II rats were randomly selected from normal rats that received only distilled water and the extract (300 mg/kg, p.o.) respectively. Group III to Group V animals were selected from the alloxanised rats. Group III animals served as diabetic control. Group IV animals received glibenclamide (600 µg/kg) and group V was treated with the extract (300 mg/kg) in a similar manner. Blood samples were collected from the tip of tail of each rat under mild ether anesthesia at 0 h, 1 h, 2 h and 4 h after the administration of test samples and tested for glucose concentration as above.

Multidose study

For multidose study, administration of test samples was continued for 10 days, once daily through oral route. Blood samples were collected from the tip of tail and the estimation of blood glucose was carried out as above on the 1, 3, 7 and 10 day of the drug administration. Body weights of all the animals were recorded just prior to and on the 10th day of the study to determine the change in the body weight, if any.

Statistical analysis

Statistical significance was determined by one way analysis of variance (ANOVA) followed by Dunnet's t-test. $P<0.05$ indicates significant difference between group means.

RESULTS AND DISCUSSION

Table 1 shows the blood glucose level of normal and experimental animals after oral administration of glucose (2 g/kg). Extract as well as standard drug treated animals showed more significant decrease in peak blood glucose level after 1 h. After 2 h, the extract treated animals tended to bring the values near normal.

The results of Table 2 reveals that the extract produced significant decrease in the blood glucose level when compared with the controls in alloxan induced hyperglycemic rats in the single dose experiment at the tested dose level and are comparable with the standard drug glibenclamide.

In the multi dose study (Table 3), the test extract constantly maintained significant reduction of the glucose level in diabetic rats throughout the experimental period suggesting the anti-hyperglycemic property of the extract. Diabetes mellitus causes failure to use of glucose for energy that leads to increased utilization and decreased storage of protein responsible for reduction of body weight essentially by depletion of the body proteins (12). In the present study, it was observed that the ethanolic extract reversed the weight loss of the diabetic rats and they returned to near normal.

During diabetes the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. The rate of glycosylation is directly proportional to concentration of blood glucose and with improvement of glycemic control glycosylated hemoglobin also decreases (13). Hence the estimation of glycosylation of hemoglobin is a well-established parameter useful in the management and prognosis of the disease (14). Our study gave a clear view that the ethanolic extract prevented significant elevation of glycosylated hemoglobin in vitro, with IC50 value being 11.25 µg/ml that is comparable with the reference drug α- tocopherol (Table 4). Further, since the non-enzymatic glycosylation of hemoglobin is an oxidative reaction (15).

Table 1: Effect of ethanolic extract of Cuscuta aerial parts (300 mg/kg, p.o.) on oral glucose tolerance test (OGTT) in normal and alloxan induced diabetic rats

S. No.	Groups	Treatment	Groups Treatment Blood sugar level (mg/dl)				
			Fasting	30 min	60 min	90 min	120 min
1	I	Normal	76.00 ±0.75	147.81 ±2.14	175.83 ± 2.11	123.19 ±2.73	83.12±1.89
2	II	Normal + Extract	73.82±0.80 ns	147.60 ±1.67ns	182.18 ±2.37ns	126.11 ±3.76ns	83.67 ±3.13ns
3	III	Diabetic control (Alloxan only)	251.34 ±4.10*	324.36 ±4.14*	373.14 ±5.13*	317.36 ±3.27*	316.85 ±2.37*
4	IV	Diabetic + Exrtact	76.53 ±1.56*	145.86 ±2.81*	175.37 ±3.51*	126.50 ±2.40*	85.52 ±1.41*
5	V	Diabetic + Glibenclamide	76.59 ±3.02*	161.55 ±3.35*	183.37 ±2.55*	125.82 ±2.41*	89.50 ±1.53*

Values are mean ± SEM for n=6; *P < 0.05 = significant; NS = Not significant;
Group II and III are compared with group I while Group IV and V are compared with group III.

Table 2: Effect of single dose treatment of ethanolic extract of Cuscuta aerial parts (300 mg/kg, p.o.) on blood glucose level in normal and alloxan induced diabetic rats

S. No.	Groups	Treatment	Blood glucose level (mg/dl)			
			Basal Value	1 h	2 h	4h
1	I	Normal	76.42 ±0.69	75.14±0.63	75.81±0.86	76.18±0.77
2	II	Normal + Extract	76.19±0.86ns	75.81±0.69ns	75.14±0.64ns	74.39±0.74ns
3	III	Diabetic control (Alloxan only)	348.69±2.84*	350.16±2.61*	348.84±2.74*	351.19±2.68*
4	IV	Diabetic + Glibenclamide	344.16±5.11ns	317.52±5.34*	297.80±3.83*	283.82±3.55*
5	V	Diabetic + Exrtact	337.56±3.17ns	2869.34±4.80*	265.84±3.36*	244.84±3.22*

Values are mean ± SEM for n=6; *P < 0.05 = significant; NS = Not significant;
Group II and III are compared with group I while Group IV and V are compared with group III.

Table 3: Effect of multiple dose treatment of ethanolic extract of Cuscuta aerial parts (300 mg/kg, p.o., once daily) on blood glucose level and change in body weight after 15 days in normal and alloxan induced diabetic rats

S. No.	Groups	Treatment	Blood glucose level (mg/dl)					Change in body weight (g)
			Basal Value	Day 1	Day 3	Day 7	Day 10	
1	I	Normal	76.42 ±0.69	76.19 ±0.46	75.86 ±0.40	76.45 ±0.67	76.47 ±0.53	(+)10.89 ±1.37
2	II	Normal + Extract	76.19 ±0.86ns	75.00 ±0.76ns	75.30 ±0.67ns	74.24 ±0.58ns	73.26 ±0.76*	(+)10.00 ±0.89ns
3	III	Diabetic control (Alloxan only)	348.69 ±2.84*	353.81 ±2.61*	353.86 ±3.18*	352.20 ±3.65*	350.16 ±3.53*	(-)7.83 ±0.87*
4	IV	Diabetic + Glibenclamide	343.19±5. 21ns	261.34±4.0 9*	235.82±3. 56*	218.32 ±4.17*	207.31 ±3.55*	(+)9.83 ±0.98*
5	V	Diabetic + Exrtact	337.56±3. 17ns	219.67±3.5 8*	207.31±3. 86*	203.30 ±2.58*	187.49 ±1.79*	(+)9.15 ±1.05*

Values are mean ± SEM for n=6; *P < 0.05 = significant; NS = Not significant;
Group II and III are compared with group I while Group IV and V are compared with group III.

Table 4: Effect of ethanolic extract of Cuscuta on percent inhibition of hemoglobin glycosylation in vitro

S. No.	Groups	Treatment	Blood glucose level (mg/dl)			
			Basal Value	1 h	2 h	4h
1	I	Normal	76.33 ±0.71	76.17±0.65	75.83±0.95	76.17±0.79
2	II	Normal + Extract	76.17±0.87ns	75.83±0.70ns	75.19±0.76ns	73.34±0.76ns
3	III	Diabetic control (Alloxan only)	349.67±2.95*	351.19±2.41*	348.84±2.66*	353.19±2.58*
4	IV	Diabetic + Glibenclamide	343.17±5.12ns	318.50±5.36*	296.82±3.91*	285.81±3.55*
5	V	Diabetic + Exrtact	338.50±3.19ns	286.34±4.89*	265.82±3.36*	246.83±3.20*

Values are Mean ± S.D. for n=3; r = regression co-efficient.

Cuscuta reflexa contains various flavonoids (kaempferol, quercetin), coumarins and flavonoid glycosides 16 kaempferol and quercetin significantly improve insulin-stimulated glucose uptake in mature 3T3-L1 adipocytes. (Fang et al., 2008).

By literature survey and results obtained justify that *Cuscuta reflexa* by traditional medicinal practitioners to treat diabetes and serve as an effective way to control blood sugar.17

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