EVALUATION OF ANTIDIABETIC POTENTIAL OF *OUGEINIA OOJEINENSIS* LEAVES IN STREPTOZOTOCIN-INDUCED-DIABETIC RATS

Jagdish Singh[1*], Ram Kumar Sahu[2], Deo Nandan Prasad[3], Rajendra Jangde[4], Rajesh Gupta[5]

1. Department of Technical Education, Govt. of Punjab, Chandigarh, India.
2. Department of Pharmacognosy, Oriental College of Pharmacy, Bhopal-462021, India.
3. Shivalik College of Pharmacy, Nangal, Dist. Ropar (Punjab), India.
4. University Institute of Pharmacy, Pt. Ravishankar Shukla University, Raipur-492010, India.
5. SRI SAI College of Pharmacy, Badhani, Pathankot (Punjab) -145001, India.

For e. mail correspondence: badhanjs@gmail.com

Summary

The aim of this study was to evaluate the antidiabetic potential of the methanol and aqueous extracts of leaves of *Ougeinia oojeinensis*. This was tested in normal and Streptozotocin (STZ)-induced diabetic rats, using oral administration of methanol and an aqueous extract (100 and 200 mg/kg body weight) of *Ougeinia oojeinensis* leaves. After the oral administration of methanol and aqueous extracts at doses of 100 and 200 mg/kg body weight, blood glucose levels were monitored at specific intervals and it was found that they were significant lowered. Glibenclamide was used as a standard drug at a dose of 0.25 mg/kg. The experimental data revealed that both extracts has significant antihyperglycemic activity in Streptozotocin-induced rats compared to the standard drug.

Keywords *Ougeinia oojeinensis*, antidiabetic, glibenclamide, lipid profile, streptozotocin

Introduction

Every year the number of diabetic patients is growing alarmingly all over the World. Diabetes is a chronic disease characterized by dearrangement in carbohydrate, fat, protein metabolism. Most of the hypoglycemic agents used in allopathic medicines are reported to have side effects in the long run. Therefore, there is a need to search for effective and safe drugs for diabetes.

Ougeinia oojeinensis (Roxb.) Hochr (Fabaceae) known in Hindi as Tinsa and in Sanskrit as Ratha is a deciduous trees, found in the outer Himalayas and sub-Himalayan tracts from Jammu to Bhutan up to an altitude of 1500m and extending through the whole of northern and central India into the greater part of Deccan peninsula[1,2]. The extract of the whole plant *O. oojeinensis* were scientifically evaluated for anti-inflammatory and analgesic activities in previous studies. The 50% of ethanolic extract of stem bark has

been reported exhibit antispasmodic action[3]. The hepatoprotective, in-vivo antioxidant, in-vitro anti-inflammatory and wound healing activity of *O. oojeinensis* bark have been reported[4-7]. Phytochemical investigated on *O. oojeinensis* have reported the presence of lupeol, hydroxlupeol, betulin and isoflavanones such as dalbergioidin, homoferreirin and ougenin[8-10]. Yet there is paucity of information regarding the activity of *O. oojeinensis* in diabetic protection. The main objectives of this study are to assess the antidiabetic potential of methanol and aqueous extracts of the leaves of *O. oojeinensis* in control of blood glucose levels and effectiveness on various biochemical parameters, namely, total cholesterol, triglycerides (TGL), high density lipoprotein (HDL), low density lipoprotein, (LDL), and very low density lipoprotein, (VLDL).

Material and Methods

Plant materials: The leaves of *Ougeinia oojeinensis* were collected from Betul district, Madhya Pradesh, India, during the months of January and February 2007. The species was identified by the local people during the time of collection and later on authentication was made by Dr. P. Jayaraman, Botanist, Plant Anatomy Research Centre (PARC), Chennai, India. The leaves were shade dried, reduced to coarse powder and stored in airtight container till further use.

Preparation of extract: 1 Kilogram of powdered drug was packed in soxhlet apparatus and extracted with petroleum ether (60-80ºC) to defat the drug. Defatted powdered drug was then extracted with methanol. The methanol extract was separated and the marc was further extracted with distilled water. The solvents were removed by distillation and the last traces of solvent being removed under reduced pressure.

Experimental animals: Male wistar albino rats having weight 180-230gm were kept in quarantine for 10 days under standard husbandry conditions (27.3^{o}C, Relative humidity 65 ±10%) for 12 hrs in dark and light cycle respectively and were given standard food and water *ad. libitum*. The project proposal was approved by the Institutional Animal Ethical Committee (1349/ac/10/CPCSEA).

Acute oral toxicity study: Acute oral toxicity was performed by following OECD guideline – 420 fixed dose procedure for methanol and aqueous extract and it was found that dose increasing up to 2000 mg/kg body wt. shown no toxicity or mortality in experimental rats. The LD_{50} of the methanol and aqueous extract as per OECD guidelines – 420 is greater then 2000 mg/kg[11,12].

Oral glucose tolerance test (OGTT): The oral glucose tolerance test was performed in overnight fasted (18 hours) normal rats. The rats were divided into three groups (n = 6) and were administered drinking water, *O. oojeinensis* methanol extract and aqueous extract at doses of 100 and 200 mg/kg body weight, respectively. Glucose (2 g/kg) was fed 30 minutes prior to the administration of the extracts. Blood was withdrawn from the retro-orbital sinus after 30, 60, and 120 minutes of extract administration, and the plasma obtained after centrifugation at 3000 rpm was estimated for fasting plasma glucose levels using a glucose oxidase–peroxidase glucose estimation kit[13].

Induction of non-insulin dependent diabetes mellitus (NIDDM): Non-insulin dependent diabetes mellitus was induced in overnight fasted adult Wistar strain albino male rats weighing 170 – 220 g by a single intraperitoneal injection of 60 mg/kg Streptozotocin, 15 minutes after i.p. administration of 120 mg/kg of nicotinamide. Streptozotocin (STZ) was dissolved in a citrate buffer (pH 4.5) and nicotinamide was dissolved in normal saline. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hours and then on day 7, after injection. The threshold value of fasting plasma glucose to diagnose diabetes was taken as > 126 mg/dl. Only those rats that were found to have permanent NIDDM were used for the study[14,15].

Experimental design: The animals were segregated into seven groups of six rats each. The extract was administered for 12 days. Group I served as normal control rats administered drinking water daily for 12 days; Group II diabetic control rats administered drinking water daily for 12 days; Group III diabetic rats administered methanol extract (100 mg/kg); Group IV diabetic rats administered methanol extract (200 mg/kg); Group V diabetic rats administered aqueous extract (100 mg/kg); Group VI diabetic rats administered aqueous extract (200 mg/kg); Group VII diabetic rats administered standard drug glibenclamide (0.25 mg/kg) for 12 days.

The fasting glucose levels were determined on days 1, 5, and 12 of extract administration. During the experimental period, the rats were weighed daily and the mean change in body weight was calculated.

Estimation of biochemical parameters: The biochemical parameters were determined on day 12 after the animals were sacrificed by cervical dislocation. Total cholesterol, triglycerides (TGL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL), were determined by the glucose oxidase method, using an auto-analyzer[16,17].

Statistical analysis: Results of estimation of biochemical and functional parameters have been reported as mean value ± SEM. The variation in a set of data has been estimated by performing one way analysis of variance (ANOVA). Individual comparisons of group mean values were done using Dunnet's test (Sigma stat 3.5). P values <0.05 were considered statistically significant.

Results

The result of acute toxicity study of methanol and aqueous extracts of *O. oojeinensis* on laboratory animals showed that the animals were safe up to a maximum dose of 2000 mg/kg body weight. The effects of methanol and aqueous extracts of *O. oojeinensis*, on the plasma glucose level are shown in table 1. Both the extracts treated rats, significant reduction in plasma glucose level, while in normal control rats, plasma glucose level was increased.

Table 1: Effect of methanol and aqueous extracts of *O. oojeinensis* on oral glucose tolerance test

Group	Plasma glucose concentration (mg/dl)		
	30 min	60 min	90 min
Normal control	79.42±2.15	115.17±2.28	108.31±1.84
Normal + MEOO (100 mg/kg)	72.35±1.23	96.18±3.41	87.62±2.39*
Normal + MEOO (200 mg/kg)	63.12±3.51*	83.32±1.65*	68.54±2.17*
Normal + AEOO (100 mg/kg)	75.84±2.64	94.29±1.71	88.14±3.24*
Normal + AEOO (200 mg/kg)	68.11±1.72	88.51±2.16*	73.19±1.91*

Methanol extract of *O. oojeinensis* (MEOO), Aqueous extract of *O. oojeinensis* (AEOO), Values are expressed as mean ± SEM (Number of animals, n=6); * Significantly different from the normal control at P<0.05

Induction of diabetes in the experimental rats was confirmed by the presence of a high fasting plasma glucose level. The effect of both extract of *O. oojeinensis*, on fasting plasma glucose level of normal and streptozotocin induced are shown in table 2. The difference between the experimental and control rats in lowering the fasting plasma glucose levels was statistically significant (*P<0.05*) in diabetic rats. From table 2 it also revealed that decreased in glucose level of both extracts are less compared to standard drug.

Table 2: Effect of methanol and aqueous extracts of *O. oojeinensis*on fasting plasma glucose level in rats

Group	Fasting plasma glucose concentration (mg/dl)		
	Day 1	Day 5	Day 12
Normal control	84.36±1.62	88.27±2.13	79.47±1.56
Diabetic control (Streptozotocin)	215.32±1.71[a]	231.14±2.42[a]	236.6±3.15[a]
Diabetic + MEOO (100 mg/kg)	212.18±2.34	189.43±3.62	120.33±1.84
Diabetic + MEOO (200 mg/kg)	218.51±1.99*	156.13±2.61*	91.58±2.74*
Diabetic + AEOO (100 mg/kg)	217.62±2.82	197.62±2.94	138.80±3.24*
Diabetic + AEOO (200 mg/kg)	215.36±3.14*	164.37±3.51*	101.42±2.28*
Diabetic + Standard Glibenclamide (0.25 mg/kg)	220.12±2.48*	142.28±2.18*	93.15±3.81*

Methanol extract of *O. oojeinensis* (MEOO), Aqueous extract of *O. oojeinensis* (AEOO), Values are expressed as mean ± SEM (Number of animals, n=6); significantly different at [a]P<0.05 when compared with normal control group, *P<0.05 when compared with diabetic control group.

The effect of the both extracts on diabetes induced hyperlipidemia was also studied. It was observed that due to diabetes there was an increase in the total cholesterol levels as well as triglyceride levels. The HDL levels were reduced in the diabetic animals and the VLDL and LDL levels were increased significantly (Table 3).

Both the doses extracts showed a significant decrease in the total cholesterol levels and triglyceride levels. In particular, the methanol extract of 200 mg/kg body weight showed a much relevant action. It also increased the HDL level and was successful in suppressing the VLDL and LDL levels as compared to the standard drug (Table 3).

Table 3: Determination of biochemical parameters after treatment with ethanol and aqueous extracts of *O. oojeinensis* and Glibenclamide

Group	Total cholesterol (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
Normal control	83.24±3.16	43.51±2.81	79.18±2.91	25.41±2.67	36.8±3.47
Diabetic control (Streptozotocin)	184.6±2.64[a]	21.32±3.41	162.5±2.38[a]	54.28±2.41[a]	151.3±3.64[a]
Diabetic + MEOO (100 mg/kg)	110.3±4.36*	23.42±3.74	121.9±3.26	41.76±2.91	91.23±2.38*
Diabetic + MEOO (200 mg/kg)	86.34±3.51*	41.62±2.17	88.5±3.67*	21.92±3.72	47.8±2.75*
Diabetic + AEOO (100 mg/kg)	119.8±2.92*	22.57±2.33	138.53±2.19	47.61±3.17	101.2±3.11*
Diabetic + AEOO (200 mg/kg)	81.74±3.85*	38.30±2.65	101.43±4.18*	29.83±4.26	59.61±3.54*
Diabetic + Standard Glibenclamide (0.25 mg/kg)	77.32±4.29*	44.5±2.53*	77.22±4.36*	26.31±2.78	34.8±2.92*

Methanol extract of *O. oojeinensis* (MEOO), Aqueous extract of *O. oojeinensis* (AEOO), Values are expressed as mean ± SEM (Number of animals, n=6); *Significantly different from the normal control at $P<0.05$

Discussion

The result of acute toxicity study of methanol and aqueous extracts of *O. oojeinensis* on laboratory animals showed that the animals were safe up to a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed as per OECD guidelines both the extracts fall under class four values LD_{50} value being 2000 mg/kg. The pharmacological evaluations were therefore carried out at doses of 100 and 200-mg/kg body weight.

The fundamental mechanism underlying hyperglycemia in diabetes mellitus involves over-production and decreased utilization of glucose by the tissues. In our study, the difference observed between the initial and final fasting plasma glucose levels of different groups under investigation revealed a significant elevation in blood glucose in diabetic control group as compared with normal animals at the end of the 12-day experimental period. When methanol and aqueous extracts of *O. oojeinensis* were administered to glucose loaded normal rats fasted for 18 h, decrease in plasma glucose level was observed after 30 min. Both the extracts reduced plasma glucose level to normal at 90 min. During study it was found that both extracts control significantly the blood glucose level on streptozotocin induced diabetic rats. The methanol and aqueous extracts induced a significant reduction on blood glucose level in STZ-induced-diabetic rats as compared to the diabetic control group. But methanol extract showed more significant antidiabetic activity as compared to aqueous extract. The possible mechanism by which *O. oojeinensis* brings about its hypoglycemic action in diabetic rat may be by potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form.

The marked increase in serum triglycerides and cholesterol observed in untreated diabetic rats. Under normal circumstances insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia. The significant control of the levels of serum lipids in the both extracts treated diabetic rats may be directly attributed to improvements in insulin levels upon *O. oojeinensis* therapy. Elevation of plasma lipid concentration in diabetes is well documented. In insulin deficient diabetics, the plasma free fatty acid concentration is elevated as a result of increased free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification–triglyceride lipolysis cycle is displaced in favour of lipolysis. Induction of diabetes with STZ is associated with the characteristic loss of body weight which is due to increased muscle wasting in diabetes. Diabetic rats treated with the extracts showed an increase in body weight as compared to the diabetic control which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis[18].

Abnormalities in lipoproteins are very common in both NIDDM and IDDM. Although lipoprotein alterations appear to be an intrinsic part of these disorders, such alterations are also induced by diabetes associated complications such as obesity and renal disease. The total cholesterol, triglyceride levels, VLDL and LDL were observed to be elevated in diabetics but reduced by both extracts as well as glibenclamide showing their beneficial effects. In the present study, HDL levels remained unchanged in diabetics compared to the other groups. These results suggest the beneficial effects of the natural extract in improving the imbalance in lipoprotein metabolism are also comparable to those of glibenclamide.

The present study has indicated the fact that the plant *O. oojeinensis*, has antidiabetic constituents and production of a safe antidiabetic drug is very much possible from the leaves part.

References

1. Kirtikar KR, Basu BD. Indian medicinal plants. Vol. I. Dehradun, India, Oriental Longman Ltd, 1998: 756.
2. Anonymous. The Wealth of India, Raw Material. Vol. VII. C.S.I.R. New Delhi, 1997: 193 –197.
3. Khare CP. Encyclopedia of Indian Medicinal Plants. Springer, 2004: 343.
4. Sahu RK, Roy A. Hepatoprotective activity of ethanolic extract of bark of *Ougeinia oojeinensis* (Roxb.) Hochr in CCl_4 treated male rats. Pharmacologyonline 2009; 2(May-August): 1-5.
5. Sahu RK, Sharma U, Roy A, Dewangan D, Namdeo KP. Antioxidant activity of ethanolic extract of bark of *Ougeinia oojeinensis* (Roxb.) Hochr on CCl_4 induced hepatotoxicity in rats. Bioscience, Biotechnology Research Asia 2008; 5(2): 783-787.
6. Sahu RK, Dewangan D, Roy A, Namdeo KP. Anti-inflammatory action of *Ougeinia oojeinensis* (Roxb.) Hochr. bark by HRBC membrane stabilization. Research Journal of Pharmacy and Technology 2008; 1(01): 57-58.
7. Sahu RK, Kulshrestha V, Kothiya S, Yadav P, Roy A. Healing potential of gel containing extract of *Ougeinia oojeinensis* on excision wounds in wistar rats. Journal of Global Pharma Technology. 2009; 2: 103-106.
8. Mukherjee DK, Barua AK, Bose PK. Chemical Investigation of Ougeinia dalbergioides Benth. Science and Culture 1963; 29: 151–152.
9. Ghosh AC, Dutta NL. Chemical Investigation of Ougeinia dalbergioides Benth. Journal of Indian Chemical Society 1965; 42(12): 831– 835.
10. Balakrishna S, Ramanathan JD, Seshadri TR, FRS, Venkataramani B. Special Chemical Components of the Heartwood of Ougeinia dalbergioides Benth. Proc. Royal Society London 1962; 268A: 1
11. Ecobichon DJ. Fixed dose procedure guidline 420. The basis of toxicity testing. 2nd ed. New York, CRC Press; 1997.
12. Ghosh MN. In: Schild HO editor. Fundamentals of Experimental Pharmacology. Calcutta: Scientific Book Agency, 1984.
13. Shirwaikar A, Rajendran K, Punitha IS. Antidiabetic activity of alcoholic stem extract of *Coscinium fenestratum* in streptozotocin-nicotinamide induced type 2 diabetic rats. J Ethnopharmacol 2005; 97: 369-374.
14. Angel I, Burcelin R, Prouteau M, Girard J, Langer SZ. Normalization of insulin secretion by selective alpha 2-adrenoceptor antagonist restores GLUT-4 glucose transporter expression in adipose tissue of type II diabetic rats. Endocrinology 1996; 137: 2022-2027.
15. Masiello P, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, *et al.* Experimental NIDDM: Development of a new model in adult rats administered streptozotocin and nicotinamide. Diabetes 1998; 47: 224-229.
16. Nyarko AK, Sittie AA, Addy ME. The basis for the antihyperglycaemic activity of *Indigofera arrecta* in the rat. Phytother Res 1993; 7: 1-4.
17. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 1972; 97: 142-145.
18. Sharma U, Sahu RK, Roy A, Golwala DK. *In vivo* antidiabetic and antioxidant potential of *Stephania hernandifolia* in streptozotocin-induced-diabetic rats. Journal of Young Pharmacists 2010; 2(3): 255-260.