ORIGINAL ARTICLE

Anti Diabetic and Hypolipidemic Activity of Bark of Ethanolic Extract of *Ougeinia Oojeinensis (ROXB.)*

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SUMMARY

The hypoglycemic and hypolipidemic effect of Ethanolic extract of *Ougeinia oojeinensis* (200mg/kg) bark was evaluated with measurements including, Body weight, blood glucose level, urine glucose and biochemical parameters. The ethanolic extracts of the powdered bark was tested for its efficacy in alloxan-induced diabetic rats. Animals were induced for diabetes with Alloxan (150 mg/kg of body weight- i.p.) and treated orally with Ethanolic extract of *Ougeinia oojeinensis*. The extracts were also evaluated for acute oral toxicity studies and its effect on different biochemical parameters. The extracts showed significant (p<0.01) antihyperglycemic and hypolipidemic activity as compared to diabetic control. The extract shows beneficial effects on blood glucose and urine glucose level. It also reduces the elevated biochemical parameters such as triglycerides (TGL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), Total Cholesterol (TC) and increased the reduced level of high density lipoprotein (HDL) and body weight, which might be due to presence of steroids, tannins, alkaloids and triterpenoids present in that extract. Thus ethanolic extract could serve as good oral hypoglycemic agents and seems to be promising for the development of phytomedicines for diabetes mellitus.

KEY WORDS:
Ougeinia oojeinensis, Anti diabetic activity, Plasma glucose level Alloxan induced diabetic rats

INTRODUCTION

According to WHO, the prevalence of diabetes is likely to increase by 35% by the year 2025. Currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 79.4 million by 2025, making it the country with the highest number of diabetics in the world [1,2]. Diabetes is a serious metabolic, disorder with micro and macrovascular complication that results in significant morbidity and mortality[3]. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries [4]. Modern medicines like biguanides, sulphonylureas and thiozolidinediones are available for the treatment of diabetes. But they also have undesired effects associated with their uses[5]. Alternative medicines particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability [6]. Medicinal plants and their products have been used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity [7, 8]. Medicinal Plants are a richsource of natural products. Medicinal plants and their products have been widely used for treatment of diabetic populace all around the world with less known scientific basis oftheir functioning [9,10]. Hence, natural products from medicinal plants need to be investigated by scientific methods for their anti-diabetic activity. The plant Ougeinia oojeinensis (Roxb.) belonging to family Fabaceae and commonly known as Atimukta in Sanskrit, Tinis in Bengali, Tinisha in Hindi, Narivengayam in Tamil, Bhinoharmo in Gujarati, Karimutale in Kannada, Malavenna in Malayalum, Tiwas in Marathi, Sandan pipli in Nepali and Tellamotuka in Telugu[11]. The ayurvedic formulation Tinisha clinically used for burning syndrome, skin disease, urinary disorder, obesity, anti inflammatory [12], anti spasmodic, and anti hypertensive activity. As per the literature review the plant having hypoglycemic and hypolipidemic property [11], which is not scientifically documented. In the present study, we reported hypoglycemic and hypolipidemic potentials of *Ougeinia oojeinensis* (Roxb.) in diabetic rat model.

MATERIALS AND METHODS

Taxonomical identification:

The bark of *Ougeinia oogeinensis* was collected and identified.

Preparation of extracts

The collected bark was shade dried completely. The dried bark was then coarsely Powdered and was sieved (sieve # 60) to get uniform coarse powdered. The extract was prepared by continuous hot extraction using ethanol as a solvent. Extracts obtained was concentrated, dried kept in a desiccator for further use. The yield was found to be 14.50%w/w.

Preliminary phytochemical screening

The Ethanolic extract of *Ougeinia oojeinensis* was screened for the presence of various phytoconstituents like steroids, alkaloids, flavonoids, saponin, mucilage, tannin and phenolic compounds [13].

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Experimental Animals
All the experiments were carried out using male, Swiss Albino mice (25-30 g) and Wister rats (150-200 g) procured from the animal house, IRT Perundurai medical college, Erode, Tamilnadu, India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24 ± 2°C and relative humidity of 30–70%. A 12:12 light: day cycle was followed. All animals were allowed free access to water and fed with standard commercial rat chaw pellets (M/s. Hindustan Lever Ltd, Mumbai). The animal experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (688/2/C-CPCSEA)

Acute oral toxicity studies
The acute toxicity study was carried out as per OECD 425 Guidelines. Mortality in each group within 24 h was recorded. The animals were observed for a further 14 days for any signs for delayed toxicity. The Ethanolic extract of *Ougeinia oojeinensis* had good margin of safety and did not shown any lethal effects on the animals up to the doses of 2000mg/kg. Hence the LD50 of *Ougeinia oojeinensis* was considered as 2000mg/kg. Studies were carried out with 1/10 of the LD50 as therapeutic dose (200mg/kg).

Anti-diabetic study [14-20]
In the experiment a total of 24 overnight fasted rats were used. The 18 rats were rendered diabetic by the intraperitoneal injection of alloxan (150 mg/kg). 48hrs after alloxan injection, the animals which did not developed hyperglycemia i.e. glucose level > 200mg/dl, were rejected and replaced with new animals. Immediately after confirmation of diabetes, rats were classified into four groups of six rats each. Evaluation of antidiabetic effect of test extracts was done by taking six rats in each groups as: Group I served as normal control (saline); Group II served as diabetic control (alloxan induced);

Group III received ethanolic extract (200mg/kg) and Group IV served as reference standards (Glibenclamide, 3 mg/kg). Treatment was continued for 14 consecutive days, with once a day dose. Before the treatment (0 day) and at the end of 5th, 10th and 14th day, blood samples were collected from the retro orbital vein of each rat under mild ether anesthesia and serum separated by centrifugation of blood at 4000 rpm for 10mins. Samples were subjected to glucose measurement using a semi auto analyzer. Urine glucose level was estimated on 0 day and 14th day by Benedict's test. Estimation of biochemical parameters were done with 1ml of blood withdrawn on 14th day, from all four groups of rats (normal, diabetic control, extracts and standard treated) under mild anesthesia, where serum was separated by centrifugation of sample at 4000 rpm for 10min and stored in a refrigerator until analyzed. And the serum was subjected for the estimation of TGL, HDL, LDL, VLDL and TC by a semi auto analyzer.

The percentage reduction /increased glucose concentration and biochemical parameters was calculated by the following formula

$$\frac{\text{Diabetic control-test}}{\text{Diabetic control}} \times 100$$

STATISTICAL ANALYSIS
One-way analysis of variance (Anova) followed by Dunnett's method of multiple comparisons was employed using Graphpad Instat 3.0 software. A probability value of $p<0.01$ was considered to be statistically significant.

RESULTS

Preliminary phytochemical screening
The preliminary phytochemical analysis of Ethanolic Extract of *Ougenina Oojeinensis* shows presence of flavonoids, saponins, alkaloids, Mucilage, tannins and phenolic compounds.

Acute toxicity
Acute oral toxicity studies following OECD guidelines-425, up and down procedure, showed that ethanolic extracts upto 2000mg/kg are non-toxic and safe.

Blood glucose level
The standard (glibenclamide 3mg/kg) and ethanolic extract (200mg/kg) treated groups, the peak values of blood sugar significantly decreased to 128 mg/dl (60.89%) and 84.17 mg/dl (74.28%) simultaneously on the 14th day (Figure 1&2). Thus, the ethanolic extract was found to be more significant ($p<0.01$) as standard drug in lowering blood glucose level compare to diabetic control.

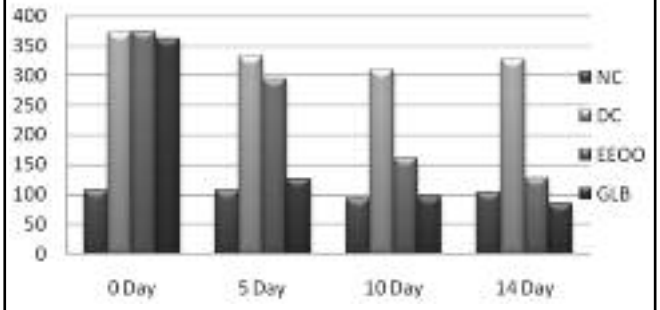
Body weight and urine glucose
The Figure 3 shows the body weight of the normal and treated groups significantly differ from diabetic control on 14th day. In the same way urine glucose level of normal and treated groups also significantly differ from diabetic control on 14th day shown in Table I.

Biochemical parameters
Figure 4 shows ethanolic extracts has significantly reversed the diabetes-induced hyperlipidemia Compared to diabetic control. A significant percentage reduction of total cholesterol level (59.91%), LDL (64.85%), TGL (22.58%) and VLDL (22.36%) in ethanolic extract treated was comparative to standard drug treated groups, total cholesterol (66.55%), LDL (79.50%), TGL (26.68%) and VLDL (26.57%) and reached normal value. However, the HDL level increased with treatment of extract and GLB group respectively. Figure 5 shows increased HDL level by extract and GLB.

Table I: Effect of oral administration of the ethanolic extract of Ougenina Oojeinensis (Roxb.) on urine sugar in severe diabetic rats

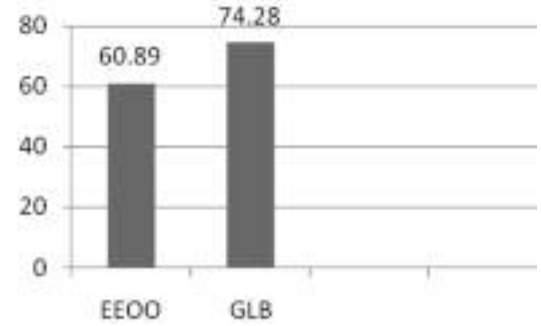
Group	Urine sugar	
	O day	14 day
Normal control	-	-
Diabetic control	+++	+++
Ethanolic extract	+++	+
Glibenclamide	+++	+

(Trace = +, significantly = +++, Nil = -)



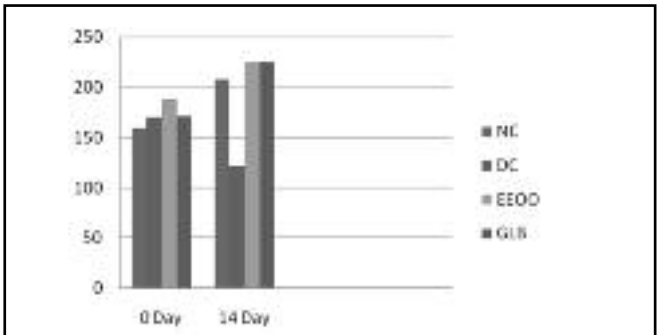
Significant (P <0.01) compared with treated diabetic groups Vs Diabetic control.

Fig. 1: Data for Blood glucose level of Ethanolic Extract of Ougenina Oojeinensis (Roxb.)



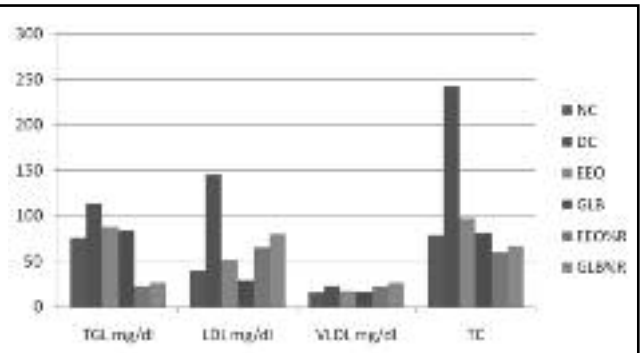
Significant (P <0.01) compared with treated diabetic groups Vs Diabetic control.

Fig. 2: Percentage reduction of blood glucose level by Ougenina Oojeinensis (Roxb.) on 14th day



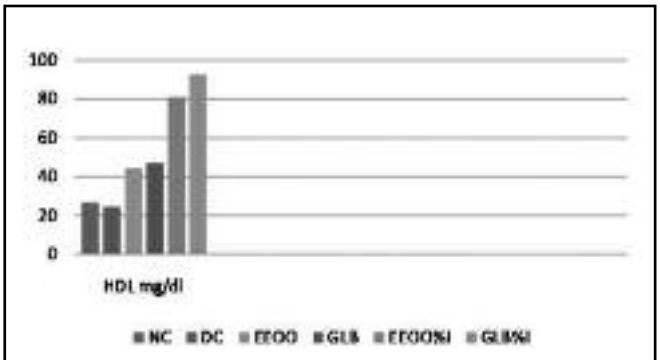
Significant (P <0.01) compared with treated diabetic groups Vs Diabetic control.

Fig. 3: Data for Body Weight of Ethanolic Extract Of *Ougenina Oojeinensis (Roxb.)*



Significant (P <0.01) compared with treated diabetic groups Vs Diabetic control.

Fig. 4: Effect of ethanolic extracts of bark of Ougenina Oojeinensis (Roxb.) on Biochemical parameters



Significant (P <0.01) compared with treated diabetic groups Vs Diabetic control.

Fig. 5: Effect of ethanolic extracts of bark of *Ougenina Oojeinensis (Roxb.)* on Biochemical parameters

DISCUSSION

The present study was undertaken to investigate the anti-diabetic and hypolipidemic effects of *Ougenina Oojeinensis* (Roxb.) in a diabetic rat model. Glibenclamide was used as a standard drug. It is well established that glibenclamide (a long lasting sulfonylurea) acts mainly by stimulating insulin secretion. Alloxan is widely used to induce diabetes in experimental animals. The mechanism of action in β-cells of the pancreas has been intensively investigated and now is quite well understood. The cytotoxic action of alloxan is mediated by reactive oxygen species. Alloxan and the product of its reduction, dialuric acid, establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter highly reactive hydroxyl radicals are formed by Fenton reaction. The actions of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration cause rapid destruction of β-cells and thus

increase the blood sugar [21, 22]. The Earlier studies reported, that saponin and combination of saponins showed significant antidiabetic activity [23, 24]. There are reports that some plants contains mucilages and minerals like calcium, zinc, magnesium, manganese and copper had remarkable hypoglycaemic activity decreasing the blood glucose levels in diabetic rats within 15days [25, 26]. In the present study indicated that daily administration of *Ougenina Oojeinensis* (200 mg/kg) up to 14 days showed anti-diabetic and hypolipidemic effects in diabetic rats. In a diabetic model rat, an increase in blood and urine sugar levels in diabetic rats was prevented by Ougenina Oojeinensis (200 mg/kg). The effects were comparable to the standard drug glibenclamide. The majority of the experiments confirmed the benefits of medicinal plants with hypoglycaemic effects in the management of diabetes mellitus.

Numerous mechanisms of actions have been proposed for these plant extracts. Some hypotheses relate to their effects on the activity of pancreatic ß cells (synthesis, release, cell regeneration/revitalization) or the increase in the protective/inhibitory effect against insulinase and the increase of the insulin sensitivity or the insulin-like activity of the plant extracts. Other mechanisms may involve improved glucose homeostasis (increase ofperipheral utilization of glucose, increase of synthesis of hepatic glycogen and/or decrease of glycogenolysis acting on enzymes, inhibition of intestinal glucose absorption, reduction of glycaemic index of carbohydrates, reduction of the effect of glutathione. All of these actions may be responsible for the reduction and or abolition of diabetic complications. The saponins will reduce the level of serum glucose levels, liver phosphorylase and glucose-6-phosphatase activities, and significantly increased the serum pyruvate level and liver glycogen. There was also marked improvement in glucose utilization in diabetic rats. Serum insulin and pancreatic cAMP levels showed significant increases in diabetic rats [23]. The accumulating evidences suggest that anti-hyperglycemic action of *Ougenina Oojeinensis* might be involved in both pancreatic and extra pancreatic mechanisms.

Since, lipid abnormalities accompanying with atherosclerosis is the major cause of cardiovascular disease in diabetes. Therefore ideal treatment of diabetes, in addition to glycemic control, should have a favorable effect on lipid profiles. High level of TC and LDL are major coronary risk factors [27]. Further, the studies suggested that TG itself is independently related to coronary heart disease [28, 29]. The abnormalities in lipid metabolism lead to elevation in the levels of serum lipid and lipoprotein that in turn play an important role in occurrence of premature and severe atherosclerosis, which affects patients with diabetes [30]. Hence, measurements of biochemical parameters are necessary to prevent cardiac complications in diabetes condition. In this study, Ougenina Oojeinensis (200 mg/kg) showed significant reduction in TC, TG, LDL, VLDL levels and increased level of HDL in diabetic model rats. However, the increased HDL (cardioprotective lipid) level by Ougenina Oojeinensis was comparable to the standard drug glibenclamide. Therefore, Ougenina Oojeinensis has potential role to prevent formation of atherosclerosis and coronary heart disease. Several authors reported that secondary metabolites, such as saponins, flavonoids, phenolic compounds, and triterpenoids, have hypolipidemic activity [31-33]. Hence, the hypolipidemic properties of Ougenina Oojeinensis may be due to different types of active secondary metabolites, each with a single or diverse range of biological activities.

CONCLUSION

The present study demonstrated that ethanolic extract of Ougenina Oojeinensis could be useful in management of diabetes associated with abnormalities in lipid profiles. Further study need to be isolate, identify the active compounds and formulation.

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