EVALUATION OF ANTIDIABETIC ACTIVITY OF LEAF EXTRACT OF OCIMUM SANCTUM LINN. IN ALLOXAN-INDUCED NON-INSULIN DEPENDENT DIABETIC RATS

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ABSTRACT

BACKGROUND

To study the antidiabetic activity of ethanolic extract of leaves of *Ocimum sanctum* Linn. (EEOS) in alloxan-induced non-insulin dependent diabetic rats.

MATERIALS AND METHODS

Wistar Albino rats weighing 150-200 grams were grouped into five equal groups taking six animals in each group. Group A served as control (Normal), Group B as diabetic control, Group C and D received EEOS at a dose of 250 mg/kg and 500 mg/kg orally respectively, Group E was given standard drug (Glibenclamide 5 mg/kg) for 28 consecutive days and the effect of the ethanolic extract of *Ocimum sanctum* leaves on blood glucose levels was measured at regular intervals. At the end of the study, blood samples were collected from all the animals for biochemical estimation.

RESULTS

The result indicate that the test drug compound EEOS has statistical significance ($p \leq 0.05$) and sustained oral hypoglycaemic activity comparable with the hypoglycaemic effect of Glibenclamide.

CONCLUSION

The study confirms that EEOS has antidiabetic activity against alloxan-induced diabetic rats. It could be a novel antidiabetic agent and also a dietary adjunct for the management of type 2 diabetes and its complication. Further studies are required to confirm the antidiabetic activities of individual phytoconstituents of *Ocimum sanctum*.

KEYWORDS

Ocimum sanctum, Antidiabetic Activity, Alloxan, Glibenclamide, Phytochemical Constituents.

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BACKGROUND

Diabetes mellitus also known as 'Madhumeha' in Sanskrit represent a heterogeneous group of disorders, which has plagued the mankind since the ancient civilisation. It is a chronic metabolic disorder with micro and macrovascular complications, characterised by chronic hyperglycaemia and disturbances of carbohydrates, fats and protein metabolism associated with absolute or relative deficiencies in insulin secretion and/or insulin action.^[1] It has become the third killer of the health of mankind along with cancer, cardiovascular and cerebrovascular disease. Diabetes mellitus is a global health crisis which has been persistently affecting the humanity, irrespective of the socio-economic profile and geographical location of the population. The prevalence of diabetes mellitus is expected to reach up to 4.4% in 2030 and highest occurrence is found in India, China and USA.^[2]

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According to International Diabetes Federation (IDF) 7th edition, the number of individuals with diabetes in 2015 crossed 415 million and by 2040 this will rise to 642 million.^[3] Though advancement is made in the field of modern medicine to cure diabetes, still there is increasing demand by patients to use natural products with antidiabetic activity due to the side effects associated with the use of insulin or oral hypoglycaemic agents. In recent findings, extracts of various plant materials are capable of decreasing blood sugar level in experimental animal models and are considered to be less toxic than synthetic ones.^[4]

Ocimum sanctum Linn., commonly known as tulsi or tulasi in hindi and holy basil or sacred basil, belonging to family Lamiaceae is a strongly scented small annual herb, about 30–60 cm tall with hairy stems and simple opposite green leaves. Leaves have petioles and are ovate, up to 5 cm long, usually slightly toothed. Leaf colour ranges from light green (Rama tulsi) to dark purple (Krishna tulsi). Flowers are purplish in elongate racemes in close whorls.^[5] It grows wild in the tropics and warm regions. In India, it is grown throughout the country from Andaman and Nicobar Island to the Himalayas up to 1800 metres above the sea level. It is also abundantly found in Malaysia, Australia, West Africa and some of the Arab countries.

Traditional Uses

Ocimum sanctum Linn. (Tulsi) is known as 'the elixir of life' since it promotes longevity. Different parts of the plant are traditionally used for common cold, headache, earache, sore

throat, bronchitis, malarial fever, antidote for snake bite and scorpion sting, fatigue, skin, wound infection, etc.^[6]

Pharmacological Activities

Several medicinal properties have been attributed to *Ocimum sanctum* Linn. Different parts of the plant, i.e. leaves, flowers, stem, root, seeds, etc. are known to possess therapeutic potential like analgesic, expectorant, anti-asthmatic, anti-microbial, anti-emetic, antidiabetic, anti-fertility, anti-cancer, diaphoretic, hepatoprotective, hypotensive, hypolipidaemic, anti-stress, etc.^[7,8]

Phytochemical Constituents

The chemical composition of *Ocimum sanctum* Linn. is highly complex, containing many nutrients and other biologically active compounds. The leaves of *Ocimum sanctum* Linn. contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpene hydrocarbon caryophyllene. Fresh leaves and stem of *Ocimum sanctum* extract yielded some phenolic compounds such as cirsilineol, circimaritin, isothymusin, ursolic acid, apigenin and rosameric acid and appreciable quantities of eugenol which exhibit anti-oxidant and anti-inflammatory activities. Two flavonoids viz. orientin and vicenin from aqueous leaf extract have been isolated that provide protection against radiation induced chromosomal damage in human blood lymphocytes.^[7,8]

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Leaves of *Ocimum sanctum* Linn. (OS) were collected from local areas of Dibrugarh in the month of April–May, identified and authenticated by Dr. L. R. Saikia, Department of Life Science, Dibrugarh University, Assam (Voucher specimen no. DULSc 448). A voucher specimen was deposited in the herbarium of the institute.

Preparation of the Plant Extract

Fresh leaves of *Ocimum sanctum* are washed thoroughly with distilled water, air dried, powdered. About 850 gms of powder was obtained, which was then packed into a Soxhlet apparatus and extraction was done by continuous hot percolation using ethanol (95% v/v). The extract was concentrated using a rotary evaporator. It was further concentrated and dried in desiccators. The final yield of ethanolic extract was found to be 7.24% (w/w).^[9] The extract collected was stored in air tight glass containers in refrigerator at 2–8°C for further use in experiments.

Phytochemical Analysis

EEOS was subjected to qualitative phytochemical analysis of alkaloids, flavonoids, tannins, saponins, sterols, terpenoids as per standard methods.^[10]

Drugs and Chemical Used

Alloxan monohydrate was obtained from G. S. Chemical testing lab and allied industries, New Delhi. Crude powder of Glibenclamide was obtained from Ranbaxy Laboratories Ltd.

Experimental Animals

Healthy Wistar albino rats (*Rattus norvegicus*) 7-8 weeks old, weighing 150–200 grams were taken from Central Animal House, Gauhati Medical College (Registration No. IAEC Regd. No. 351/CPCSEA-3/1/2001). The animals were housed in

standard cages under standard conditions of 12 hours light and dark cycle and normal room temperature. Animals were fed with normal diet and water ad libitum before starting the study permission from the Institutional Animal Ethics Committee was taken. The study was conducted according to CPCSEA guidelines.

Acute Oral Toxicity Test

Acute oral toxicity test was done following OECD guidelines 425 (Up and down method). EEOS was found safe at 2000 mg/kg dose.^[11] Two arbitrary doses 250 mg/kg and 500 mg/kg were selected for the study.

Experimental Design

Animals are randomly assigned into five groups with six animals in each group (n = 6).

Group A - Normal group (Given only saline 10 mL/kg/ day).

Group B - Diabetic control (Given saline 10 mL/kg + Alloxan).

Group C - Diabetic treated with EEOS (250 mg/kg/day).

Group D - Diabetic treated with EEOS (500 mg/kg/day).

Group E - Diabetic treated with Glibenclamide (5mg/kg/day).

Standard drug Glibenclamide (5 mg/kg) and ethanolic extract of *Ocimum sanctum* (EEOS) were given orally with the help of feeding cannula daily for 4 weeks.

Induction of Diabetes in Experimental Animals

Wistar albino rats were made diabetic by a single intraperitoneal injection of Alloxan monohydrate (150 mg/kg).^[12] Alloxan is first weighed individually for each animal according to the body weight and then solubilised with 0.2 mL saline (154 mM NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels > 200 mg/dL were included in the study. Treatment with plant extract was started 48 hours after alloxan injection.^[13]

Collection of Blood Sample and Blood Glucose Estimation

Blood samples were drawn from tail tip of rat at weekly intervals till the end of the study (i.e. 4 weeks). Fasting blood glucose estimation and body weight measurement were done after 48 hours, 7th, 14th, 21st and 28th day. Blood glucose estimation was done by One Touch electronic glucometer; Johnson and Johnson Company, USA using glucose strip. On the 28th day, blood was collected from retro-orbital plexus under mild ether anaesthesia after overnight fasting and fasting blood sugar and other biochemical parameters were estimated. Total cholesterol was estimated by CHOP/PAP method,^[14] TG by GPO/PAP method,^[15] HDL-C measured by PEG/CHOD-PAP method,^[16] LDL-C measured by Friedwald's Formula.^[17]

Statistical Analysis

All the values of body weight, fasting blood sugar and lipid profile were expressed as mean $\pm$ standard error of mean (S.E.M) and analysed by using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. 'P' value of < 0.05 were considered significant.

RESULTS

Acute Toxicity Test

No mortality was recorded among the rats at a dose of 2000 mg/kg. Hence, EEOS at doses of 250 mg/kg and 500 mg/kg was found to be safe. This selected dose was also confirmed by Subramani Parasuraman et al.^[18]

Phytochemical Analysis

Phytochemical analysis of leaves of *Ocimum sanctum* Linn. has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and terpenoids.

Effect of Extract Treatment on the Body Weight

The results of body weight are shown in Table 1. In normal (Control) group, there is slight increase in body weight. In the diabetic control group, there is significant reduction in body weight compared to group A and in groups C, D and E there is significant increase in body weight compared to Group B.

Group	Day 1	Day 28
Normal Control	154.8±1.515	156.7±1.229
Diabetic Control	155.8±0.9458 ^a	134.0±0.894 ^a
Diabetic Test (250 mg/kg)	155.2±0.8724 ^b	141.7±0.802 ^b
Diabetic Test (500 mg/kg)	156.0±1.125 ^b	146.7±1.606 ^b
Diabetic Standard	155.5±0.9574 ^b	147.7±0.918 ^b

Table 1. Effect of Ethanolic Extract of Leaves of *Ocimum sanctum* on Body Weight (Grams) of Alloxan-Induced Diabetic Rats

All values are expressed in mean ± SEM. Analysed by One-Way ANOVA followed by Dunnett's multiple comparison tests a = p < 0.05 when compared to normal control Group b = p < 0.05 when compared to diabetic control group.

Effect of Extract Treatment on Fasting Blood Sugar Level

The results of fasting blood sugar are shown in Table 2. On repeated administration of the extract for 4 weeks, a significant (p < 0.05) decrease in blood sugar was found in Groups C, D and E compared to Group B. Group B showed significant rise in blood sugar as compared to Group A.

Group	Mean Blood Glucose Level (mg/dL)				
	After 48 Hours	Day 7	Day 14	Day 21	Day 28
Normal Control	87.67±1.17	87.33±1.52	88.83±1.30	90.33±1.22	91.83±1.01
Diabetic Control	210.3±0.80 ^a	225.7±0.802 ^a	243.3±0.95 ^a	240.3±0.95 ^a	246.5±1.78 ^a
Diabetic Test (250 mg/kg)	222±1.65 ^b	168.7±3.007 ^b	141.8±1.27 ^b	121.3±1.33 ^b	104.7±1.52 ^b
Diabetic Test (500 mg/kg)	220.7±1.52 ^b	170.3±1.49 ^b	133.3±1.43 ^b	116.3±2.49 ^b	103.7±1.82 ^b
Diabetic Standard	219.7±1.40 ^b	161±3.75 ^b	135.3±3.49 ^b	116.7±2.90 ^b	99.67±0.95 ^b

Table 2. Effect of Ethanolic Extract of Leaves of *Ocimum sanctum* on Fasting Blood Glucose Level of Alloxan-Induced Diabetic Rats

All values are expressed in Mean ±SEM. Analysed by One-Way ANOVA followed by Dunnett's multiple comparison tests. A = p < 0.05 when compared to normal control group b = p < 0.05 when compared to diabetic control group.

Effect of Extract Treatment on Fasting Lipid Profile

The results of fasting lipid profile are shown in Table 3. On repeated administration of the extract for 4 weeks, a significant (p < 0.05) decrease in total cholesterol, serum LDL, serum TG and significant increase in serum HDL was found in Groups C, D and E compared to Group B. Group B showed significant rise in total cholesterol, serum LDL and serum TG and decrease in serum HDL as compared to Group A.

Group	Serum Total Cholesterol (mg/dL)	Serum TG (mg/dL)	Serum HDL (mg/dL)	Serum LDL (mg/dL)
Normal Control	67.17±2.056	81.83±2.626	25.50±1.478	25.30±1.185
Diabetic Control	135.3±1.745 ^a	151.2±1.195 ^a	17.67±0.667 ^a	87.43±1.926 ^a
Diabetic Test (250 mg/kg)	94.17±2.344 ^b	92.67±1.308 ^b	33.33±1.308 ^b	41.87±2.720 ^b
Diabetic Test (500 mg/kg)	87.67±1.202 ^b	89.50±1.648 ^b	35.33±1.926 ^b	34.93±1.153 ^b
Diabetic Standard	88.50±1.875 ^b	89.33±2.186 ^b	35.33±1.606 ^b	35.30±1.865 ^b

Table 3. Effect of Leaves of *Ocimum sanctum* on Lipid Profile of Alloxan-Induced Diabetic Rats

All values are expressed in mean ± SEM. Analysed by One-Way ANOVA followed by Dunnett's multiple comparison tests; a = p < 0.05 when compared to normal control group b = p < 0.05 when compared to diabetic control group.

DISCUSSION

Alloxan monohydrate is commonly used to induce non-insulin dependent diabetes mellitus in many experimental animals. The mechanism of action of Alloxan has been thoroughly studied. Alloxan, a cytotoxic agent induces diabetes in various animal species through destruction of islets of Langerhans of the pancreas. After administration it is rapidly and selectively taken up by the β cells of the pancreas, following which there is formation of redox cycle for generation of Reactive Oxygen Species (ROS), superoxide radicals and hydrogen peroxide.^[19] Another mechanism is the effect of ROS on the DNA of the pancreatic islets. The fragmentation of DNA takes place in the beta cells exposed to alloxan that causes DNA damage, which stimulates poly ADP-ribosylation, a process participating in DNA repair. Antioxidants like superoxide dismutase, catalase and the non-enzymatic scavengers of hydroxyl radicals have been found to protect against alloxan toxicity.^[20] In addition, the disturbances of intracellular calcium homeostasis has also been responsible for diabetogenic action of alloxan, as it elevates cytosolic free Ca²⁺ concentration in the beta cells of the pancreatic islets. Increased concentration of Ca²⁺ and ROS ultimately damages the beta cells of the pancreatic islets.^[21]

In our study, we observed that EEOS decreased blood glucose in alloxan-induced diabetic rats comparable with the oral hypoglycaemic agent, Glibenclamide (a sulphonylurea). The mechanism of action of the extract could be similar to that of sulphonylurea, which promote insulin secretion by closure of K⁺ ATP (Adenosine 5-monophosphate) channels. This results in membrane depolarisation and increased Ca²⁺ influx, which is a key initial step in insulin secretion.^[22,23]

The antidiabetic effect of EEOS also may be due to the effect of active flavonoids, phenols, steroids and saponins which scavenges free radicals liberated by alloxan in diabetic rats. Similar hypoglycaemic effects have been reported for several plants that contain flavonoids.^[24]

Apart from the regulation of carbohydrate metabolism, insulin also plays an important role in the metabolism of lipids. Insulin is a potent inhibitor of lipolysis, because it inhibits the activity of hormone sensitive lipases in adipose tissue and suppresses the release of free fatty acid into circulation. As a result of insulin resistance in adipose tissue, lipolysis and free fatty acid flux from the adipocytes are increased, leading to increased lipid (very low density

lipoprotein and triglyceride) synthesis in the hepatocytes. This is responsible for dyslipidaemia found in type 2 Diabetes mellitus.^[25]

The hypocholesterolaemic activity of EEOS may be due to presence of flavonoids and related phenolic compounds. Similar results has also been reported by Khan et al.^[26] Flavonoids significantly increased LDL receptor mRNA levels, which in turn increase hepatic uptake and degradation of LDL causing a decrease in serum LDL levels.^[27,28]

CONCLUSION

It is thus concluded that EEOS at a dose of 250 mg/kg and 500 mg/kg body weight produced significant antidiabetic activity in alloxan-induced NIDDM in rats. Also it produced significant antihyperlipidaemic effect and thus proves to be effective in preventing and managing complications of diabetes, i.e. hyperlipidaemia and related consequences. Therefore, *Ocimum sanctum* leaves could be a novel antidiabetic agent and also a dietary adjunct in the management of diabetes mellitus. However, further studies have to be undertaken to find out the exact mechanism of antidiabetic activity of *Ocimum sanctum*.

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