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Evaluation of antidiabetic activity of ethanolic extract of *Ocimum sanctum* Linn. leaves in alloxan induced diabetic albino rats

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Abstract

Background: The study of plants having Antidiabetic activity may give a new approach in the treatment of diabetes mellitus. The present research study was aimed to evaluate Antidiabetic effect of ethanolic extract on leaves of *Ocimum sanctum* Linn. (EEOS) in alloxan induced diabetic albino rats.

Material and Method: Albino rats weighing 150- 250 grams were grouped into five (5) equal groups taking six animals in each group (n=6). Group I served as control (normal control), whereas Group II was served as diabetic control, where as Group III and IV received ethanolic extract of leaves of *Ocimum sanctum* Linn. (EEOS) at a doses of 250 mg/kg and 500 mg/kg orally respectively, Group V was given standard drug (Glibenclamide 5mg/kg) for 28 consecutive days and the antidiabetic effect of the *Ocimum sanctum* Linn. leaves ethanolic extract on blood serum glucose levels was measured at regular intervals. At the end of the study blood samples were collected from all the animals for biochemical estimation.

Result: The present study clearly indicate that the test drug compound EEOS has statistically significant ($p \leq 0.05$) and sustained oral hypoglycaemic activity which is comparable with the hypoglycaemic effect of the standard drug Glibenclamide.

Conclusion: The present research study confirms that EEOS has antidiabetic activity against alloxan induced diabetic albino 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 activity of individual phytochemical constituents of *Ocimum sanctum*.

Keywords: *Ocimum sanctum*, glibenclamide, antidiabetic, ethanolic extract, diabetic albino rats, serum glucose, herbal remedy

Introduction

Diabetes mellitus is also known as 'Madhumeha' in Sanskrit represent a heterogeneous group of disorders which has plagued the mankind since the ancient civilization. It is a chronic metabolic disorder with micro and macrovascular complications, characterised by chronic hyperglycaemia and disturbances of protein, carbohydrates and fats metabolism associated with absolute or relative deficiencies in insulin secretion and/ or insulin action [1]. It has become the 3rd killer of the health of mankind along with cancer, cardiovascular & cerebrovascular disease. Diabetes mellitus is almost a global health crisis which has been persistently affecting the humanity, irrespective of the socio- economic profile & geographical location of the population. The prevalence of diabetes mellitus is expected to reach upto 4.4% in 2030, and highest occurrence is found in India, China and USA [2]. 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 upto 642 million [3]. Though here 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 which are 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, upto 5 cm long, usually slightly toothed. Leaf colour ranges from light green which is (Rama tulsi) to dark purple (Krishna tulsi). Flowers of *Ocimum sanctum* Linn. 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 meters above the sea level. It is also abundantly found in Malaysia, Australia, West Africa and some of the Arab countries.

Materials and Methods

Wistar albino rats weighing 150- 200 grams were grouped into five(5) equal groups taking six animals in each group(n=6). Group I served as control (normal), Group II served as diabetic control, Group III & IV received EEOS at a dose of 250 mg/kg & 500 mg/kg b.w. orally respectively, where as the Group V was given standard drug (Glibenclamide 5mg/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.

Collection and authentication of plant material

Leaves of *Ocimum sanctum* Linn. (OS) were collected from the local regions near Khammam city at rotary nagar in the month of April – May, and are identified and are authenticated by Assistant Professor and Head, Department of Botany, Govt. SRBJNR PG College, Khammam. A spicemen voucher was deposited in the herbarium of the institute.

Preparation of the plant extract

Fresh leaves of *Ocimum sanctum* are thoroughly washed 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 of Leaves of *Ocimum sanctum* Linn. was found to be 7.24% (w/w) [6]. 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 [7].

Drugs and chemical used

Alloxan monohydrate was obtained from (Sigma Chemicals St.louis, USA.). Crude powder of Glibenclamide was obtained from Aventis Pharma Ltd, Ankles war.

Experimental animals

Healthy albino rats (*Rattus norvegicus*) 7- 8 weeks old weighing 150 – 250 grams were taken from Central Animal House, Mamata Medical College (IAEC) at Mamata Medical College, Khammam, Telangana state. The animals were housed in standard cages under standard conditions of 18 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 [8] Two arbitrary doses 250mg/kg and 500mg/kg

were selected for the present research study.

Experimental Design

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

Group I – Normal group (given only saline 10 ml/kg/ day)

Group II – Diabetic control (given saline 10 ml/kg + Alloxan)

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

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

Group V - 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 the feeding canula 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) [9]. 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 the 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 [10].

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 serum glucose estimation were done on 14th, 21st and 28th day. Blood glucose estimation was done by diagnostic Kits (Roche, Mumbai), using glucose strip. On the 28th day, blood was collected from retro orbital plexus under mild ether anaesthesia after overnight fasting biochemical parameter like fasting blood serum glucose levels was estimated.

Statistical analysis

All the values of fasting serum blood glucose were expressed as mean±SD and analysed by using one way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. 'p' value of <0.05 (p <0.05) were considered significant.

Results

The present investigation revealed that the antidiabetic activity of ethanolic extract of *Ocimum sanctum* Linn. leaves on intra peritoneal injection of alloxan at a dose of 150mg/kg b.w. caused significant elevation of blood serum glucose in untreated groups (diabetic rat group) when compared to control group was shown in (Table 2). Treatment of diabetic rats with *Ocimum sanctum* Linn. leaves ethanolic extract for 28 days caused dose dependent decrease in blood serum glucose levels in diabetic albino rats. Glibenclamide treated diabetic rats also showed significant ($P < 0.00$) decrease in blood serum glucose levels after 28 days of treatment as shown in (Table 2).

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.* [11].

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 fasting blood serum sugar level

The results of fasting blood serum glucose are shown in Table

2. On repeated administration of the extract for 4 weeks, a significant ($p < 0.05$) decrease in blood serum glucose levels was found in Groups III, IV and V compared to Group II. Group II showed significant rise in blood sugar as compared to Group I.

Table 1: Blood glucose levels of albino rats of normal control group

S. No.	Animal (albino rats) weight (in grams)	Normal control group albino rats blood glucose levels (mg/dl)		
		14 th day	21 st day	28 th day (final)
1.	168	90	95	90
2.	180	95	98	96
3.	172	100	100	93
4.	165	105	105	95
5.	160	98	100	105
6.	170	96	99	100

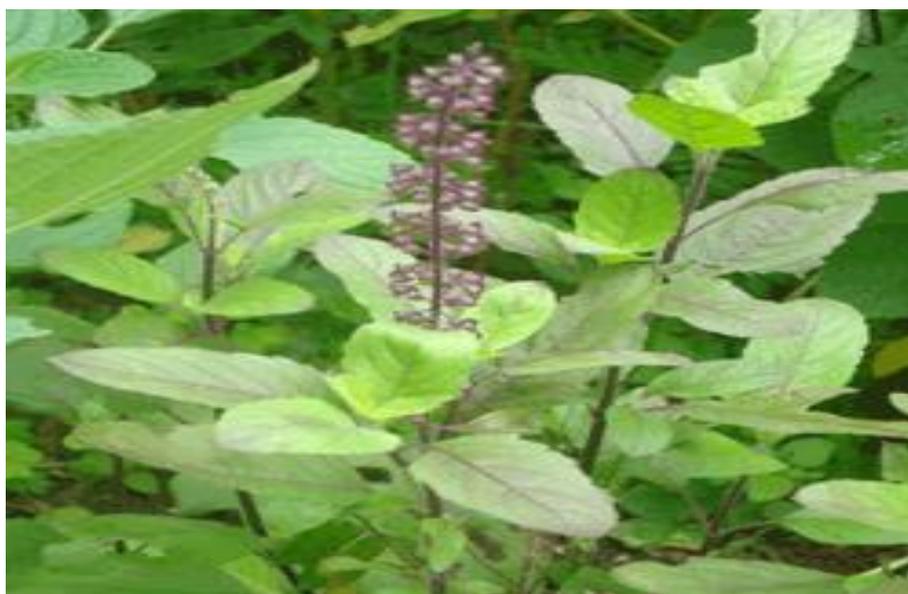


Fig 1: *Ocimum sanctum* Linn. Plant

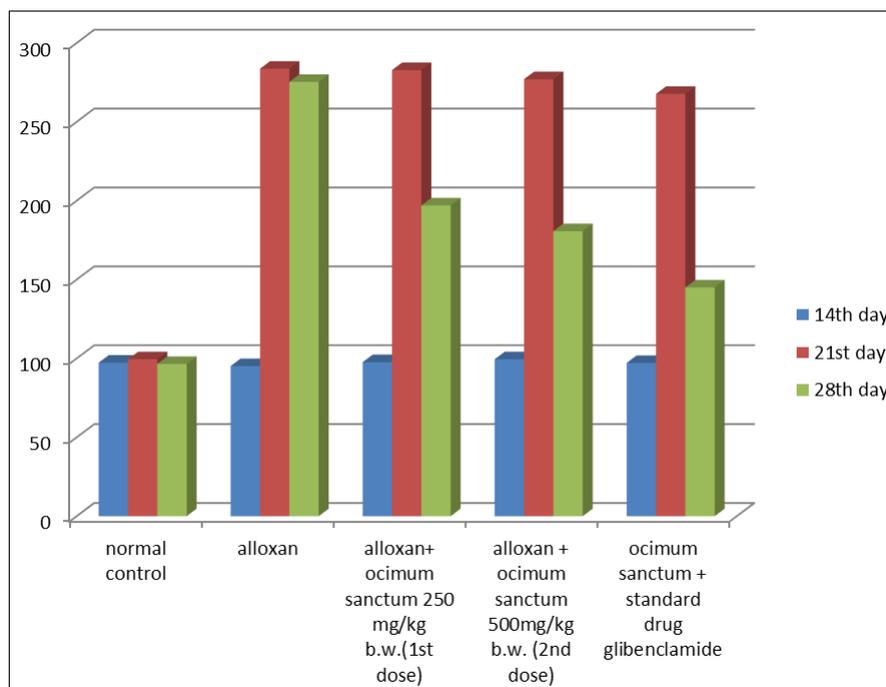


Fig 2: Effect of ethanolic extract of leaves of *Ocimum sanctum* on fasting blood serum glucose level of alloxan induced diabetic albino rats.

Table 2: Effect of *Ocimum sanctum* (os) linn. Leaves ethanolic extract on fasting blood serum glucose levels in alloxan induced diabetic albino rats.

Group treatment	Fasting blood serum glucose (mg/dl)		
	14 th day	21 st day	28 th day (final)
Normal control group (given only saline 10 ml/kg/ day)orally	97.3±5.0	99.5±3.2	96.5±5.3
Diabetic control (given saline 10 ml/kg + Alloxan (150 mg/kg) in a single i.p. dose	95.17±7.4	283.7±5.9	275.3±4.8
Diabetic+ OS Linn. leaves ethanolic extract (250mg/kg/ b.w. day) orally	97.67±7.9	282.8±5.2*	197±5.9**
Diabetic+ OS Linn. leaves ethanolic extract (500mg/kg/ b.w. day) orally	99.5±3.2	277±3.1*	180.7±6.0**
Diabetic+standard drug Glibenclamide 5mg/kg/ b.w. /day orally	97.17±4.4	267.7±6.3*	145±3.8**

All values are expressed in Mean±SD. Analyzed by one way ANOVA followed by Dunnet's test for multiple comparison tests. * = p<0.05 when compared to normal control group (significant p value). ** = p<0.00 when compared to diabetic control group (Highly significant p value).

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 [12]. 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 [13]. In addition, the disturbances of intracellular calcium homeostasis has also been responsible for diabetogenic action of alloxan as it elevates cytosolic free Ca^{2+} concentration in the beta cells of the pancreatic islets. Increased concentration of Ca^{2+} and ROS ultimately damages the beta cells of the pancreatic islets [14]. In present study observed that EEOS decreased blood glucose in alloxan induced diabetic albino 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 Ca^{2+} influx which is a key initial step in insulin secretion [15, 16]. 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 albino rats. Similar hypoglycaemic effects have been reported for several plants that contain flavonoids [17].

Conclusion

Study conclude that EEOS at a dose of 250mg/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. Thus, it also supported the folklore claim of anti- diabetic activity of plant. However, further studies have to be undertaken to find out the exact mechanism of antidiabetic activity of *Ocimum sanctum*.

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Declarations

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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