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An evaluation of anti-hyperglycemic activity of *Ocimum sanctum* Linn (leaves) in Wister rats

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Abstract

Objective: Blood glucose control remains a main focus in the managing of patients with type 2 diabetes. Pioglitazone is one of the insulin sensitizer with limitations like weight gain and peripheral edema, increase the incidence of heart failure and increase the risk of bone fractures. To this purpose we evaluated anti hyperglycemic activity of *Ocimum sanctum* Linn leaf in comparison with Pioglitazone in alloxan induced diabetic rats.

Methods: This comparative study was conducted in 16 male Wister rats for the period of 3 months. *Ocimum* leaves were dried; Soxhlet apparatus was used for extraction. Diabetes mellitus was induced in rats by single intraperitoneal injection of Alloxan monohydrate dissolved in sterile normal saline at a dose 160 mg/kg body weight. DM mellitus was confirmed by measuring fasting blood glucose level using tail vein blood sample. After 1 week, rats with fasting blood glucose of more than 300 mg/dl were considered as diabetics and used for further experiment. The data was expressed as mean \pm SEM, Statistical difference in mean were analyzed by one way ANOVA.* $P < 0.05$ was considered as significant.

Results: Administration of the test drug i.e aqueous extract of *Ocimum sanctum* Linn (300 microgram/gram body weight), produced statistically significant reduction in the blood glucose level ($p < 0.0001$) which was 345 mg/dL before treatment and reduced to 263 mg/dL after treatment.

Conclusion: Aqueous extract of leaves of *Ocimum sanctum* Linn has got significant anti-diabetic activity by its antihyperglycemic action and insulin sensitizing activity greater than control and lesser than reference drug pioglitazone.

Keywords: Diabetes Mellitus, *Ocimum sanctum* Linn, Pioglitazone, Insulin Sensitizer

1. Introduction

It is anticipated that currently 19.4 million people are affected by diabetic disease, which can likely to go up to 57.2 million by the year 2025 in India [1] Blood glucose control remains a main focus in the managing of patients with type 2 diabetes. Studies have conclusively determined that reducing body blood sugars decreases the onset and progression of microvascular complications [2-3]. Personalization of blood sugar control, balancing of glycemic control with its potential risks, taking into account the adverse effects of glucose-lowering drug agents. Pioglitazone is one of the insulin sensitizer, which is a substitute to metformin, but limitations like weight gain and peripheral edema, increase the incidence of heart failure and increase the risk of bone fractures, predominately in women [4-5]. This emphasizes the urgent need for supplementary effective anti-hyperglycemic agents. Herbal drugs also comprise a vital part of traditional medicine shows that there are more than four hundred plant species showing antidiabetic activity [6]. *Ocimum sanctum* Linn belongs to the family of Lamiaceae, popularly known as Tulsi which is also a sacred plant for many Hindus in India. Fresh leaves of *Ocimum* commonly used as home remedy or ayurvedha for the treatment of cough, cold, abdominal pain, skin diseases, arthritis and diarrhea. The preclinical evaluation on various extracts of different parts of *Ocimum Sanctum* Linn and other varieties showed anti-fertility, anti-cancer, anti-diabetic, anti-fungal, hepatoprotective and cardioprotective actions [7]. To this purpose we have evaluated anti hyperglycemic activity of *Ocimum* leaf in comparison with Pioglitazone in alloxan induced diabetic rats.

Materials and Methods

This comparative study was conducted at Madras Medical College, Chennai for three months, between august 2008 to October 2008. *Ocimum sanctum* Linn plant was taxonomically identified from the rural parts of Chennai and it was endorsed by certified Taxonomist from

Tamilnadu Agricultural University, Chennai. The plant leaves were dried under the shade for 10 days and made coarse powder using electrical grinder. Soxhlet apparatus was used for extraction. The extraction procedure was carried out for next 24 hours at about 55–60 °C; then it was filtered through muslin cloth. The filtrate was concentrated to a dry mass by evaporation under reduced pressure. The hydro extract of leaves of *Ocimum sanctum* Linn was stored in a desiccator at room temperature till further analysis. Wistar albino rats were obtained central animal house, Madras Medical College, Chennai, the animals were housed as per CPCSEA guidelines and study was approved by the Institute Animal Ethics Committee.

Healthy, adult male Wistar albino rats, weighing between 160 ± 20 grams were used for this experiment. Diabetes mellitus was induced in overnight-fasted rats by single intraperitoneal injection of freshly prepared Alloxan monohydrate dissolved in sterile normal saline at a dose 160 mg/kg body weight. Diabetes mellitus was confirmed by measuring fasting blood glucose level using tail vein blood sample. After 1 week, rats with fasting blood glucose of more than 300 mg/dl were considered as diabetics and used for further experiment [8].

- Group I: Normal control.
- Group II: Diabetic control (Alloxan - 160mg/kg/i.p)
- Group III: Diabetic animals treated with Pioglitazone (1 mg/kg) [9].
- Group IV: Diabetic animals treated with aqueous leaf extract of *Ocimum Sanctum* Linn (300 micrograms/ gram body weight)

This study was done to determine the minimum dosages of extract to be administered and hypoglycemic activity of aqueous extracts was evaluated. A total of 16 Wistar rats were divided into four equal groups. The animals of group I served as untreated control whereas the other three groups II, III, and IV, were administered with the aqueous extract at a single dose of 300 mg/kg respectively. Plasma glucose was estimated 4 hours after the administration of test drug and calculations done in each group using following formula [10].

$$\% \text{ variation of glycaemic} = \frac{Gi - Gt}{Gi} \times 100$$

Where Gi and Gt were the values of initial glycaemia (0 hour) and glycaemia at 4, 8 and 12 hours respectively. The plasma glucose levels of different groups at different time intervals were also compared. The extract dose that lowered the glucose level by 25% at 4 hours was considered the minimum hypoglycemic dosage. At the end of seven days of extract administration, 1ml of blood samples were collected from the tail directly into anticoagulant bottles contains sodium fluoride. The plasma was separated after centrifugation. One touch ultra diagnostic reagent kit [11] was used to estimate plasma glucose concentration.

Statistical Analysis

The data was expressed as mean ± SEM, Statistical difference in mean were analyzed by one way ANOVA. *P < 0.05 was considered as significant.

Results

In the control group, the blood glucose values were 345 mg/dL and 356 mg/dL respectively before and after administration of control. There was an increase in the blood glucose level

indicating that vehicle used to dissolve test and standard drugs was not having any blood glucose lowering action. Pioglitazone produced maximum reduction in blood glucose level to 220 mg/dL and was statistically highly significant ($p < 0.001$) compared to the value of 345 mg/dL before drug administration. Administration of the test drug i.e aqueous extract of *Ocimum sanctum* Linn (300 microgram/gram body weight), produced statistically significant reduction in the blood glucose level ($p < 0.0001$) which was 345 mg/dL before treatment and reduced to 263 mg/dL after treatment. Thus, overall the blood glucose lowering action was observed more with pioglitazone followed by *Ocimum sanctum* Linn and then control group.

Discussion

Rao SA *et al.* [12] and Suanarunsawat *et al.* [13] in their ethanolic extract of *O. Sanctum* showed significant anti-diabetic effect in alloxan induced diabetes mellitus in rats, and the fixed oil of *O. sanctum* significantly reduced hyperlipidemia induced by high fat diet fed Wistar rats. Vats V *et al* in leaves of *Ocimum sanctum* Linn commonly known as Tulsi are similarly studied for their hypoglycemic and antioxidative properties [14] *Ocimum tenuiflorum* is known to have many pharmacological activities and it is traditionally used as an anti-tussive agent. In this present investigation, we predicted the biological activities of phytoconstituents of *Ocimum sanctum* Linn. Which indicated pharmacological actions as insulin promoter activity, known to have anti-diabetic, cardioprotective, wound healing, anti-oxidant, hypolipidemic, anti-microbial, gastroprotective, immunomodulatory, anti-nociceptive and anti-cancer effects [15].

Conclusion

Aqueous extract of leaves of *Ocimum sanctum* Linn has got significant anti-diabetic activity by its antihyperglycemic action and insulin sensitizing activity greater than control and lesser than reference drug pioglitazone. In addition to regular antidiabetic drugs, *Ocimum sanctum* Linn leaves can also be considered as add on/adjutant agent in the treatment of type-2 diabetes management. Further studies are required to confirm the anti-diabetic activities of individual phytoconstituents of *Ocimum Sanctum* Linn in human studies were warranted.

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Conflict of Interest: None declared.

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