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EVALUATION OF THE ANTIDIABETIC EFFECT OF *OCIMUM SANCTUM* IN TYPE 2 DIABETIC PATIENTS.

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ABSTRACT

This study was carried out to find out the antidiabetic effect of *Ocimum Sanctum* (*O. sanctum*) in type 2 diabetes patients as so many studies have showed its antidiabetic effect experimentally but very few studies are performed to investigate the efficacy in humans. The study was performed in two different groups for a period of 90 days. One group received Glibenclamide and other one received Glibenclamide plus *O. sanctum*. Fasting blood glucose (FBG) and Post prandial Blood Glucose level (PPBG) were estimated at the time of enrolment in the study and then after 30 days and 90 days. The group which received Glibenclamide, showed a significant drop in the mean FBS as well as PPBS. Fasting Blood glucose level was 174.35 gm/dl on day 1, which dropped significantly to 114.50 gm/dl on 90th day. Post prandial blood glucose levels was 247.31 gm/dl, on day 1, which dropped significantly to 152.02 gm/dl on 90th day. The group which received Glibenclamide plus *O. sanctum* also showed a significant drop in the FBS as well as PPBS level. Fasting blood glucose level on day 1 was 171.53 gm/dl, which dropped significantly to 103.50 gm/dl on day 90 and post prandial blood glucose was 254.13 gm/dl on day 1, which dropped significantly to 143.12 gm/dl on day 90. The drop in both FBG and PPBG levels was more in the group which received Glibenclamide plus *O. sanctum*. The group which received Glibenclamide, showed a significant drop in the Glycosylated Haemoglobin (HBA1c) levels when compared between day 1 to day 90. The group which received Glibenclamide plus *O. sanctum* also showed a significant drop in the Glycosylated Haemoglobin (HBA1c) level when compared between day 1 to day 90. The drop in HBA1c levels was more in the group which received Glibenclamide plus *O. sanctum*. All of the patients were observed for hypoglycaemic episodes at every visit from Day 1 to Day 90. More than 80% of the subjects who received only Glibenclamide and Glibenclamide plus *O. sanctum* did not have any hypoglycaemic episodes. Thus from the results we can conclude that Ocimum sanctum can be used as an adjuvant in Type 2 Diabetes Mellitus patients.

Key words: Alloxan, Antioxidant, Ocimum Sanctum, Glibenclamide, Type 2 diabetes Mellitus.

INTRODUCTION

Diabetes Mellitus is a spectrum of common metabolic disorders, arising from a variety of pathogenic mechanisms, all resulting in hyperglycaemia.[1] India is facing a diabetic explosion, according to the World Health Organization (WHO) estimates, India had 32 million diabetic subjects in the year 2000 and this number would increase to 80 million by the year 2030.[2] The International Diabetes Federation (IDF) also reported that the total number of diabetic subjects in India is 41 million in 2006 and that this would rise to 70 million by the year 2025.[3] The major sources of morbidity of diabetes are chronic complications that arise from prolonged hyperglycaemia, including retinopathy, neuropathy, nephropathy and cardiovascular disorders. Fortunately these chronic complications can be reduced in many cases by sustained control

of blood glucose. There are three types of diabetes mellitus out of which Type 2 Diabetes mellitus is most common type, which was known earlier as Non-Insulin Dependent Diabetes Mellitus (NIDDM), accounts for about 90% of all the diabetes among different populations of the world.[4] Currently used most of the oral hypoglycaemic agents produce serious side effects like hypoglycaemic coma and hepatorenal disturbances. Also safety of most of the presently used drugs is not established during pregnancy.[1,5] Hence, there is urgent need for the search for safer and more effective hypoglycaemic agents. Plant based control to different diseases can serve as an alternative in the areas where there is difficulty of availability of modern treatment. In large areas of developing world, numerous plant species are used as folk medicine to Diabetes Mellitus. Many Indian medicinal plants are recommended for the treatment of Type 2 Diabetes Mellitus. *O. Sanctum*, commonly known as *Tulasi,* is one of the plants that has long been used in traditional herbal medicine against Diabetes.[6,7,8,9] However, only few scientific attempts have been made to correlate scientifically. Hence, the present study was planned to validate the antidiabetic effect of *O. Sanctum*, scientifically and also its effect as add on therapy when combined with oral hypoglycaemic agents in patients of Type 2 Diabetes Mellitus.

MATERIALS AND METHODS

1.1 Study Population

The patients who visited tertiary care hospital were included in the study. A total of 60 patients were selected for the study. A Randomised control trial conducted in the tertiary care hospital, Puducherry. This study was carried in a semi-urban population. The study included 30 Type 2 diabetic cases who Received Only Glibenclamide and 30 Type 2 diabetic cases received Glibenclamide plus *O. Sanctum* tablet. The baseline examinations were carried out in the population. A total of 60 patients with type 2 diabetes mellitus were included in the study.

1.2 Ethical Clearance

Protocol was duly submitted to Institutional Human ethics committee and approval was taken before starting the study. The study was performed under the supervision of the Physicians. All the procedure was informed to the patient in his native language and informed written consent was taken from them.

1.3 Diagnosis

Type 2 Diabetes mellitus was diagnosed according to American Diabetes Association (ADA) criteria 2000 by demonstrating any one of the following.[10]

Fasting plasma glucose level ≥ 7.0 mmol/L (126 mg/dL).

Plasma glucose ≥ 11.1 mmol/L (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test.

Symptoms of hyperglycaemia and casual plasma glucose ≥ 11.1 mmol/L (200 mg/dL).

Glycosylated haemoglobin (HbA_{1C}) $\geq 6.5\%$.

1.4 Inclusion Criteria

Uncomplicated Diabetes Mellitus Type 2 Patients on monotherapy.

HbA_{1c} level at the screening visit between 6.5 to 10.5%.

Patients of either sex (male or Female) between the ages 40-60 years.

Patients on Glibenclamide at dose of 5 mg per day.

1.5 Exclusion Criteria

Type 1 diabetic patient

HbA_{1c} level at the screening visit more than 10.5%.

Patients requiring concomitant medication for other disorders, patients with history of angina, ketoacidosis, myocardial infarction, abnormal thyroid and liver function tests and patients with other endocrine metabolic disorder as well as those with micro or macro vascular complications.

2.1 Initial Assessment

The patients diagnosed as type 2 diabetes mellitus by physician of the hospital underwent full physical examination. The physical examination consisted of assessment of the parameters like Height, weight, Body composition, Body mass Index, Waist circumference, Vital signs heart rate, blood pressure, CVS Examination, Respiratory examination, abdominal examination, sensory neurological examination of extremities.

Information related to pharmacological treatment (Oral hypoglycaemic agents, Insulin), pre-existing health or diabetic complications, smoking status as well as demographic information was also recorded at the initial assessment.

2.2 Administration of O. Sanctum

Test drug in capsule form of 250 mg was procured from Himalayan Drug Company Bangalore. Eligible Patients, after doing randomisation were divided into two groups. One group received Only Glibenclamide at 5 mg per day with food, while the other group received *O. Sanctum* Capsule for 30 days in addition to Glibenclamide. Patients who have been prescribed *O. Sanctum* were advised to consume one capsule half an hour before breakfast and another capsule half an hour before dinner. Prior to the start of the study, pre-treatment measurements were recorded for each patient, after the completion of the 30 day course they were requested to attend the scheduled follow up appointments for general check-up and post treatment assessment.

2.3 Follow Up Appointments

Follow up assessment were scheduled every month till the completion of duration of study (i.e. upto 90 days) following the initial assessment. At subsequent visits, a complete physical examination was performed. Diabetes related pharmacologic treatment was reassessed based on previous laboratory investigations (Blood Glucose and HbA1c levels) and patient feedback related intolerance or side effects of current drug treatment. All Pertinent laboratory investigations such as haemoglobin HbA1c, blood glucose tests were collected and the patients were given *O. sanctum* capsule for next 30 days.

2.4 Drop Outs

No dropouts recorded in the study.

2.5 Laboratory investigations

Blood samples (3-5 ml) were drawn from each patient by venepuncture through plastic disposable syringes. The blood samples were collected in a clean oven dried glass bottles All Laboratory investigations were conducted at central research laboratory using the set protocol and procedures established by the hospital.

2.5.1 Fasting and Post Prandial Blood Glucose

The Fasting Blood Glucose, post prandial blood glucose tests were carried out for every patient initially and subsequent visits. The concentration of glucose in the plasma sample was determined by an auto analyser modification of ferricyanide reduction method.

2.5.2 Haemoglobin A_{1c}

Assessment of HbA_{1c} was performed following the initial assessment and at the time of end of the study. HbA_{1c} allows a long term assessment of the patient's blood sugar control. As opposed to a fasting glucose test which simply gives a snapshot of glucose control at a given time, HbA_{1c} levels represent the management of blood sugar levels over the past ninety days. Thus, assessment of HbA1c provides a more accurate representation of glycaemic control, particularly when assessments are more than 60 days apart.

3.1 Statistical Analysis

Statistical analysis was done on Statistical Package for Social Sciences (SPSS, Chicago, Version 15). Unpaired student's t test was done. The control group is compared with the diabetic group. Data were expressed as mean ± Standard deviation. The results were rationally analysed. $P < 0.05$ was considered statistically significant.

RESULTS

4.1 Demographics

The Study group comprised of both males and females with the age ranging from 30 to 65 years having type 2 diabetes. The total number of patients enrolled in the study was 60, of which 39 were males and 21 were females. The patient suffering from diabetes having family history of diabetes either maternal or paternal found to be in large proportion than compared with no family history of either type of diabetes.

5.1. Effect of O. Sanctum on Fasting Blood Glucose Level

The group which received Glibenclamide, showed a sustained drop in the mean Fasting blood glucose levels when compared between day 1 to day 90. On day 1 it was 174.35 gm/dl, which dropped significantly to 114.50 gm/dl on 90th day. The group which received Glibenclamide plus *O. Sanctum* also showed a sustained drop in the Fasting mean blood glucose level when compared between day 1 to day 90. On day 1 it was 171.53 gm/dl, which dropped significantly to 103.50 gm/dl on day 90. The drop in the Fasting mean blood glucose levels obtained by Glibenclamide plus *O. Sanctum* was more than Glibenclamide, when used alone. The mean Fasting blood glucose fall percentage in the Glibenclamide group when compared between day 1 to day 30 was 15.48% which further increased when the treatment was continued for 90 days and was 34.32%. The mean Fasting blood glucose fall percentage in the Glibenclamide plus *O. Sanctum* group when compared between day 1 to day 30 was 21.75% which further increased when the treatment was continued for 90 days and was 39.66%. ((Table 1 and 2).

5.2. Effect of Ocimum Sanctum on Post Prandial Blood Glucose Level

The group which received Glibenclamide, showed a sustained drop in the mean Post prandial blood glucose levels when compared between day 1 to day 90. On day 1 it was 247.31 gm/dl, which dropped significantly to 152.02 gm/dl on 90th day. The group which received Glibenclamide plus *O. Sanctum* also showed a sustained drop in the Post prandial mean blood glucose level when compared between day 1 to day 90. On day 1 it was 254.13 gm/dl, which dropped significantly to 143.12 gm/dl on day 90. The drop in the Post prandial mean blood glucose levels obtained by Glibenclamide plus *O. Sanctum* was more than Glibenclamide, when used alone. The mean Post prandial blood glucose fall percentage in the Glibenclamide group when compared between day 1 to day 30 was 19.87% which further increased when the treatment was continued for 90 days and was 38.53%. The mean Fasting blood glucose fall percentage in the Glibenclamide plus *O. Sanctum* group when compared between day 1 to day 30 was 28.29% which further increased when the treatment was continued for 90 days and was 43.68%. ((Table 1 and 2).

Table 1: ***Mean values of Fasting and Post Prandial blood glucose levels and standard deviations in different group***

	Parameters	Day 1	Day 30	Day 90
GLIB	FBG	174.35 ± 54.26	147.36 ± 31.91	114.50* ± 16.87
	PPBG	247.31 ± 59.01	198.16 ± 35.70	152.02** ± 33.61
GLIB + OS	FBG	171.53 ± 52.15	134.22 ± 26.91	103.50$^{\#}$ ± 14.74
	PPBG	254.13 ± 60.21	182.23 ± 31.62	143.12$^{\#\#}$ ± 27.43

Results were expressed in Mean ± SD;
****P < 0.05 when FBS of Glibenclamide group compared between Day 1 and Day 90***
***** P < 0.05 when PPBS of Glibenclamide group compared between Day 1 and Day 90***
$^{\#}$P < 0.05 when FBS of Glibenclamide + OS group compared between Day 1 and Day 90
$^{\#\#}$* P < 0.05 when PPBS + OS of Glibenclamide group compared between Day 1 and Day 90

Table 2: ***Comparison of percentage reduction in Fasting and Post Prandial blood glucose levels in case of Glibenclamide group and group receiving OS plus Glibenclamide***

	Parameters	Day 30	Day 90
GLIB	FBG	15.48%	34.32%*
	PPBG	19.87%	38.53%**
GLIB + OS	FBG	21.75%	39.66%$^{\#}$
	PPBG	28.29%	43.68%$^{\#\#}$

Results were expressed in Mean ± SD;
****P < 0.05 when % Reduction of FBS of Glibenclamide group compared between Day 1 and Day 90***
***** P < 0.05 when % Reduction PPBS of Glibenclamide group compared between Day 1 and Day 90***
$^{\#}$P < 0.05 when % Reduction FBS of Glibenclamide + OS group compared between Day 1 and Day 90
$^{\#\#}$* P < 0.05 when % Reduction PPBS + OS of Glibenclamide group compared between Day 1 and Day 90

6.1. Effect of Ocimum Sanctum on Glycosylated Haemoglobin (HBA1c)

The group which received Glibenclamide, showed a significant drop in the Glycosylated Haemoglobin (HBA1c) levels when compared between day 1 to day 90. On day 1 it was 7.62, which dropped significantly to 5.31 on 90th day.

The group which received Glibenclamide plus *O. Sanctum* also showed a significant drop in the Glycosylated Haemoglobin (HBA1c) level when compared between day 1 to day 90. On day 1 it was 7.76, which dropped significantly to 4.98 on day 90. ((Table 3)

Table 3: ***Comparison of HbA1c levels in case of Glibenclamide group and group receiving OS plus Glibenclamide.***

	Day 1	Day 90	Percentage Drop
GLIB	7.62 ± 0.68	5.31 ± 0.55*	30.31
GLIB + OS	7.76 ± 0.73	4.98 ± 0.39$^{\#}$	35.82

Results were expressed in Mean ± SD;
****P < 0.05 when HbA1c of Glibenclamide group compared between Day 1 and Day 90***
$^{\#}$P < 0.05 when HbA1c FBS of Glibenclamide + OS group compared between Day 1 and Day 90

7.1. Safety Parameters

All the patients were observed for hypoglycaemic episodes at every visit from Day 1 to Day 90. More than 80% of the subjects who received only Glibenclamide did not have any hypoglycaemic episodes. Occurrence of single hypoglycaemic episode was observed on 5 (16.66%) occasions. Occurrence of two hypoglycaemic episodes was observed on 4 (13.33%) occasions

The patients who received Glibenclamide plus *O. Sanctum* did not have any hypoglycaemic episodes in more than 85% of the patients. Occurrence of single hypoglycaemic episode was observed on 5 (16.66%) occasions. Occurrence of two hypoglycaemic episodes was observed on 2 (6.66%) occasions. None of the hypoglycaemic episode led to hospitalization or hypoglycaemic coma. (Table 4).

Table 4: ***Comparison of Hypoglycaemic Episodes in case of Glibenclamide group and group receiving OS plus Glibenclamide.***

	No of Hypoglycaemic Episodes	Day 1-30	Day 30- 90
GLIB	0	25 (83.33%)	26 (86.66%)
	1	02 (6.66%)	03 (10.00%)
	2	03 (10%)	01 (03.33%)
GLIB + OS	0	26 (86.66%)	27 (90%)
	1	02 (6.66%)	03 (10%)
	2	02 (6.66%)	00 (0%)

DISCUSSION

O. sanctum is a medicinal plant distributed mainly in the tropical and subtropical regions including India. It is widely used for its hypoglycaemic and antidyslipaedimic effects in diabetes. However these effects were displayed only in short term studies

In the present study, the combination of Glibenclamide plus *O. Sanctum* was found more effective in lowering both fasting and post prandial blood glucose levels in the patients of type 2 diabetes mellitus than who consumed only oral hypoglycaemic agents. Assessment of blood glucose levels was performed on the 1st day, 30th day and at the end of therapy (90th day).

The current study additionally evaluated change in HbA_1C levels. The add on therapy of Glibenclamide plus *O. Sanctum* showed a significant drop in the Glycosylated Haemoglobin (HBA1c) level when compared between day 1 to day 90 and was found more effective than the group which received only Glibenclamide.

All the patients were observed for hypoglycaemic episodes at every visit from Day 1 to Day 90. Add on therapy of *O. Sanctum* resulted to be effective as well as tolerated in the patients with type 2 diabetes mellitus with mild and infrequent adverse effects.

These results were related with those of Agarwal et al who reported the hypoglycaemic activity of leaves of *O. Sanctum* in their randomised, placebo controlled, crossover single blind trial in patients with non-insulin dependent diabetes mellitus where in represented reductions of mean fasting and post prandial blood glucose levels were 17.6 % and 7.3 % respectively.[11] The mean fasting and post prandial blood glucose level reductions in the present study were 39% and 49% respectively. The significant reduction in the present study may be due to the concomitant use of oral antidiabetic agents.

Rai et al had studied the effect of *O. Sanctum* powder supplementation on glycaemic control in 27 non-insulin dependent diabetes mellitus subjects. All the patients were on the hypoglycaemic drugs. The above parameters were monitored at the start and on the 30th day. After 1 month of supplementation, a significant lowering of the blood glucose (20.8%) was seen.[12] The current study also confirms with the study of Rai et al. The more significant results were obtained in the current study because the study duration was comparatively longer.

In conditions of sustained hyperglycaemia such as diabetes mellitus, the proportion of haemoglobin that is glycosylated is increased substantially. This glycosylation is the result of post translation modification of haemoglobin A molecules. The amount of Glycosylated haemoglobin in a patient reflects the glycaemic control during the previous 6-8 weeks.[13] Even though the improvement of diabetic control can be monitored in terms of blood glucose levels, HbA1c is the only measure which truly reflects long term euglycaemic control in patients with secondary failure to oral hypoglycaemic agents. An improvement of HbA1c by 1% even in this group of patient is therefore encouraging. In the present study, *O. Sanctum* significantly reduces glycosylated haemoglobin level (mean reduction of about 30%), which is an indication of its overall euglycaemic control. There were no Side effects of the drug. This study hence confirms the earlier observations regarding the efficacy and safety of Ocimum sanctum in the treatment of type 2 diabetes mellitus.

Furthermore the results correlate with all the preclinical studies reported so far carried out by vast et al and Chattopadhyay, Eshrat Halim and Narendhikaran et al who used aqueous and alcoholic extract of *O. Sanctum* in rat models showed significant decrease in the levels of blood glucose and glycosylated haemoglobin.[14,15,16].

O. Sanctum leaves have been reported to contain several chemicals including ursolic acid, apigenin, luteolin, orientin and moludistin. It is widely known that Ocimum sanctum leaves are rich in essential oils particularly eugenol. Moreover it is not clear which of its constituents or combinations are responsible for the antihyperglycaemic effects. Although the preliminary reports shows that *O. Sanctum* possesses a hypoglycaemic effect when used as an adjuvant, it was possible to reduce the dosage of the oral hypoglycaemic agents. In the present study, significant glycaemic control was observed in all the 30 patients. The exact mechanism of action of *O. Sanctum* has been recently established that Ocimum sanctum leaf extract stimulates insulin secretion from pancreatic Beta cells.

Weather *O. Sanctum* normalises hyperglycaemia after longer periods of diabetes is not known. A Larger group of patients and longer duration of study suggested to conclusively proving its effect as the mainstay of drug therapy in type 2 Diabetes patients. Its efficacy in arresting diabetic complications also needs to be further evaluated. However, considering the fact that in majority of patients enrolled, blood glucose levels were poorly controlled on previous drug/drugs therapy, the results of this add on therapy appears to be efficacious and safe in treating type 2 Diabetes Mellitus.

Thus, it can be concluded from the study that *O. Sanctum* has therapeutic usefulness in type 2 diabetes Mellitus as adjunct drug. Further elaborative detailed clinical studies required to potentiate this claim.

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