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Evaluation of Antihyperglycemic Activity of Ocimum Sanctum Leaves in Alloxan Induced Diabetic Rats

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ABSTRACT: *Biological evaluation of antihyperglycemic activity of Ocimum sanctum leaves in alloxan induced diabetic rats was carried out. Preliminary phytochemical analysis indicated the seeds extract of Ocimum sanctum leaves contain sterols, flavonoid glycosides, carbohydrates, phenolics and tannins. MEOC (200 mg/kg) showed significant decrease in serum glucose level at 2, 4 and 6 h. Continuous treatment with MEOC (200 mg/kg) for a period of 28 days showed a significant decrease in the serum glucose level in diabetic rats. Maximum reduction of serum glucose level in acute and subacute study occurred at the dose of 200 mg/kg. Subacute treatment for 28 days with the MEOC in the treated doses brought about improvement in body weights indicating its beneficial effect in preventing loss of body weight in diabetic rats. The ability of MEOC to prevent body weight loss seems to be due to its ability to reduce hyperglycemia. Thus it may be concluded that the methanolic extract of leaves of Ocimum sanctum is endowed with significant Antihyperglycemic activity justifying its use in traditional system of medicine.*

Keywords*- Ocimum sanctum, antihyperglycemic activity, flavonoid, gram –ve, MEOC, glycosides.*

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I. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels[1,2].

Diabetes mellitus is one of the main threats to human health in the 21st century. The World Health Organization (WHO) estimated that there were 135 million diabetics in 1995 and this number would increase to 300 million by the year 2025. India leads the world today with the largest number of diabetics in any given country. Due to increasing obesity, sedentariness and dietary habits in both Western and developing countries, the prevalence of type 2 DM is growing at an exponential rate[3].

The literature indicates that the prevalence of the metabolic syndrome and particularly diabetes is high among migrant Asian Indians and is rising very rapidly even within the Indian subcontinent. Recent WHO reports show that, India already has the largest number of diabetic patients compared to any given country. It has also been reported that Indians have very high rate of premature coronary artery diseases[4].

It has been further reported that an increase in family income was associated with diabetes and cardiovascular diseases are the major cause of death in people with both types of diabetes as per the studies conducted in 10 countries.

One of the studies conducted in south India reported an increase in ischemic heart diseases (IHD) in NIDDM compared to control population. Overall 17.8% of the patients out of a cohort of 60597 had IHD . 95% of the population had NIDDM one the studies of the south Indian population (Mohan et. al., 1991). The various factors like serum cholesterol, diastolic blood pressure, post pyramidal plasma glucose, nephropathy, retinopathy had a positive association with IHD. The IHD is higher among the migrant Asian Indians compared to Europeans and occurs at younger age in migrant Indians in UK[5].

Insulin requirements are high during normal late pregnancy and differ only slightly between normal and gestational diabetic women. However, in contrast to healthy women, GDM women consistently show reduced insulin responses to nutrients. When insulin levels and responses are expressed relative to each individual's degree of insulin resistance, a large defect in pancreatic β-cell function is consistently found in women with prior GDM. The majority of women with GDM appear to have β-cell dysfunction that occurs on a background of chronic insulin resistance already present before pregnancy. Both lean and obese women developing GDM show distinct resistance to the ability of insulin to stimulate glucose disposal and to suppress both glucose production and fatty acid levels[6].

Defects in the binding of insulin to its receptor in skeletal muscle do not appear to be involved in the state of insulin resistance in GDM women. Many other defects, such as alterations in the insulin signalling pathway, reduced expression of PPARγ and reduced insulin-mediated glucose transport have been found in skeletal muscle or fat cells of women with GDM. Whether any of these defects are primary or the result of more fundamental defects in insulin action is currently unknown. It has recently been suggested that post-receptor defects are present in the insulin signalling pathway in the placenta of women with pregnancies complicated by diabetes and obesity. In addition, expression studies demonstrate that post-receptor alterations in insulin signalling may be under selective maternal regulation and are not regulated by the foetus[7,8].

II. Experimental

Plant collection and authentication:

The leaves of *Ocimum sanctum* were collected from our own surrounding areas. The leaves were shade dried and then milled into powder by a mechanical grinder. The powder was passed through sieve and used for further studies.

Drugs and Chemicals

Metformin (Bright Pharma. Ltd. India), Alloxan monohydrate (NP CHEM, India), Glucose estimation kit (Vijay Biomedical Pvt.Ltd., India) and D –glucose (S.D.Fine Chem. Ltd, India) and Ethanol (Merck, Mumbai, India) were purchased from respective vendors.

Preparation of the extracts

The dried powder of *Ocimum sanctum* leaves was subjected to hot continuous extraction with methanol in a Soxhlet extractor and filtered. The filtrate was evaporated at room temperature and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in desiccators. The extract was dissolved in distilled water to prepare the drug solution of concentration of 100 mg/ml and used for pharmacological studies.

Animals

The Wistar rats weighing 200-300 gm were obtained from, Sainath Agencies, Uppal, Hyderabad. All the animals were stored in standard cages and maintained at 27ºc ± 2ºc under 12hrs dark/light cycle. The animals were fed with standard rat feed and water was given *ad libitum.* Ethical clearance for handling of animals and the procedures used in the study was obtained from the institutional animal ethical committee prior to the beginning of the study.

Induction of experimental diabetes

Diabetes was induced in rats by a single intravenous injection of aqueous Alloxan monohydrate (80 mg /kg i.v.). After 72 h, the animals showing serum glucose levels above 180 mg /dl (diabetic) were selected for the study. All the animals were allowed free access to water and pellet diet.

Collection of blood and determination of serum glucose

Blood samples were collected by retro-orbital puncture (ROP) technique. The collected blood samples were analyzed for glucose levels by the glucose oxidase peroxidase (GOD/POD) method and serum glucose levels were expressed in mg/dl.

Effect of MEOC on serum glucose in alloxan-induced diabetic rats

The diabetic rats were divided into four groups (n =6), viz.: group I-vehicle or Normal Control(distilled water, 10 ml/kg); group II-Diabetic Control; group III- MEOC (200 mg/kg); group IV- Metformin or Standard (10 mg/kg). All drugs were given orally.

The acute study involved estimation of serum glucose at 0, 2, 4, 6 and 24 h after MEOC and Metformin administration.

In subacute study involved repeated administration of drug for 28 days at prefixed times and serum glucose levels were estimated on the 7th, 14th, 21st day. At the end of 21 days the drug administration was stopped and a rest period of 7 days was given to the animals to study effect of drug treatment on blood glucose after 7 days i.e. on 28th day. The data were represented as mean serum glucose level and standard error of mean (SEM).

Effect of MEOC on body weight in Alloxan-induced diabetic rats

The rats were weighed daily during the study period of 28 days and their body weights were noted and presented as mean change in body weights.

III. Results And Discussions

Statistical analysis

The results are expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by Dunnet's test.

Table 1: Effect of methanolic extract of *Ocimum sanctum leaves* in alloxan-induced diabetic rats (acute study).
Groups Treatment (mg/kg) Mean Fasting Serum Glucose Level (mg/Groups g/dl) ± SEM

GROUP	0hr	2hr	4hr	6hr	24hr
Group I (Vehicle)	**227.68±9.106**	**238.45±8.978**	**242.3±8.481**	**244.8±8.326**	**250.47±7.436**
Group II (Diabetic Control)	**250.83±4.93*** **	**208.78±2.574***	**224.4±4.016**	**230.28±2.578**	**237.88±2.245**
Group III (MEOC-200)	**148.55±1.689*** **	**175.45±6.952*** **	**228.68±1.216**	**247.33±.581**	**264.87±2.376**
Group IV (Metformin 10)	**145.17±6.194*** **	**230.12±1.591**	**241±1.07**	**248.58±2.998**	**302.97±3.323*** **

*Values are mean± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Dunnet's test. *P<0.05, ***P<0.001 as compared with vehicle treated group (distilled water 10ml/kg).*

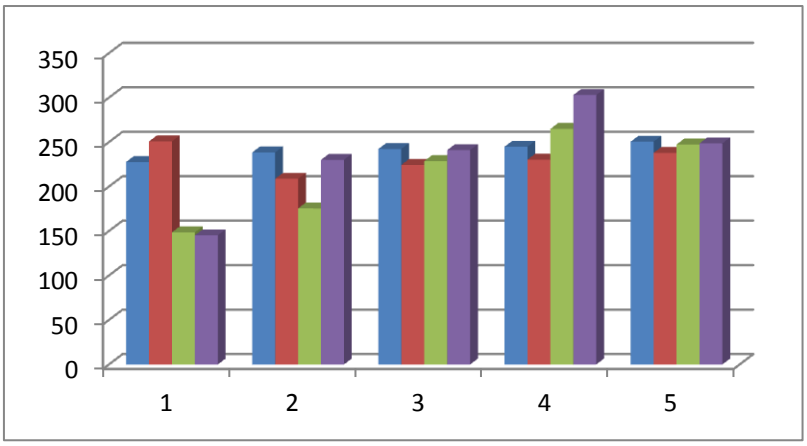

***Fig: 1.* Effect of methanolic extract of *Ocimum sanctum leaves* in alloxan-induced diabetic rats (acute study).**
(Note: Blue- Control, Maroon- Diabetic, Green- MEOC, Violet- Standard)

Table 2: Effect of methanolic extract of *Ocimum sanctum leaves* on serum glucose level in alloxan-induced diabetic rats (subacute study).
Groups Treatment (mg/kg) Mean Fasting Serum Glucose Level (mg/Groups g/dl) ± SEM

GROUP	Day 0	Day 7	Day 14	Day 21	After 7 rest period
Group I (Vehicle)	**115.5±2.391**	**117.67±1.585**	**117±1.751**	**115.33±1.606**	**154.97±1.8**
Group II (Diabetic Control)	**238.03±2.605**	**225.72±2.029**	**220.52±0.863**	**192.48±1.452**	**138.28±.68**
Group III (MEOC-200)	**294.88±4.690****	**261.13±3.399****	**197.6±2.733**	**161.9±2.089**	**148.02±.649****
Group IV (Metformin 10)	**259.8±.2.168*** **	**213.17±2.578***	**204.62±2.629*** **	**144.02±1.043*** **	**135.7±.3225*** **

*Values are mean± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Dunnet's test. *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle treated group (distilled water 10ml/kg).*

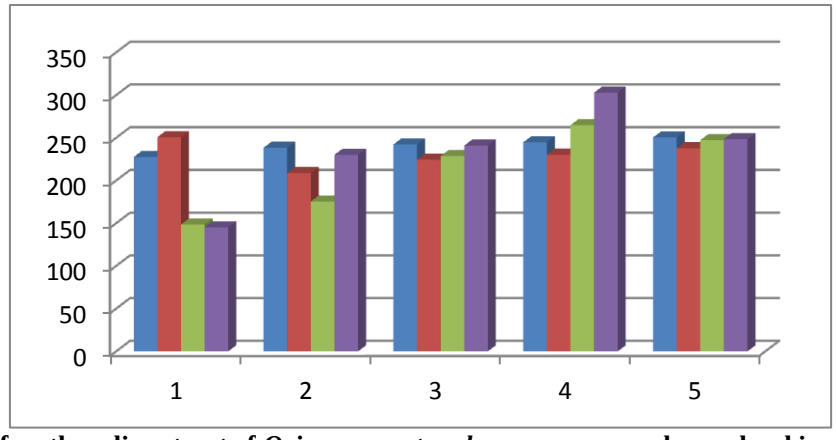

***Fig: 2.* Effect of methanolic extract of *Ocimum sanctum leaves* on serum glucose level in alloxan-induced diabetic rats (subacute study).**

Table 3: Effect of methanolic extract of *Ocimum sanctum leaves* on body weight in alloxan-induced Diabetic rats.
Groups Treatment (mg/kg) Mean Fasting Serum Glucose Level (mg/Groups g/dl) ± SEM

GROUP	Day 0	Day 7	Day 14	Day 21	After 7 rest period
Group I (Vehicle)	**133.33±4.364**	**135.67±4.849**	**138±3.587**	**139.67±3.913**	**180.67±2.275**
Group II (Diabetic Control)	**155.83±4.470**	**163.5±0.619**	**168.17±.477**	**175.17±1.400**	**221±1.653**
Group III (MEOC-200)	**173±7.519****	**178.17±4.901**	**177.67±3.043**	**180.83±4.472**	**178.33±4.12**
Group IV (Metformin 10)	**179.5±4.470*****	**194.83±2.257*****	**202.17±1.470*** **	**215.33±1.726*** **	**203.5±.885*** **

*Values are mean± S.E.M., n = 6 in each group, data were analyzed by one way ANOVA followed by Dunnet's test **P<0.01, ***P<0.001 as compared with vehicle treated group (distilled water 10ml/kg).*

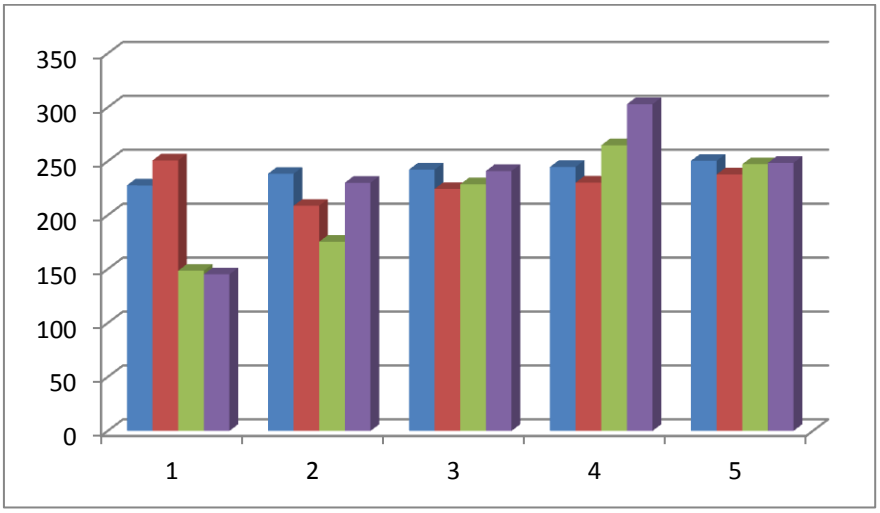

***Fig: 3.* Effect of methanolic extract of *Ocimum sanctum leaves* on body weight in alloxan-induced Diabetic rats.**

Effect of MEOC on serum glucose in alloxan-induced diabetic rats:

In acute study, administration MEOC (200 mg/kg) as well as Metformin (10 mg/kg) in diabetic rats, showed significant reduction of serum glucose levels at 2, 4, and 6 h interval. The onset of reduction of serum glucose of MEOC (200 mg/kg) treated rats was observed at 4h and peak effect at 6 h from basal value 262.3 mg/dl) but effect was waned at 24 h after administration. The onset of Antihyperglycemic effect of Metformin (10mg/kg, p.o) was at 2 h (213.5 mg/dl) the peak effect was at 6 h (231.5 mg/dl) (Table 1).

In the **subacute study**, repeated administration (once a day for 28 days) of the MEOC as well as Metformin caused significantly reduced in the serum glucose level as compared with vehicle treated group. Significant decreases in serum glucose of the diabetic rats were seen at doses of 200 mg/kg in dose-dependent manner as compared with vehicle treated group. Maximum reduction in serum glucose level from basal value was observed (128 mg/dl) on 28th day in the diabetic rats treated with MEOC 200mg/kg. Metformin treated animals showed maximum reduction in serum glucose level from basal value (135.3 mg/dl) on 28th day (Table 2).

Effect of MEOC on body weight in alloxan-induced diabetic rats:

Administration of vehicle (distilled water, 10ml/kg.p.o.) in alloxan induced diabetic rats resulted in gradual decrease in body weight during the study period. Body weight of MEOC (200 mg/kg) treated diabetic rats prevented further loss of body weight in diabetic rats. On the other hand, rats gained body weight which indicated beneficial effect of MEOC **(Table 3).**

IV. Conclusion

Biological evaluation of antihyperglycemic activity of Ocimum sanctum leaves in alloxan induced diabetic rats was carried out. Preliminary phytochemical analysis indicated the seeds extract of Ocimum sanctum leaves contain sterols, flavonoid glycosides, carbohydrates, phenolics and tannins. MEOC (200 mg/kg) showed significant decrease in serum glucose level at 2, 4 and 6 h. Continuous treatment with MEOC (200 mg/kg) for a period of 28 days showed a significant decrease in the serum glucose level in diabetic rats. Maximum reduction of serum glucose level in acute and subacute study occurred at the dose of 200 mg/kg.

Subacute treatment for 28 days with the MEOC in the treated doses brought about improvement in body weights indicating its beneficial effect in preventing loss of body weight in diabetic rats. The ability of MEOC to prevent body weight loss seems to be due to its ability to reduce hyperglycemia. Thus it may be concluded that the methanolic extract of leaves of Ocimum sanctum is endowed with significant Antihyperglycemic activity justifying its use in traditional system of medicine.

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