



DPP4 INHIBITORS AS MODERN ARMAMENTS IN THE MANAGEMENT OF DIABETES USING *OCIMUM SANCTUM*

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Abstract:

The present work deals with investigation of DPP-IV inhibition activity and Antidiabetic activity of, Aqueous and Ethanolic extracts of plants; *Ocimum sanctum* (Tulsi) leaves. We investigate the DPP-IV inhibition activity and Antidiabetic effect using different extract of *Ocimum sanctum* at dose levels of 200,400 and 600 mg/kg/day for 28 days in rats.

During investigation it was found that Ethanolic extract of *Ocimum sanctum* inhibited DPP-IV activities ($66.81 \pm 0.05\%$) at greater extent than that of Aqueous extract ($53.25 \pm 0.04\%$) and was more effective to reduce hyperglycemic condition (1.5 times) in experimental animals. The result of present study reveals that *O. sanctum* extracts contain some novel DPP-IV inhibitors with hypoglycemic potential and could be developed as therapeutic armament for diabetes mellitus.

Index Terms - Dipeptidyl peptidase-IV (DPP 4), glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), insulin, diabetes.

I. INTRODUCTION

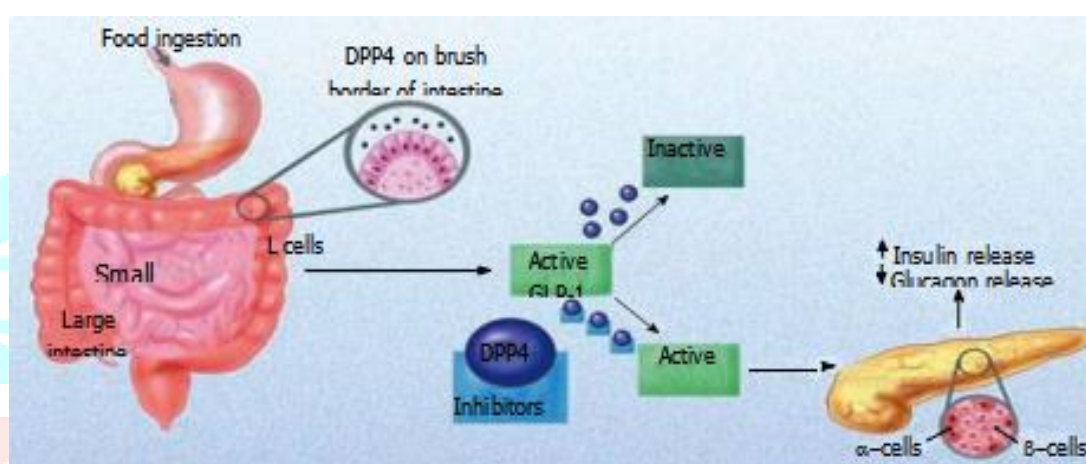
Diabetes mellitus, commonly well known as diabetes. It is a cluster of metabolic disorders that are distinguished by high blood glucose levels over a prolonged period of time and associated with scanty production of insulin by the pancreatic β -cells and insulin resistance. According to World Health Organization it is the World's fifth leading cause of death and it is estimated that it will surpass 366 million populations worldwide by the year 2030.^[1,2,3]

Therefore a novel approaches required in the treatment of Type2 diabetes Dipeptidyl peptidase-IV (DPP 4) enzyme inhibitor as modern armaments can use in management of diabetes can use.^[4,5]

Role of DPP4 Inhibitors in Diabetes

DPP-4 is a omnipresent enzyme, present on epithelial and endothelial cells, and this enzyme expressed in numerous tissues including the liver, gut, placenta, lung, and kidney. The enzyme is drop from the plasma membrane as soluble circulating DPP-4 in blood.

DPP-4 inhibitors work by blocking the action of DPP-4, an enzyme which destroys the hormone incretin.^[6] Incretins are gut hormones that are secreted from enteroendocrine cells into the blood within minutes after taking food. Physiological roles of incretins are to regulate the amount of insulin that is secreted after food eating. Incretin hormones from the gut during food ingestion mainly glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), these incretin hormones potentiate the glucose-stimulated insulin secretion.^[7]



(Dipeptidyl peptidase-IV (DPP4) inhibitors role in insulin release)

Material and Methods

Collection and preparation of Plant materials-In the present work plant was identify as *ocimum sanctum* for cultivation and sample herbarium was prepared for plant identification and authentication from Central Ayurveda Research Institute Jhansi UP (284003) with reference code; 272 and Dried, coarsely powdered *ocimum sanctum* leaves were defatted using petroleum ether in soxhlet apparatus, and extract with using **distilled water and 95% ethanol** as solvent in the soxhlet apparatus. Extracts were concentrated in a water bath at temperature of 55 ° C then concentrated extract was stored in cool place for further study.

Experimental animals

In the study sprague dawley (SD) rats of both sex were selected . The experimental protocols and procedures used in this study was approved by Ethical Committee of the Institute of Pharmacy BU Jhansi reference no BU/Pharm/IAEC/July/19/01.

Induction of Hyperglycemia - Hyperglycemia was induced experimentally by repeated two dose of fresh prepared Streptozotosin (STZ) in sodium citrate buffer and administered intraperitoneal (i. p.) at dose level of 60 mg/kg/day ,control group received equal volume of sodium citrate buffer solution on the 6th day of

STZ administration blood glucose level was measured using glucometer strips rats with glucose level 200mg/dl were consider diabetic and used in this study.^[8]

Experimental design

Experimental rats were randomly divided into 8 groups of six animals each. Group1 to Group3 animals were administered orally with aqueous extract of *Ocimum sanctum* leaves (OSAE) with 200,400 and 600 mg/kg/day, Group 4to Group6 animals were administered orally with Ethanolic Extract of *Ocimum sanctum* leaves (OSEE) with 200,400 and 600 mg/kg/day, Group 7 animals were treated with Metformin (standard drug) using 400 mg/kg/day, Group 8 animals were orally administered with 0.1ml vehicle daily at 10.30am up to 30 days.

Blood Sampling

Blood sampling was done by sterilizing the tail of the experimental rat animals with 10% alcohol and then nipping the tail at the start of the experiment and repeated after 1, 2,3 and 24 hours.^[9]

Blood glucose estimation

Bleeding was enhanced by gently “squeeze” the tail from the body towards the tip. The blood glucose levels were determined with ACCU-CHECK Compact Plus.

On last day (28th) day blood sample for observation of DPP4 inhibition activity, using retro-orbital route blood sample of 1ml from each rat was collected and stored at -20°C until the assay process completed

DPP-IV enzyme inhibition assay

DPP-IV assay was performed in research laboratory of Aakar Biotechnologies Privet Limited Lucknow following the protocol originally described by Sedo A *et.al.* as earlier workers as routinely followed in laboratory. Diprotein A was used as a standard. A decrease in DPP-IV level was measure for the inhibition activity.^[10,11]

Statistical analyses

Data are expressed as mean \pm S.E.M. for statistical evaluation of the data, analysis of variance (ANOVA) followed by the post hoc Newman–Keuls multiple comparison tests using a trial version of Prism software for Windows was used. The % inhibition was calculated using the formula, $\frac{\text{O. D. of Control} - \text{O. D. of Sample}}{\text{O. D. of Control}} \times 100$.

Results:

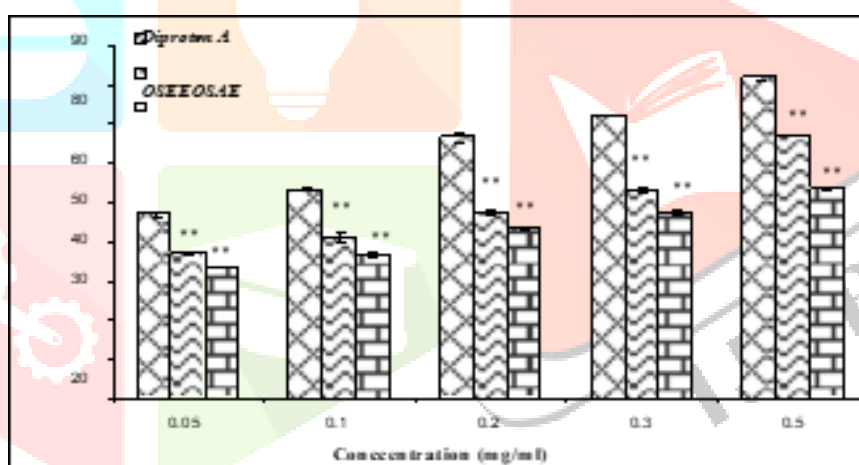
Orally administered *O. sanctum* Aqueous and Ethanolic leaves extract decrease blood glucose level at all three doses level 200, 400 and 600 mg/kg/day (Table-1) This occurred in different phases, in the first one hour the extract caused a steep decline in blood glucose levels, followed by a steady decline up to the third hour. After this, a gradual increase was recorded in the twenty fourth hour. However, the sugar levels were reduced in a dose dependent manner. *O. sanctum* Ethanolic leaves extract show higher hypoglycemic effect.

Results from DPP-IV inhibition assay revealed that *O. sanctum* Ethanolic extract (OSEE) inhibited enzyme activity at greater extent ($65.81 \pm 0.04\%$) than that of *O. sanctum* Aqueous extract OSAE ($53.25 \pm 0.04\%$) as compared to standard; Diprotein A (Figure-2)

Table-1

Group No.	Treatment(mg/kg/day)	Blood Glucose level (mg/dl)				
		0 hr	1 hr	2hr	3hr	24hr
Group1	OSAE 200	200±2.12 ^a	156±0.15 ^{ba}	156±0.41 ^b	160±0.22 ^b	190±0.12 ^b
Group2	OSAE400	198±0.22 ^a	160±0.11 ^b	150±0.12 ^b	158±0.23 ^{ab}	169±0.14 ^b
Group3	OSAE600	201±0.15 ^a	159±0.22 ^b	160±2.11 ^{ab}	160±4.11 ^a	161±2.24 ^{ca}
Group4	OSEE200	210±0.22 ^a	151±0.24 ^{ba}	152±0.42 ^b	150±0.25 ^{ab}	158±0.41 ^b
Group5	OSEE400	205±4.22 ^a	154±0.44 ^b	154±0.22 ^b	150±0.10 ^a	159±0.20 ^b
Group6	OSEE600	202±0.20 ^a	150±0.23 ^{ba}	155±0.21 ^{ab}	148±0.42 ^{ab}	149±0.25 ^{ca}
Group7	Metformin(Standard drug) 400	205±0.24 ^a	158±0.22 ^a	154±0.45 ^a	152±0.48 ^a	150±3.24 ^b
Group8	Control group	98±1.14 ^b	99±0.14 ^b	97±0.15 ^b	100±0.12 ^b	97±2.08 ^c

Above Results are expressed as Means ± SD for six rats per group. Values followed by the same superscript are not statistically different ($P \leq 0.05$; analyzed by ANOVA followed by Tukey's post hoc test).



Conclusion:

The present work provide the evidence that the Ethanolic leaves extract of the *Ocimum sanctum* exhibit more hypoglycemic activity and DPP4 inhibition activity compare to Aqueous leaves extract on experimental Streptozotosin induced diabetic rats when the drug administered using oral route. Therefore *Ocimum sanctum* can use as modern armament in management of diabetes.

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