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Herbal medicine as a source of DPP-4 inhibitor for the treatment of Type 2 Diabetes

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Abstract: Insulin resistance and poor secretion lead to the metabolic condition known as type 2 diabetes. One of the protein enzymes involved in the control of blood glucose levels is dipeptidyl peptidase-4 (DPP-4), which is also known as adenosine deaminase complexing protein 2 (CD26) or glucagon like peptide -1 (GLP-1).While synthetic DPP-4 inhibitors are a powerful antidiabetic treatment for type 2 diabetes, they are still prohibitively costly and come with side effects. Herbal medicines are the rich source for diabetes treatment, in present investigation antidiabetic and DPP-4 inhibitory activity of aqueous and ethanolic extract of *Ocimum sanctum* leaves and *Momordica charantia* fruits were investigated on dose levels of 200,400 and 600 mg/kg/day for 28 days. In the study we revealed thatassessment of blood glucose levels in diabetic rats following treatment with different plant extracts we uncovered was truly remarkable. The Aqueous Extract of Ocimum sanctum leaves (OSAE), Ethanolic Extract of Ocimum sanctum leaves (OSEE), Aqueous Extract of Momordica charantia fruits (MCAE), and Ethanolic Extract of Momordica charantia fruits (MCEE) all exhibited distinct, yet potent, antidiabetic activity on dose dependent manner. MCEE (159±6.00 mg/dL) was1.16 times more effective comparative to OSEE (186±3.00 mg/dL), combinations of extracts shown synergistic effect (133±3.00 mg/dL) on 28th day compared with control untreated group, and combination of OSEE&MCEE ($P<0.0001$) and OSAE&MCAE($P<0.001$) improved insulin levels after treatment compared to the drug solution group. OSAE and MCAE have more inhibitory effect on DPP-4 level compared to OSEE and MCEE however combinations have lesser inhibitory effect.

Keywords: Dipeptidyl peptidase-4, Type 2 diabetes, glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), pancreatic β-cells, *Ocimum sanctum* leaves, *Momordica charantia* fruits etc.

Introduction

Diabetes is become one of India's most pressing health concerns [1]. Almost 70 million individuals in India will have diabetes by 2025, up from an estimated 40 million in 2007, according to the International Diabetes Federation's (IDF) Diabetes Atlas. By 2030, the United States, India, and China will have the highest prevalence of diabetes worldwide. One in five people diagnosed with diabetes will be of Indian descent. These staggering figures put India's economic burden from diabetes among the world's biggest [2, 3]. Complications from the condition are the true cause of its impact, since they raise the risk of morbidity and mortality. In 2005, diabetes was associated with a disproportionate share of the cardiovascular disease and stroke deaths in India, which cost the country an estimated $210 billion, according to the World Health Organisation. The World Health Organisation predicts that India would incur a total of around $333.6 billion in costs due to diabetes, cardiovascular disease, and stroke in the next decade [4,5]? More than half of all diabetics in India have poorly regulated blood sugar levels (HbA1C > 8%), untreated high blood pressure and dyslipidemia, and many more suffer from diabetic vascular consequences, according to research. The situation has become even more complex due to factors such as increased mental stress, fast urbanisation and industrialization, the shift from traditional to contemporary lifestyles, and the intake of calorie-and fat-rich meals [6,7].

Diabetes present scenario

One of the biggest problems in global health, diabetes mellitus affects more people than any other non-communicable illness. [8, 9, 10]In a prior study, the National Health and Nutrition Examination Survey (NHANES) in the US put the prevalence of diabetes at 9.3%.[11] Other European nations with similarly high percentages were Poland (15%) and Spain (10.3%). From 2001 to 2008, a prior research in the Asia-Pacific area detailed a steadily increasing trend of diabetes in Hong Kong, with the frequency of the disease increasing with age.[12] In mainland China, another study found that 9.7 percent of adults had diabetes, indicating an epidemic proportions in the incidence of the disease among those aged 20 and up. [13]Worldwide, 552 million people will be affected by diabetes by 2030, according to a new analysis from the International Diabetes Federation. [14]The predicted prevalence of the disease rose from 2.8% in 2000–2007 to 9.9% in 2025–2030. Such epidemiological information points to a dramatic increase in diabetes cases

predicted for the next decades. [15]The fast increasing incidence of diabetes has placed a heavy financial strain on society as a whole, particularly on healthcare systems, according to credible research. About 11% of all adult health care spending in 2011 was attributable to diabetes, which cost an estimated $465 million worldwide. Diabetes also increases the risk of death and morbidity, which may lower health-related quality of life and shorten life expectancy. [16]The most prevalent form of diabetes, type 2, affects around 90% of people with the disease. It is linked to changes in lifestyle choices, the environment, and the ageing population. [17]Consequently, optimally controlling type 2 diabetes has thus become one of the most difficult problems confronted by doctors and politicians throughout the globe.

Role of DPP4 in diabetes

Adenosine deaminase complexing protein 2, also known as CD26 or dipeptidyl peptidase-4 (DPP4), is a protein that is expressed in humans by the DPP4 gene. Hopsu-Havu and Glenner found the enzyme in 1966; it was named dipeptidyl peptidase IV (DP IV) after its discovery and subsequent chemistry research. [18]The liver, intestines, placenta, lungs, and kidneys are just a few of the many organs that produce this ubiquitous protenious enzyme, which is found on epithelial and endothelial cells. It is easy to measure the activity of the enzyme in human plasma and serum because it is lost from the plasma membrane as soluble circulating DPP-4. DPP4 play pivotal pathological role and involve in type 2 diabetes, cancer, thyroid, colon, breast, prostate, inflammation, severe acute respiratory syndrome [19-20].
In the human body to control or prevent the pathological effect of DPP4 no biomolicule is available.

Present therapy for Type2 diabetes using synthetic medicines

Incretins are a class of metabolic hormones that promote a drop in blood glucose levels; the word "incretin" was first used by Starling in the early 1900s, and in the 1930s, La Barre, Still, and Heller refined the concept by observing that experimental animals with gut extract had lower blood glucose levels. This work laid the groundwork for the development of DPP4 inhibitors. After a meal, the pancreatic beta cells in the islets of Langerhans secrete more insulin through a process that depends on blood sugar[21, 22]. This secretion is facilitated by incretins. Ileal peptides glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP), also called glucose-dependent insulinotropic polypeptide, are the two primary peptides that meet the requirements for an incretin. GLP-1 is released into the bloodstream by L cells in the mucosa of the small and large intestines of the lower gastrointestinal tract, and GIP is made and released into the bloodstream by K cells, which are single cells in the duodenum and upper jejunum of the upper gastrointestinal tract. These cells are located in the islets of Langerhans [23-24].
Type 2 diabetes mellitus was treated with medications that were based on incretins [25]. Because DPP-4 is an efficient cleaver of GLP-1 and GIP, blocking its activity increases the quantities and effects of these hormones. This supports the idea that DPP-4 is a promising therapeutic target for T2DM treatment [26-27].
The treatment of type 2 diabetes is now supported by the approval of many DPP-4 inhibitors, including gliptin. After sitagliptin, the FDA authorised vildagliptin, saxagliptin, alogliptin, and linagliptin as DPP-4 inhibitors. [28]All of the DPP-4 inhibitors are highly selective for the enzyme, have a high affinity for it, and are orally active; however, their binding characteristics and pharmacokinetic features differ [29-30]. DPP-4inhibitos are widely used in therapy but different study report about the adverse effect of the drugs [31] and these may cause gastrointestinal infection, cutaneous sensitivity, and musculoskeletal disorders, FDA report shown about the severe joint pain as side effect [32].Type2 diabetic patients are treated with synthetic DPP-4 inhibitors along with required drugs recently have nearly five times higher cost [33-34-35]. There has been a dearth of research into the DPP-4 inhibitor functions of medicinal plants, despite the fact that herbal remedies are superior sources for diabetic therapy [36-37]. Present study assisted antidiabetic and DPP-4 inhibition effect of aqueous and ethanolic extract of *ocimum sanctum* leaves *and momordica charantia* fruits using rat animal model.

Material and methods

In the present work herbal plants were identify as *Ocimum sanctum* belong to family Lamiaceae having Report Code;272 and *Momordica charantia* member of family Cucurbitaceae with Report Code:232 for cultivation and sample herbarium were prepared for plant identification and authentication from Botany department, Central Ayurvedic Research Institute CCRAS, Jhansi, U.P.(284003) and deposited for further references.
Dried, coarsely powdered *Ocimum sanctum* leaves (2000 g) and *Momordica charantia* fruits (2400 g) were defatted using petroleum ether in soxhlet apparatus, the defatted powdered were dried (1000g and 1100g each) then extracted with using distilled water and 95% ethanol as solvent in the soxhlet apparatus (500g for OS and 550g for MC in each). Extracts were concentrated in a water bath at temperature of 55^{o} C then concentrated extracts accurately weighted OSAE (150g), OSEE (100g), MCAE (100g) and MCEE (75g) were stored in cool place for further study.

Experimental design

For In vivo evaluation of plants extract activity using rat animal model, meeting of IAEC for approval of protocol was conducted on 16 April 2021and uses of 120 Sprague dawley (SD) rats weighing about 150-200 gm both sex were

approved for project title **"Pharmacological and Phytochemical Investigation for Type 2 diabetes of some herbals."** accepted with reference number **"Bu/Pharm/IAEC/July/19/01"** experimental animals were purchased from **Central Drug Research Institute Lucknow Reg. No.34/GO/ReBI/S/99 CPCSEA.**
Experimental animals were housed in Animal house of the Institute of Pharmacy B.U. Jhansi Uttar Pradesh under the standard laboratory conditions, at 25± 2^0C temperature, 40-60% humidity with natural 12:12 hrs light and dark cycle and given standard laboratory food and water*ad libitum.*
All the observations were conducted as per CPCSEA guideline as approved protocol in the IAEC meeting.

Induction of experimental Hyperglycemia:
In this experiment, rats were given a single dose of freshly prepared Streptozotosin (STZ) in a sodium citrate buffer with a pH of 4.5. The rats were then injected intraperitoneally (i. p.) with 60 mg/kg of nicotinamide, 110 mg/kg of which was prepared in normal saline. On the sixth day of the STZ administration, the rats in the control group received an equal volume of sodium citrate buffer solution. Blood glucose levels were measured using glucometer strips, and rats with glucose levels of 200 mg/dl were considered diabetic.

Animal grouping and Experimental protocol:
Nineteen groups consisting of six rats each were randomly allocated to the diabetic animals.
Group1-3: were treated orally with aqueous leaves extract of *Ocimum sanctum* (OSAE) with 200, 400 and 600 mg/kg/day respectively for 28 days.
Group 4-6: were treated orally with Ethanolic leaves extract of *Ocimum sanctum* (OSEE) with 200,400 and 600 mg/kg/day respectively for 28 days.
Group 7-9: were treated orally with aqueous fruit extract of *Momordica charantia*
(MCAE) with 200, 400 and 600 mg/kg/day respectively for 28 days.
Group 10-12: were treated orally with Ethanolic fruit extract of *Momordica charantia* (MCEE) with 200,400 and 600 mg/kg/day respectively for 28 days.
Group13-15: were treated orally with combination of aqueous leaves extract of *Ocimum sanctum* (OSAE) and fruit extract of *Momordica charantia* (MCAE) in equal ratio with 200,400 and 600 mg/kg/day respectively for 28 days.
Group 16-18: were treated orally with combination of Ethanolic leaves extract of *Ocimum sanctum* (OSEE) and fruit extract of *Momordica charantia* (MCEE) in equal ratio with 200,400 and 600 mg/kg/day respectively for 28 days.
Group 19: Serve as standard group and treated orally with Metformin 400mg/kg /day for 28 days
Group 20: Serve as control group and was administered orally with 0.4 ml distilled water once daily.

Estimation of blood glucose level
The rats' glucose levels were measured using a glucometer (ACCU-CHECK Compact Plus). To read the glucometer, blood samples were taken from the rats' severed tail tips and placed onto a test strip that had been saturated with the blood. Daily doses of aqueous and ethanolic extracts of *Momordica charantia* fruits and *Ocimum sanctum* leaves, as well as medication extract combinations, were given for 28 days. On days 7, 21, and 28, blood glucose values were measured (Table No-)

Biochemical estimation
Blood samples were collected from different animals groups using retro orbital puncture of rat eye and collected in heparinized tube then sample kept on the ice for maximum 2 hours prior use for various biochemical assays.

Estimation of insulin: To measure insulin levels in serum and blood, we followed the instructions provided by the manufacturer and utilised a Rat Insulin ELISA Kit (Thermo scientific). In a nutshell, the standard solutions with concentrations of 0, 8, 16, 32, 80, and 140 µIU/mL were pipetted into the wells. The test sample wells were successively filled with 50 µL of diluted serum and 50 µL of an anti-Rat Insulin antibody conjugated with HRP. After 60 minutes of covering and incubation at 370C, the liquids in the wells were removed from the plate. Tris buffer was used three times after the plate was slapped on filter paper. For three minutes, each wash was gently vortexed. In each well, 50 µL of TMB substrate A was added, followed by 50 µL of TMB substrate B, and the mixture was gently shaken for 30 seconds. After covering the plate, it was left to incubate in the dark at 370C for 15 minutes. Each well was supplemented with 50 µL of stop solution and stirred well. In order to get the data needed for computation, the optical density was measured at an absorbance of 450 nm using a microplate reader.

Estimation of Dipeptidal peptidase4 (DPP-4) inhibitor activity: DPP-4 ELISA Kit (Biosource, San Diego,USA) MBS2020584 96 Tests, was used for DPP-4 assay. All reagents, samples, and standards were produced according to the manual's recommendations. After that, 100 µL of the standard or sample was added to each well, and after an hour of incubation at 37°C, the aspiration was done, and 100 µL of the prepared detection reagent A was added. Add 100 µL of

the prepared detection reagent B after incubating for 1 hour at 37°C, aspirating, and washing three times. After 30 minutes of incubation at 37°C, aspirate and wash the mixture five times. Then, add 90 µL of substrate solution and incubate for 10-20 minutes at 37°C. Finally, add 50 µL of stop solution. Data from the samples were immediately measured at λ=450 nm using a microplate reader.

Statistical analysis
The data was analysed using Prism 8 software, which included a one-way ANOVA and a Dunnet's comparison test.

Results
Blood glucose level
As shown in the figures (1A-1F) and tables (1A-1F) how different plant extracts and combinations of extracts affected blood glucose levels in diabetic rats over the course of 28 days. The treatments included Aqueous Extract of *Ocimum sanctum* leaves (OSAE), Ethanolic Extract of *Ocimum sanctum* leaves (OSEE), Aqueous Extract of *Momordica charantia* fruits (MCAE), and Ethanolic Extract of *Momordica charantia* fruits (MCEE). Additionally, we examined the combined effects of these extracts.
Effects of OSAE at dose of 600mg/kg/day on 28th day shown significant changes comparative to diabetic control($P<0.05$),the dose of OSEE 400 and 600mg/kg/day on 28th day shown significant changes comparative to diabetic control($P<0.05$), MCAE 400 and 600mg/kg/day on 28th day shown significant changes comparative to diabetic control($P<0.001$),MCEE 200 and 400 mg/kg/day on 28th day and 600mg/kg/day on 21st and 28th day shown significant changes comparative to diabetic control($P<0.0001$),The combination of OSAE and MCAE at 200,400 and 600 mg/kg/day shown significant changes on 21st day onwards compare to diabetic control(),the combination of OSEE and MCEE at 200, 400, 600 mg/kg/day displayed the most prominent on 21st as well as 28th day blood glucose-lowering effects

Insulin level
Shown in the figure-2 and table-2 OSAE shown non-significant changes, OSEE shown significant effect at dose level 600mg/kg, MCAE & MCEE shown significant changes at dose level 400 & 600 mg/kg, OSAE & MCAE combination and OSEE & MCEE combination shown significant effect compare to control group

DPP4 level
Shown in figure-3 OSAE and OSEE having inhibitory effect but value are not significant, MCAE & MCEE at dose level 400 and 600 mg/kg have significant inhibitory effect, Combination of OSAE & MCAE as well as OSEE & MCEE at all three dose level shown significant effects compare to control group

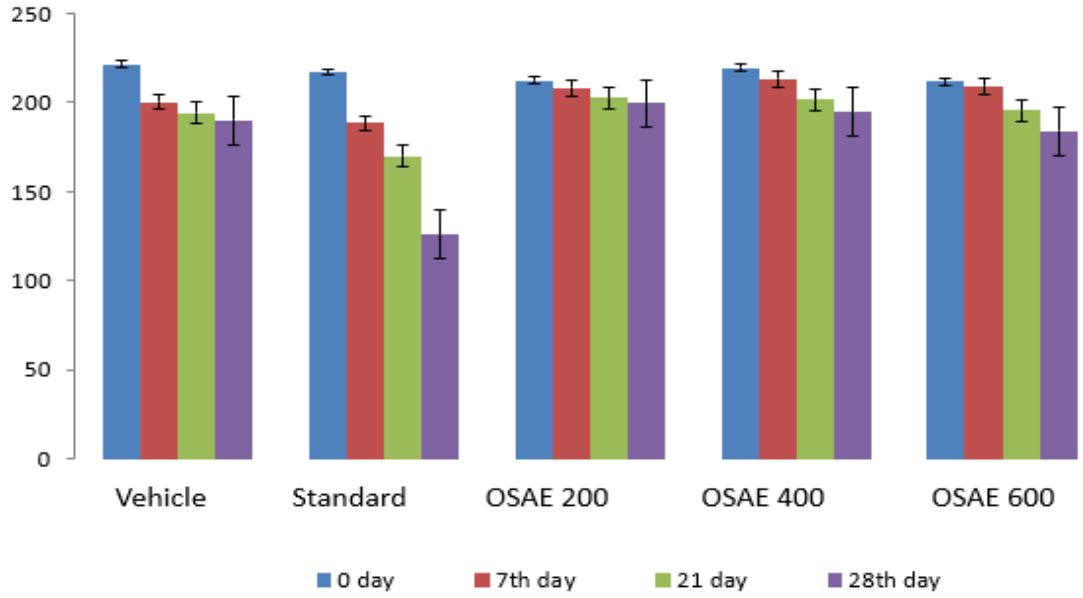

Figure 1A: Effect of Aqueous extract of *Ocimum sanctum* leaves (OSAE) on Blood glucose level (n=6 in each group)

Table 1A: Effect of Aqueous Extract of Ocimum sanctum leaves (OSAE) on Blood Glucose Levels

Drug Treatment (mg/kg/day)	Blood glucose observation of Aqueous extract of *Ocimum sanctum* leaves (n=6 in each group), in mg/dL, values are in Mean ±SEM			
	0 day	**7th day**	**21st day**	**28th day**
Vehicle (Diabetic Control)	221±2.33	200±3.90	194±4.80	189±5.40
Standard (Metformin) 400	217.5±3.90	188±2.20	169±9.00*	126±2.60***
OSAE 200	212.3±2.00	208±1.80	202.6±1.30	199.5±0.99
OSAE 400	219.8±5.10	213±4.00	201±3.40	194.6±2.10
OSAE 600	211±6.00	209±5.10	195±3.30	184±5.00*

*P<0.05 ***P<0.0001; Compared to control (Dunnett's Comparison Test)

Effects of OSEE on Blood Glucose Levels:

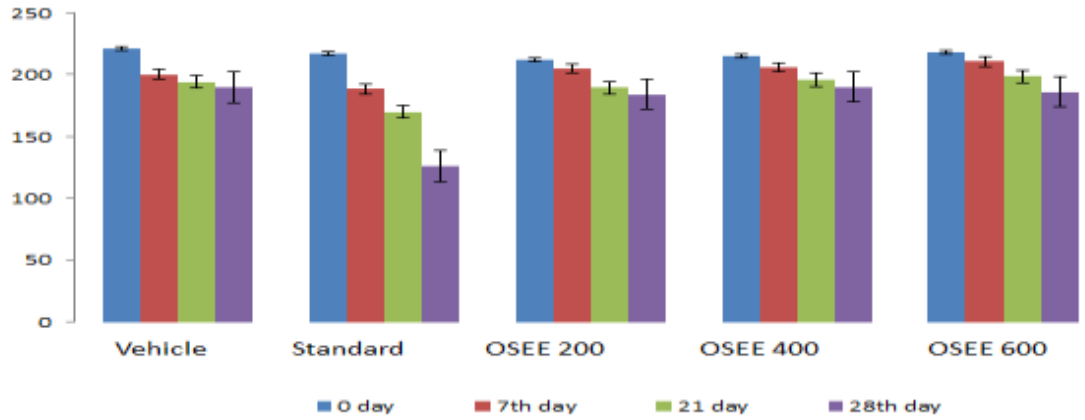

Figure 1B: Blood glucose observation of Ethanolic extract of Ocimum sanctum leaves on Blood glucose level (n=6 in each group)

Table 1B: Effect of Ethanolic Extract of Ocimum sanctum leaves (OSEE) on Blood Glucose Levels:

Drug Treatment (mg/kg/day)	Blood glucose observation of Ethanolic extract of *Ocimum sanctum* leaves (n=6 in each group), in mg/dL ,values are in Mean ±SEM			
	0 day	**7th day**	**21st day**	**28th day**
Vehicle (Diabetic Control)	221±2.33	200±3.90	194±4.80	189±5.40
Standard (Metformin) 400	217±3.90	188±2.20	169±9.00*	126±2.60***
OSEE 200	212±1.80	204±1.70	189±4.50	184±3.30
OSEE 400	215±2.30	206±1.40	195±1.00	190±0.60*
OSEE 600	218.6±6.50	210±6.40	198±3.60	186±3.00*

*P<0.05 ***P<0.0001; Compared to control (Dunnett's Comparison Test)

Effects of MCAE on Blood Glucose Levels:

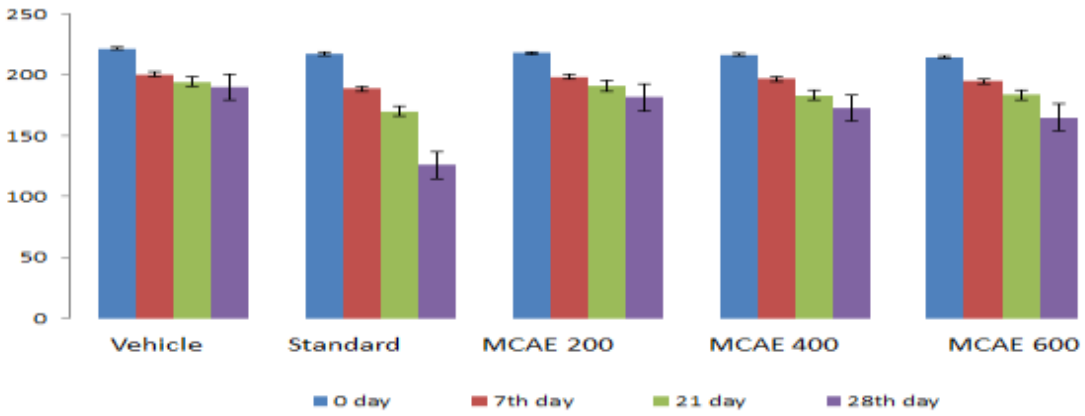

Figure 1C: Blood glucose observation of Aqueous extract of *Momordica charantia* fruits on Blood glucose level (n=6 in each group)

Table 1C: Effect of Aqueous Extract of *Momordica charantia* fruits (MCAE) on Blood Glucose Levels

Drug Treatment (mg/kg/day)	Blood glucose observation of Aqueous extract of *Momordica charantia* fruits (n=6 in each group), in mg/dL, values are in Mean ±SEM			
	0 day	**7th day**	**21st day**	**28th day**
Vehicle (Diabetic Control)	221±2.33	200±3.90	194±4.80	189±5.40
Standard (Metformin) 400	217±3.90	188±2.20	169±9.00*	126±2.60***
MCAE 200	217±3.30	198±4.00	190±3.40	181±1.70
MCAE 400	216±3.10	196±1.80	183±2.50	172±2.80**
MCAE 600	214±2.00	194±1.20	183±2.10	165±2.40**

*P<0.05, **P<0.001, ***P<0.0001; Compared to control (Dunnett's Comparison Test)

Effects of MCEE on Blood Glucose Levels:

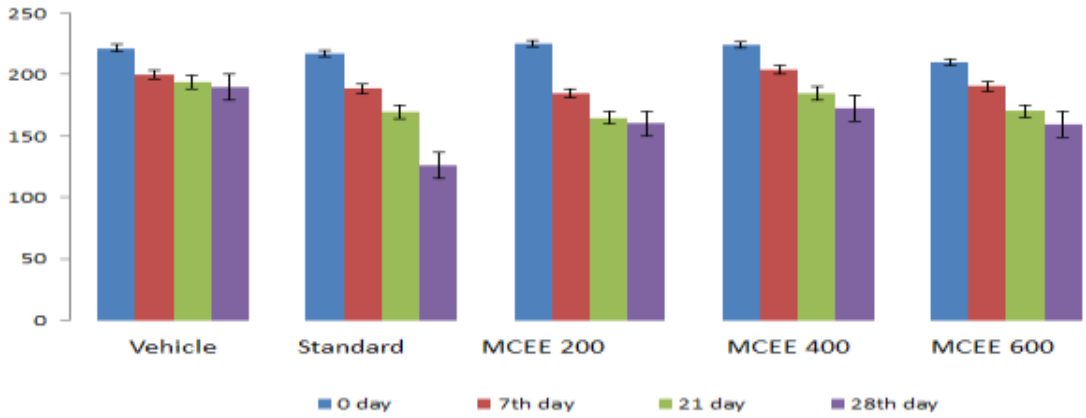

Figure 1D: Blood glucose observation of Ethanolic extract of *Momordica charantia* fruits on Blood glucose level (n=6 in each group)

Table 1D: Effect of Ethanolic Extract of *Momordica charantia* fruits (MCEE) on Blood Glucose Levels

Drug Treatment (mg/kg/day)	Blood glucose observation of ethanolic extract of *Momordica charantia* fruits (n=6 in each group), in mg/dL, values are in Mean ±SEM			
	0 day	**7th day**	**21st day**	**28th day**
Vehicle (Diabetic Control)	221±2.33	200±3.90	194±4.80	189±5.40
Standard (Metformin) 400	217±3.90	188±2.20	169±9.00*	126±2.60***
MCEE 200	225±4.10	185±8.30	165±7.70	160±3.70***
MCEE 400	224±4.50	204±5.30	185±4.10	172±4.10**
MCEE 600	209.6±2.10	190.3±3.20	170±5.00*	159±6.00***

*P<0.05, **P<0.001, ***P<0.0001; Compared to control (Dunnett's Comparison Test)

Effects of Combinations of OSAE and MCAE on Blood Glucose Levels:

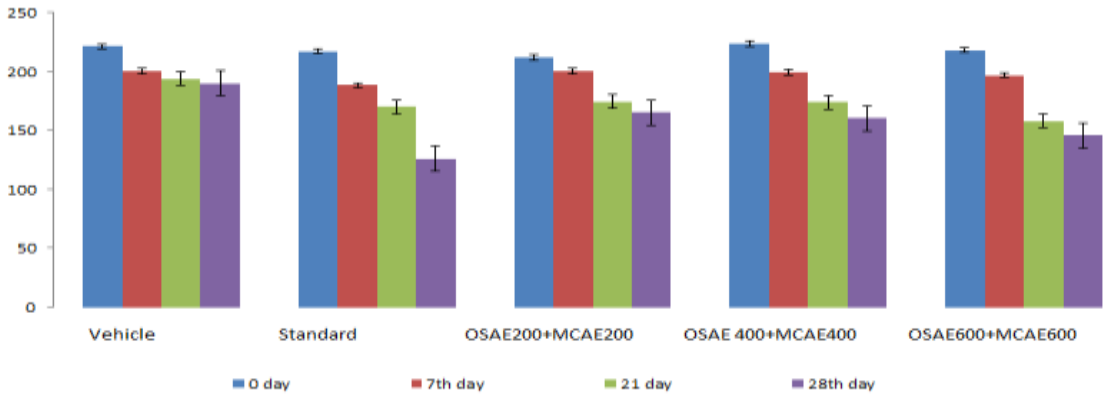

Figure 1E: Effect of combination of Aqueous extract of Ocimum sanctum leaves (OSAE) and Momordica charantia fruits MCAE) on Blood glucose level (n=6 in each group)

Table 1E: Combination of Aqueous Extracts (OSAE) and Aqueous Extracts (MCAE)

Drug Treatment (mg/kg/day)	Effect of combination of Aqueous extract of *Ocimum sanctum* leaves (OSAE) and *Momordica charantia* fruits on Blood glucose level (n=6 in each group), in mg/dL, values are in Mean ±SEM			
	0 day	**7th day**	**21st day**	**28th day**
Vehicle (Diabetic Control)	221±2.33	200±3.90	194±4.80	189±5.40
Standard (Metformin) 400	217±3.90	188±2.20	169±9.00*	126±2.60***
OSAE&MCAE 200	212±4.00	200±4.2	174±5.50	165±3.80***
OSAE&MCAE 400	223±6.10	199±2.50	173±3.40*	160±3.00***
OSAE&MCAE 600	218±2.80	196±3.00	158±4.40***	145±4.90***

*P<0.05, ***P<0.0001; Compared to control (Dunnett's Comparison Test)

Effects of Combinations of OSEE and MCEE on Blood Glucose Levels:

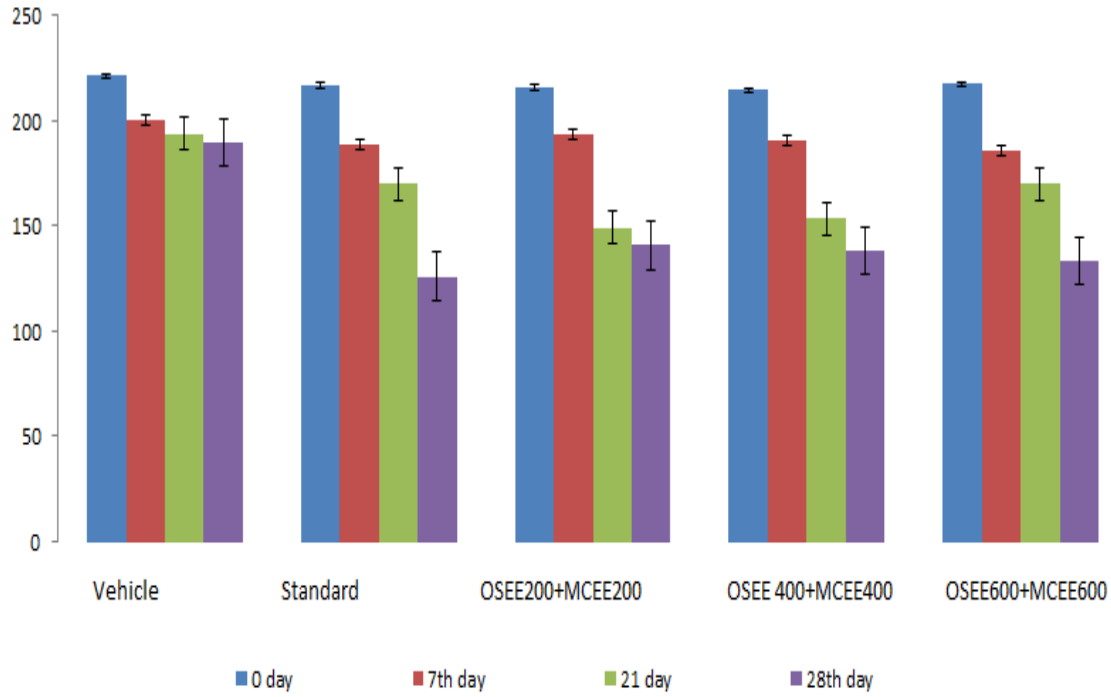

Figure 1F: Effect of combination of ethanolic extract of *Ocimum sanctum* leaves (OSEE) and *Momordica charantia* fruits on Blood glucose level (n=6 in each group)

Table 1F: Combination of Ethanolic Extracts (OSEE) and Ethanolic Extracts (MCEE)

Drug Treatment (mg/kg/day)	Effect of combination of Ethanolic extract of *Ocimum sanctum* leaves and *Momordica charantia* fruits on Blood glucose level (n=6 in each group), in mg/dL, values are in Mean ±SEM			
	0 day	**7th day**	**21st day**	**28th day**
Vehicle (Diabetic Control)	221±2.33	200±3.90	194±4.80	189±5.40
Standard (Metformin) 400	217±3.90	188±2.20	169±9.00*	126±2.60***
OSEE&MCEE 200	216±3.60	193±3.70	149±4.10***	140±1.50***
OSEE&MCEE 400	214.4±4.60	190±2.40	153±5.90***	138±2.60***
OSEE&MCEE 600	217.3±3.80	186±2.70	170±6.20***	133±3.00***

*P<0.05, ***P<0.0001; Compared to control (Dunnett's Comparison Test)

Insulin

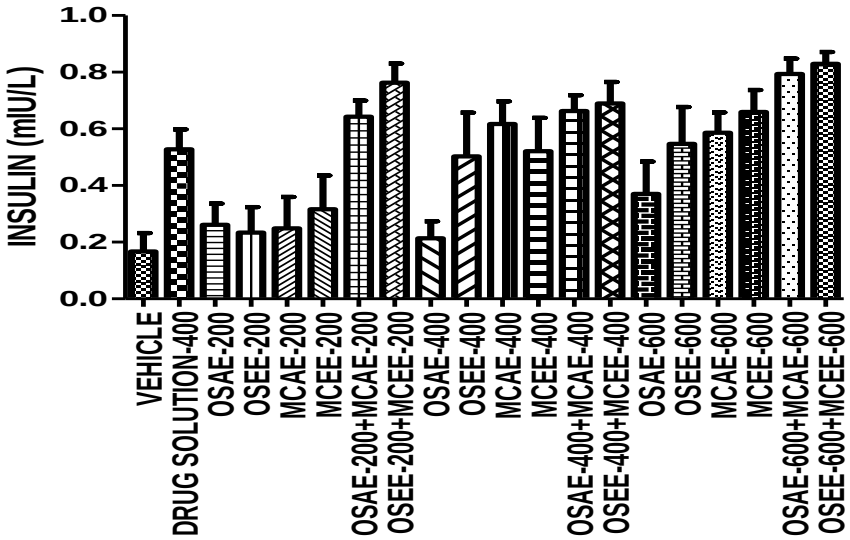

Figure 2: Effect of drug Extracts and Their Combinations using 200,400 and 600mg/kg/day dose for 28 days, on Blood Serum insulin level (n=6 in each group) in mlU/L

Table 2: Effect of drug Extracts and Their Combinations on Blood Serum insulin level (n=6 in each group)

Treatment	OSAE	OSEE	MCAE	MCEE	OSAE&MCAE (1:1)	OSEE&MCEE (1:1)	Vehicle (control)	Drug solution (400mg/kg/day)
Dose (mg/kg/day)	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM
200	0.26 ±0.07	0.23 ±0.09	0.24 ±0.11	0.31 ±0.11	0.64 ±0.05**	0.76 ±0.06***	0.16 ±0.06	0.52 ±0.06
400	0.21 ±0.06	0.50 ±0.15	0.61 ±0.08**	0.52 ±0.11	0.66 ±0.05**	0.69 ±0.07**	0.16 ±0.06	0.52 ±0.06
600	0.37 ±0.11	0.54 ±0.13*	0.58 ±0.7*	0.66 ±0.07**	0.79 ±0.05***	0.83 ±0.04***	0.16 ±0.06	0.52 ±0.06

*P<0.05, **P<0.001 ***P<0.0001Compared to control (Dunnett's Comparison Test)

DPP4inhibition

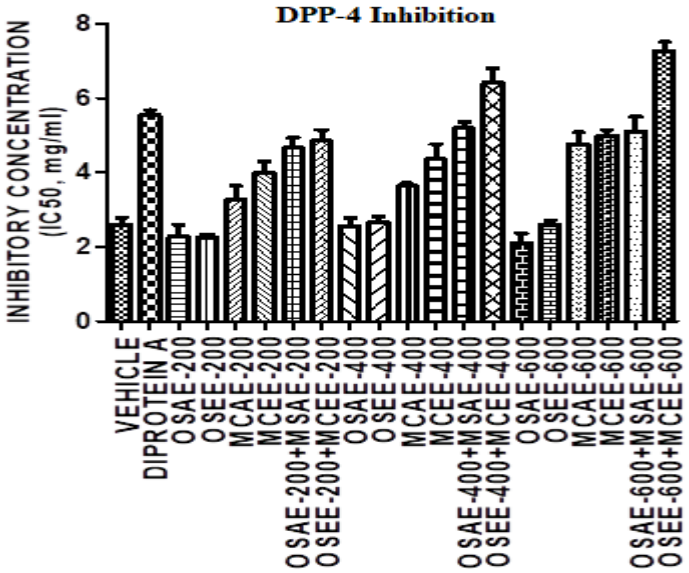

Figure 3: Effect of drug Extracts and Their Combinations using different doses on DPP-4 Inhibition level (n=6 in each group)

Table 6.21: Effect of drug Extracts and Their Combinations using 200mg/kg/day, 400mg/kg/day and 600mg/kg/day dose on DPP-4 Inhibition level (n=6 in each group)

Treatment	OSAE	OSEE	MCAE	MCEE	OSAE&MCAE (1:1)	OSEE&MCEE (1:1)	DIPROTEIN A	Vehicle (control)
Dose (mg/kg/day)	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM	Mean ±SEM
200	2.27 ±0.32	2.24 ±0.094	3.26 ±0.37	3.99 ±0.30**	4.66 ±0.27***	4.84 ±0.30***	5.53 ±0.15***	2.6 ±0.18
400	2.56 ±0.20	2.67 ±0.14	3.63 ±0.07	4.35 ±0.41***	5.19 ±0.17***	6.41 ±0.38***	5.53 ±0.15***	2.6 ±0.18
600	2.09 ±0.27	2.58 ±0.13	4.76 ±0.31***	4.99 ±0.15***	5.10 ±0.38***	7.27 ±0.23***	5.53 ±0.15***	2.6 ±0.18

**P<0.001 ***P<0.0001Compared to control (Dunnett's Comparison Test)

Discussion

The results of this study indicate that the plant extracts, OSAE, OSEE, MCAE, and MCEE, have significant blood glucose-lowering effects in diabetic rats, Ocimum sanctum leaf extracts, both the Aqueous Extract (OSAE) and the Ethanolic Extract (OSEE) demonstrated significant blood glucose-lowering effects. OSEE appeared slightly more effective, Momordica charantia fruit extracts, both the Aqueous Extract (MCAE) and the Ethanolic Extract (MCEE) exhibited substantial reductions in blood glucose levels, MCEE shown superior efficacy.The observed blood glucose-lowering effects of these plant extracts may be attributed to the presence of bioactive compounds, such as flavonoids, alkaloids, terpenoids, and phenols, which have been reported to have antidiabetic properties.

The outcomes were positive. The plant extracts showed good results in modifying insulin levels and DPP-4 inhibition, especially when taken in conjunction. These results imply that these extracts may be involved in improving insulin action, which is crucial for the management of glucose levels and diabetes. Person with type 2 diabetes having less sensitivity for insulin or less secretion of insulin from β-cells of islets. During observation, OSAE and OSEE with alkaloid, flavonoid, saponin and tannin content may stimulate insulin secretion and improve β-cells secretion.OSAE and OSEE having flavonoids work as antioxidant and inhibit dipeptidyl peptidase (DPP-4). Alkaloid, glycosides also play important role as antioxidant and involve reducing DPP-4 activity.DPP4 inhibitor responsible to increase incretin glucose like peptide & gastric inhibitory peptide (GLP-1 & GIP) in blood circulation responsible for insulin secretion from β cell of islets.MCAE and MCEE administration increase GLP-1 level as reported previously for DPP-4 inhibition activity well observed in present investigation.Combination of both shown synergistic effect and show more inhibitory effect compare to diprotein A as standard.

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Conflict of Interests

Authors have no conflicts of interests.

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