IJBPAS, February, 2026, 15(2): 554-565

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTIDIABETIC ACTIVITY OF EXTRACTS OF *A. OBESUM*, *C. GLANDULOSUM*, AND *S. SATIVA* IN EXPERIMENTAL DIABETIC RATS

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Received 15th Oct. 2024; Revised 5th Dec. 2024; Accepted 3rd Feb. 2025; Available online 1st Feb. 2026

https://doi.org/10.31032/IJBPAS/2026/15.2.9741

ABSTRACT

Diabetes mellitus is a chronic metabolic disorder, and the rising incidence of diabetes worldwide is a significant concern in both developed and developing countries. The limitations in efficacy and safety of currently available oral antidiabetic medications, along with the global epidemic of diabetes, have contributed to the growing interest in alternative therapies for managing the disease more safely and effectively. Herbal medicines are increasingly recognized as a viable alternative for controlling blood sugar levels. The objective of the study was to evaluate the antidiabetic activity of three medicinal plants (*A. obesum, C. glandulosum, and S. sativa*) using streptozotocin-induced non-insulin-dependent diabetes mellitus (NIDDM) in Wistar rats. The study investigated the effects of plant extracts on blood glucose, insulin levels, and various biochemical markers. In addition, the in vitro enzyme inhibitory activity of the plant extracts was studied. The administration of polyherbal extract orally at doses of 200 and 400 mg/kg significantly decreased the blood glucose levels compared to the control diabetic rats. The results indicate significant antidiabetic activity in the plant extracts, confirming their potential use as natural treatments for diabetes.

Keywords: Antidiabetic activity, *A. obesum*, *C. glandulosum*, *S. sativa*, streptozotocin, NIDDM, enzyme inhibitory activity

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, continues to be a global health challenge due to its rising prevalence. It is characterized by hyperglycemia, which

results from defects in insulin secretion, insulin action, or both. Glucose homeostasis involves maintaining a balance between hepatic glucose production and peripheral glucose uptake and utilization, with insulin playing a central role in regulating this balance **[1-4]**. Diabetes mellitus is broadly classified into several types, including type 1, type 2, gestational diabetes, and other specific forms. Type 1 diabetes results from the destruction of beta cells, leading to an absolute insulin deficiency. Type 2 diabetes occurs due to a progressive insulin secretory defect paired with insulin resistance. Gestational diabetes mellitus (GDM) typically develops during the second or third trimester of pregnancy and is not overtly diagnosed before this stage. Other, less common forms of diabetes include monogenic diabetes syndromes like neonatal diabetes and maturity-onset diabetes of the young (MODY), as well as diabetes arising from pancreatic diseases such as cystic fibrosis, or induced by drugs or chemicals, such as in the treatment of HIV/AIDS or after organ transplantation (American Diabetes Association) **[1, 5]**.

Diabetes mellitus is a serious metabolic disorder with profound impacts on patients' health, quality of life, and life expectancy **[6]**. The World Health Organization reports a rapid rise in diabetes in India, foreshadowing a potential epidemic. Over 62 million individuals are currently diagnosed with diabetes in India, and future projections predict a continued sharp increase **[7]**. In 2000, India led the world with 31.7 million cases of diabetes mellitus, followed by China (20.8 million) and the United States (17.7 million). By 2030, the global prevalence of diabetes is expected to rise from 171 million in 2000 to 366 million, with India anticipated to experience the largest surge, approaching 80 million cases **[8]**.

Although various synthetic drugs are available for managing hyperglycemia, they often carry significant side effects, including hyperglycemia at higher doses, dermatological reactions, liver problems, nausea, vomiting, generalized hypersensitivity, lactic acidosis, and diarrhea. These drugs can also contribute to weight gain, exacerbating type 2 diabetes. The secondary complications associated with synthetic drugs limit their utility in diabetes management **[1, 9]**. Therefore, there is an increasing demand for safe, economical alternatives to treat diabetes mellitus.

Medicinal plants have gained significant attention due to their natural origin and reduced side effects **[10]**. The World Health Organization has also endorsed the use of herbal medicines, particularly in regions where access to conventional diabetes treatments is limited. In comparison to single-plant remedies, polyherbal

formulations—combinations of various plants—are considered more effective due to their synergistic action. These formulations enhance potency and reduce the required concentrations of individual herbs, thereby minimizing adverse effects **[11-13]**.

Despite advancements in synthetic antidiabetic drugs, the search for alternative treatments using medicinal plants is ongoing, especially in traditional systems of medicine. This study focuses on three plants, *A. obesum, C. glandulosum, and S. sativa* that have been traditionally used for their potential antidiabetic properties.

MATERIALS AND METHODS

Materials

Collection and authentication of Plant Materials

The isolation of active chemical constituent of *A. obesum, C. glandulosum and S. sativa* flowers were done by collecting them form the local botanical garden.

Chemicals

Methanol, ethyl acetate, hexane, chloroform and butanol were purchased from Merck (Dramstadt, Germany). Sodium sulfate and charcoal were purchased from Sigma-Aldrich. Vaccum liquid chromatography was performed on silica gel $60GF_{254}$ and flash column chromatography on silica gel 60 (E. Merck 1.09385, Model Aldrich), while size exclusion chromatography was achieved by using Sephadex LH-20 (Ameshem Bioscience), pre-swollen in the specified solvent before loading on to the column.

Animal Selection and Care

Male Wistar rats (150–230 g) were chosen for this study and housed in polyacrylic cages under a controlled 14-hour light-dark cycle. Standard laboratory pellet food and water were provided. Rats were acclimated for ten days prior to the experiments. All procedures were approved by the institutional ethical committee, following the animal care guidelines.

Methods

Extraction, Fractionation and Purification

Powder of flower of *A. obesum C. glandulosum and S. sativa* were extracted in methanol and the crude extract was portioned between aqueous, ethyl acetate, butanol, methanol and 70: 30 (methanol: water). The EA-phase was dried with sodium sulfate (anhydrous), charcoaled and evaporated invacuo to give a thick residue which was subjected to normal pressure column chromatography on gradient system by using hexane, chloroform and methanol and afforded to five pure compound Honghelin, Obeside B and C, Betulin, Rosmerinic acid, Quercetin and flavones.

Preliminary Phytochemical Screening of Extract

The methanolic extracts of flowers of *A. obesum C. glandulosum and S. sativa* were

subjected to preliminary phytochemical tests to detect the presence of alkaloids, steroids, saponins, glycosides, anthraquinones, tannins, terpenoids, coumarins, carbohydrates and flavonoids using standard techniques.

Antidiabetic activity

A diabetic rat model produced by streptozotocin was used to evaluate the antidiabetic efficacy of *A. obesum C. glandulosum and S. sativa*

Selection of animals

Animal care and handling

Wistar male rats weighing between 150 and 230 g were used in the tests. Six rats or less per cage, each measuring 38 by 23 by 10 centimetres, were kept in the polyacrylic enclosures. A fourteen-hour light-dark cycle was employed to maintain the cages' consistent temperature of 25±2˚C. A normal dry pellet food and water were given to the rats on an as-needed basis. The experiment was scheduled to start after the rats had had ten days to get used to the lab setup. The animal ethics committee's rules were adhered to and the institutional ethical committee gave its approval to all experimental operations.

NIDDM

To induce non-insulin dependent diabetes mellitus, rats weighing between 150 and 230 g were starved for the whole night.

Diabetes was induced with the medication streptozotocin. After 15 minutes of nicotinamide treatment (120 mg/kg i.p.), one intraperitoneal dosage of 60 mg/kg streptozotocin was administered. Regular saline was used to dissolve the nicotinamide, whereas citrate buffer, which had a pH of 4.5, was used to dissolve the STZ. Hyperglycemia was seen in plasma glucose measurements done 72 hours and 7 days after the induction. A fasting plasma glucose level of >126 mg/dl was used to diagnose diabetes. Rats with chronic NIIDM were the only ones selected for the research.

Experimental design

The animals were segregated into four groups, each consisting of six animals.

Group I: Normal control rats, administered with 25% Tween 20 in distilled water.

Group II: Diabetic control rats, treated with 25% Tween 20 in distilled water.

Group III: Diabetic rats receiving an effective dose of flowers extract in 25% Tween 20 in distilled water.

Group IV: Diabetic rats receiving Metformin (250 mg/kg body weight) in 25% Tween 20 in distilled water.

Blood glucose levels will be monitored every week on the seventh, fourteenth, and twenty-first days of the experiment. Using an Acuchek-sensor glucometer, blood glucose levels will be determined via tail tipping method. The rats in group II will be given an oral dosage of thousand mg/kg of flowers extract for a period of 21 days. The Glucometer with Accuchek sensor will be

used to estimate the blood glucose levels. ANOVA will be used to analyse the data, which will then be tabulated and visually presented before the Dennett test is performed.

Estimation of insulin level

Blood samples were taken from the animals on the twenty-first day of the therapy in order to measure their insulin levels. To measure the serum insulin levels, the GLAZYME INSULIN-EIA TEST was utilised.

Estimation of biochemical parameters

In order to assess several biochemical markers, the animals were put down on the twenty-first day via cervical dislocation. TGL, HDL, LDL, and HDL were measured using the glucose oxidase protocol. Triglyceride and cholesterol test kits (Minias Globe Diagnostic kit) and a semi-automated analyzer were used in the analysis.

Additionally, using commercially available kits from Crest Biosystems (India), serum samples from both groups were examined for the presence of AST, ALT, ALP, LDH, urea, and creatinine.

Statistical Analysis

The findings are reported as the mean ± SEM derived from six distinct experiments. Statistical evaluation was conducted through ANOVA along with Dunnett's test to determine the significance of variations among the groups. A meaning threshold was set at $P < 0.05$.

In vitro enzyme inhibitory activity

The medicinal plant has antidiabetic qualities because of its strong phytoconstituent composition. The secondary metabolites in the plant have the ability to increase the release of insulin in addition to improving glucose transport and metabolism in muscles. The action of natural products on carbohydrate-binding sites is thought to be the mechanism. The key to this process is the well-known debranching capacity of the enzymes α-glucosidase and α-amylase. Before being absorbed, these enzymes hydrolyze dietary polysaccharides into disaccharides and monosaccharides. Bioactive substances like flavonoids and polyphenols inhibit these enzymes, delaying the digestion and absorption of carbs and so reducing postprandial glucose levels.

α-glucosidase enzyme inhibitory activity

Assay Procedure: The assessment of the α-glucosidase enzyme's inhibitory activity involved monitoring the release of 4-nitrophenol from p-nitrophenyl-D-glucopyranoside. A mixture of 3.3 milliliters of 10 mM p-nitrophenyl α-D-glucopyranoside, 0.1 milliliters of potassium phosphate (pH 6.8), and the enzyme α-glucosidase was prepared. Acarbose, LE, and ODE solutions at various concentrations (0.2 ml) were added to the

mixture as supplements. In every test tube, there was 1.7 cc of liquids.

Following the incubation at 37°C for 30 minutes, the reaction mixtures were stopped by adding 2.0 ml of 100 mM sodium carbonate. The measurement of the released p-nitrophenol was conducted at 400 nm using a spectrophotometer. The % inhibition rates were then calculated using the formula below:

%Inhibition = $Abs_{control}$ – Abs_{sample}/ $Abs_{control}$ X 100

RESULT AND DISCUSSION

Preliminary Phytochemical Screening of Extract

The extract obtained after extraction of the plant material viz., *A. obesum, C. glandulosum and S. sativa* (Flower) were subject to phytochemical screening which revealed the present of various active phytocontituents. The result were presented in **Table 1, 2 and 3**.

Table 1: Preliminary Phytochemical Screening of *A. obesum*

S. No.	Chemical Constituents	Methanolic	Aqueous	Pet. Ether	Chloroform
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Glycosides	+	+	+	+
4	Steroids	+	+	+	+
5	Flavonoids	+	+	+	+
6	Saponins	+	+	+	+
7	Fixed oils and fats	-	-	-	-
8	Tannins	+	+	+	+
9	Proteins and amino acids	+	-	+	-
10	Terpenoids	+	-	-	-

Abbrevations: (+) is positive; (-) is Negative

Table 2: Preliminary Phytochemical Screening of *C. glandulosum*

S. No.	Chemical Constituents	Methanolic	Aqueous	Pet. Ether	Chloroform
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Glycosides	+	+	+	+
4	Steroids	+	+	+	+
5	Flavonoids	+	+	+	+
6	Saponins	+	+	+	+
7	Fixed oils and fats	-	-	-	-
8	Tannins	+	+	+	+
9	Proteins and amino acids	+	-	+	-
10	Terpenoids	+	-	-	-

Abbrevations: (+) is positive; (-) is Negative

Table 3: Preliminary Phytochemical Screening of *S. sativa*

S. No.	Chemical Constituents	Methanolic	Aqueous	Pet. Ether	Chloroform
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Glycosides	+	+	+	+
4	Steroids	+	+	+	+
5	Flavonoids	+	+	+	+
6	Saponins	+	+	+	+
7	Fixed oils and fats	-	-	-	-
8	Tannins	+	+	+	+
9	Proteins and amino acids	+	-	+	-
10	Terpenoids	+	-	-	-

Abbrevations: (+) is positive; (-) is Negative

Quantitative Analysis

Plotting concentration versus absorbance allowed for the calculation of the entire phenolic and flavonoid content of *A. obesum, C. glandulosum and S. sativa* using the cal. curves shown in **Figure 1 and 2**.

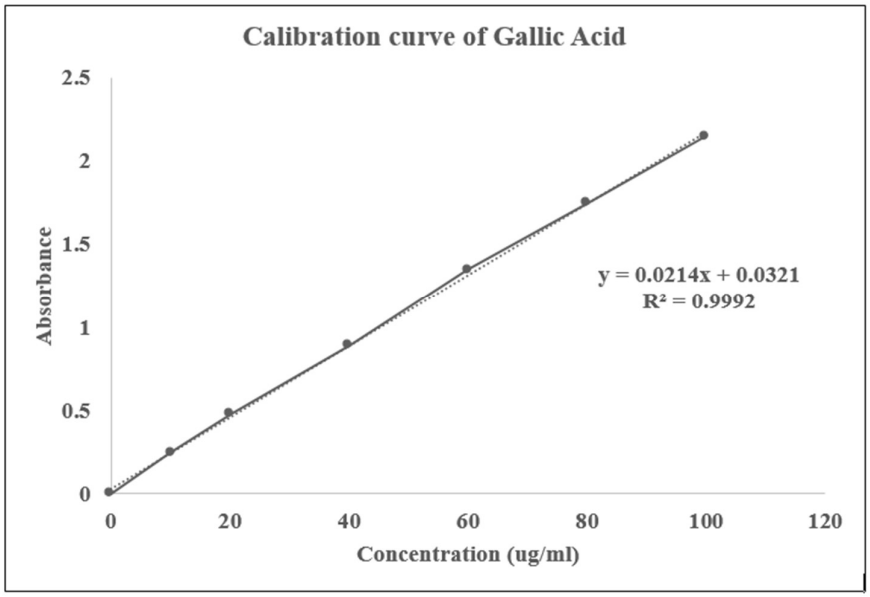

Figure 1: Calibration Curve of Gallic acid

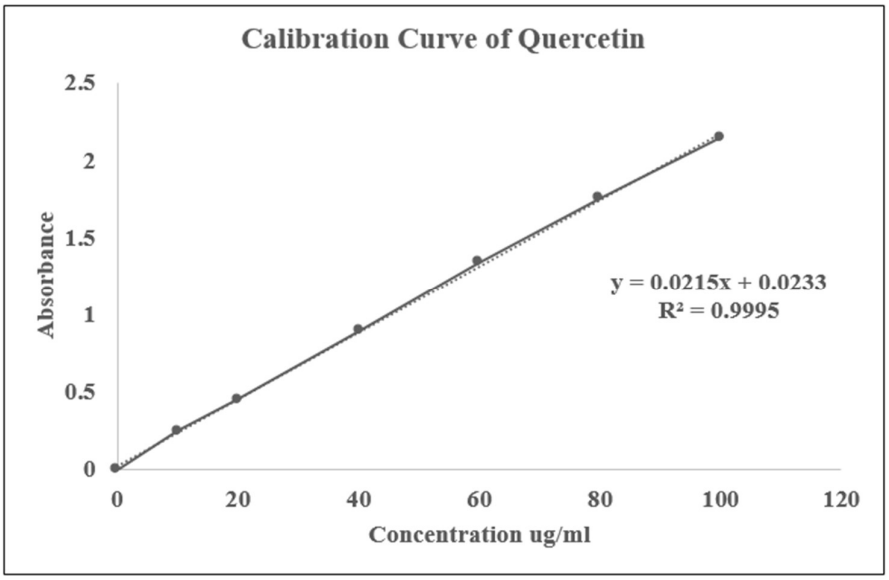

Figure 2: Calibration curve of Quercetin

The total phenolic content of the methanolic and ethyl acetate extracts of *A. obesum, C. glandulosum and S. sativa* (at concentrations of Ten µicrog/mL, twenty µicrog/mL, forty µicrog/mL, sixty µicrog/mL, eighty µicrog/mL, and hundred µicrog/mL) was determined to be 360.25±10.12 and 295.89±11.25 mg per

Gallic acid equal (mg/g GAE), correspondingly, through the utilization of a calibration curve and absorbance values.

A calibration curve was created using the absorbance values of the methanolic and ethyl acetate extracts of *A. obesum, C. glandulosum and S. sativa* (Ten µicrog/ml, twenty µicrog/ml, forty µicrog/ml, sixty µicrog/ml, eighty µicrog/ml, and hundred µicrog/ml). This enabled for the calculation of the total flavonoid content. These values were derived by using the extracts' absorbance measurements and a calibration curve.

Acute Toxicity Study of Extract (LD50)

A. obesum, C. glandulosum and S. sativa (leaves) were the plant material(s) whose methanolic extract was evaluated for acute toxicity and LD50 using OECD guideline no. 423. The results showing the effective dosage (ED50) are shown in **Table 4**.

Table 4: LD50 and ED50 of *A. obesum, C. glandulosum and S. sativa*

Plant Name	LD50	ED50
A. obesum	5000mg/kg	50mg/kg
C. glandulosum	5000mg/kg	50mg/kg
S. sativa	5000mg/kg	50mg/kg

Antidiabetic activity

In comparison to the non-diabetic control group, the diabetic control rats exhibited significantly elevated levels of serum alanine transaminase, total cholesterol, glucose, and aspartate transaminase.

Effects of *A. obesum, C. glandulosum and S. sativa* in oral glucose acceptance in normal rats

Table 5 presents the blood glucose levels after the administration of oral glucose (2 g/kg), Metformin (250 mg/kg), and methanolic extract of *A. obesum, C. glandulosum and S. sativa* (200 mg and 400 mg/kg) at different time intervals (0, 30, 60, 120, and 150 minutes) in the control group. The blood glucose levels in all groups peaked at the 30-minute mark. Notably, at the 150-minute mark, the blood glucose levels were lower in the 400 mg MEAO, MECG and MESS treated groups compared to the control group.

Table 5: Result of MEAO, MECG and MESS in oral glucose acceptance in normal fasted rats

S. No.	Group	Blood Glucose level mg/dl (Mean±SD)				
		Fasting	Post Treatment			
			30 min	60min	90min	120min
1	Control 0.3% CMC	92.45±1.23	122.25±1.53	132.15±1.13	120.45±1.23	93.22±0.22
2	Metformin (250mg/kg) + Glucose	93.35±0.21	292.15±1.67	192.45±1.64	120.45±1.91	98.45±1.24
3	MEAO+MECG +MESS (200mg/Kg)+ Glucose	94.25±1.15	295.45±1.23	198.25±0.83	118.45±0.73	97.15±0.23
4	MEAO+MECG +MESS (400mg/Kg)+ Glucose	93.15±0.23	296.05±0.73	195.45±0.67	112.05±0.76	95.45±0.23

There is a mean ± SD for values. Dunncan's numerous range tests come after the ANOVA. P < 0.05 indicates a substantial difference between values without a common superscript.

Effect of MEAO, MECG and MESS on plasma glucose level in usual fasted rats

In euglycemic rats, **Table 6** displays the plasma glucose levels at 0, 1, 2, and 4 hours post-injection. It's interesting to note that euglycemic rats administered two hundred mg and four hundred mg/kg of the methanolic extract of MEAO, MECG and MESS, as well as 250 mg/kg of metformin, did not exhibit any significant changes after 1 hour, but the group did at 4 hours ($p < 0.05$).

Table 6: Effect of MEAO, MECG and MESS on plasma glucose level in usual rats

S. No.	Group	Blood Glucose level mg/dl (Mean±SD)			
		Fasting	Time (h) after Treatment		
			1h	2h	4h
1	Control 0.3% CMC	92.45±1.23	132.15±1.13	93.22±0.22	92.12±0.12
2	Metformin (250mg/kg) +Glucose	93.35±0.21	192.45±1.64	98.45±1.24	96.05±0.24
3	MEAO+MECG +MESS (200mg/Kg)+Glucose	94.25±1.15	198.25±0.83	97.15±0.23	95.55±0.23
4	MEAO+MECG +MESS (400mg/Kg)+ Glucose	93.15±0.23	195.45±0.67	95.45±0.23	93.12±0.13

The mean ± SD is used to express values. Duncan's multiple range tests are conducted after an ANOVA. Values that do not have a common superscript show a significant difference at P < 0.05.

Effect of *A. obesum, C. glandulosum and S. sativa* on blood glucose level in alloxin induced diabetic rats

Rats with diabetes and normal rats' blood glucose levels are displayed in **Table 7** for the 0, 1, 2, and 4-hour periods following treatment. It is evident that diabetic rats' blood glucose levels were notably higher than those of normal control rats. Significantly, administration of Metformin (250 mg/kg) and the methanolic extract of MEAO, MECG and MESS (200 mg and 400 mg/kg) led to a decrease in plasma glucose levels in diabetic rats associated to the control group. Moreover, a notable hypoglycemic effect was observed in the diabetic group on the fourth day of treatment with 400 mg of MEDH and Metformin.

Table 7: Effect of *A. obesum, C. glandulosum and S. sativa* in blood glucose levels in alloxan induced diabetic rats

S. No.	Group	Blood Glucose level mg/dl (Mean±SD)			
		Fasting	Time (h) after Treatment		
			1h	2h	4h
1	Control 0.3% CMC	92.45±1.23	132.15±1.13	93.22±0.22	92.12±0.12
2	Diabetic control (Alloxan)	93.75±1.23	133.17±1.03	94.02±0.12	93.16±0.18
3	Metformin (250mg/kg) + Glucose	93.25±0.21	190.27±0.64	97.15±1.24	95.05±0.22
4	MEAO+MECG +MESS (200mg/Kg)+ Glucose	94.25±1.15	198.25±0.83	97.15±0.23	93.55±0.23
5	MEAO+MECG +MESS (400mg/Kg)+ Glucose	93.15±0.23	195.45±0.67	95.45±0.23	93.12±0.13

The mean ± SD is used to express values. Duncan's multiple range tests are conducted after an ANOVA. Values that do not have a common superscript show a significant difference at P < 0.05.

Result of *A. obesum, C. glandulosum and S. sativa* on serum AST and ALT and liver glycogen in alloxan encouraged diabetic rats

Table 8 displays the hepatic glycogen content and blood AST and ALT concentrations in both experimental and normal rats. Interestingly, the diabetic control rats' blood levels of AST and ALT dramatically rose, whereas the non-diabetic control rats' liver glycogen content dropped. In diabetic rats, however, Metformin (250 mg/kg) and the methanolic extract of MEAO+MECG+MESS (two hundred mg and four hundred mg/kg) meaningfully red. The levels of AST and ALT while concurrently increasing the body's glycogen content proportional to the control group.

Table 8: Result of *A. obesum, C. glandulosum and S. sativa* on serum ALT and AST and liver glycogen in alloxan encouraged diabetic rats

S. No.	Group	AST (U/L)	ALT(IU/L)	Glycogen (g/100g)
1	Control 0.3% CMC	8.25±0.21	29.27±0.64	3.0±1.24
2	Diabetic control (Alloxan)	94.25±1.15	92.25±0.83	1.1±0.23
3	Metformin (250mg/kg) + Diabetic	32.25±0.21	30.27±0.64	3.1±1.24
4	MEAO+MECG +MESS (200mg/Kg)+ Diabetic	30.25±1.15	30.25±0.83	4.1±0.23
5	MEAO+MECG +MESS (400mg/Kg)+ Daibetic	28.25±0.21	29.27±0.64	3.5±1.24

There is a mean ± SD for values. Dunncan's numerous range tests come after the ANOVA. P < 0.05 indicates a substantial difference between values without a common superscript.

Effect of *A. obesum, C. glandulosum and S. sativa* on serum cholestrol and triglycerides alloxan induced diabetic rats

In comparison to control rats, diabetic rats exhibited significantly elevated levels of triglycerides and cholesterol. However, as indicated in **Table 9**, the dose of Metformin (250 mg/kg) and the methanolic extract of MEAO+MECG+MESS (200 mg and 400 mg/kg) effectively normalized the triglyceride and fat levels in the rats.

Table 9: Effect of *A. obesum, C. glandulosum and S. sativa* on serum lipid and triglycerides in alloxan encouraged diabetic rats

S. No.	Group	Cholesterol (mg/dl)	Triglyceride(mg/dl)
1	Control 0.3% CMC	120.25±0.21	101.27±0.64
2	Diabetic control (Alloxan)	230.25±1.15	182.25±0.83
3	Metformin (250mg/kg) + Diabetic	190.25±0.21	108.27±0.64
4	MEAO+MECG +MESS (200mg/Kg)+ Diabetic	140.25±1.15	112.25±0.83
5	MEAO+MECG +MESS (400mg/Kg)+ Daibetic	125.25±0.21	102.27±0.64

There is a mean ± SD for values. Dunncan's numerous range tests come after the ANOVA. P < 0.05 indicates a substantial difference between values without a common superscript.

CONCLUSION

The antidiabetic effects observed in the polyherbal extracts may be attributed to the chemically active compounds present in the studied plants. The hypoglycemic activity of these formulations is likely due to the presence of glycosides, flavonoids, tannins, and saponins in the extracts. Based on the findings of this study, it can be concluded that polyherbal formulations containing extracts from *A. obesum, C. glandulosum and S. sativa* demonstrated significant antidiabetic effects by effectively regulating blood glucose levels.

In rats with alloxan-induced diabetes, a seven-day combination therapy of *A. obesum, C. glandulosum and S. sativa* in a 1:1:1 ratio markedly enhanced pancreatic β-cell function and considerably decreased fasting blood glucose levels. Furthermore, the lipid profile stayed within typical bounds. It was shown that a single oral dosage of up to 500 mg/kg BW of the combination of *A. obesum, C. glandulosum and S. sativa* was safe. When likened to the control group, there were no appreciable vicissitudes in the following parameters: body weight, comparative organ weight (heart, liver, and kidneys), or haematological and biochemical indicators.

For this reason, *A. obesum, C. glandulosum and S. sativa* together appear to have promise as a safe anti-diabetic drug. Additional education is obligatory to elucidate the antidiabetic mechanism of this combination and look into potential adjustments to the dosage ratio of *A. obesum, C. glandulosum and S. sativa*. In addition, for a thorough safety assessment, sub-chronic and chronic toxicity studies are advised.

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