



TO EVALUATE THE ANTI-OBESITY ACTIVITY OF *SALVIA SPLENDENS* EXTRACT IN HIGH FAT DIET INDUCED RAT MODEL.

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Abstract: Obesity is a global epidemic with far-reaching health consequences, driving the need for effective treatment options. This study aimed to investigate the anti-obesity potential of *Salvia Splendens*, a medicinal plant, through comprehensive phytochemical screening and pharmacological evaluation. The phytochemical analysis revealed the presence of diverse bioactive compounds, including alkaloids, flavonoids, terpenoids, tannins, saponins, and cardiac glycosides, which have been associated with various health benefits. The pharmacological evaluation in a high-fat diet-induced obese rat model demonstrated that the methanolic extract of *Salvia Splendens* (MESS) significantly reduced body weight, improved blood lipid profiles, and exhibited protective effects on the liver, compared to the positive control group. The findings suggest that *Salvia Splendens* possesses multifaceted therapeutic potential in the management of obesity and related metabolic disorders, warranting further investigations to elucidate the underlying mechanisms and explore its clinical applications.

Index Terms - Obesity, Global epidemic, Health consequences, Effective treatment options, *Salvia Splendens*, Medicinal plant, Anti-obesity potential.

I. INTRODUCTION

Obesity is a serious and growing global health concern that has reached epidemic proportions worldwide. The term "obesity" is derived from the Latin word "Obesitas," which means "heavy" or "overweight." It is characterized by an excessive accumulation of body fat, which can significantly impact an individual's overall health and well-being [1].

Body mass index (BMI), calculated by dividing an individual's weight in kilograms by the square of their height in meters, is the commonly used metric to classify obesity. According to the World Health Organization (WHO), a BMI of 30 kg/m² or higher is considered obese [2].

Obesity is not just a cosmetic issue; it is a complex, multifactorial condition that is closely linked to various chronic health problems. Individuals with obesity are at an increased risk of developing a wide range of comorbidities, including type 2 diabetes, cardiovascular diseases, hypertension, certain types of cancer, respiratory disorders, and musculoskeletal problems [3,4]. The harmful effects of obesity on an individual's physical and mental health, as well as the considerable socioeconomic burden it imposes, have made it a global public health priority [5].

The rising prevalence of obesity is a global phenomenon, with significant variations across different regions and populations. In recent decades, the prevalence of obesity has increased dramatically, not only in developed countries but also in developing nations. The WHO estimates that by the year 2025, approximately 2.3 billion adults will be overweight, and more than 700 million will be obese [6].

The etiology of obesity is complex and multifactorial, involving the interplay of genetic, environmental, behavioral, and socioeconomic factors [7,8]. Effective management of obesity requires a multifaceted approach that addresses the underlying causes and associated comorbidities.

In recent years, there has been a growing interest in exploring the potential of natural, plant-based remedies for the management of obesity and related metabolic disorders. One such plant that has garnered attention for its potential antiobesity properties is *Salvia Splendens*, a member of the Lamiaceae family [9].

II. Method and Materials:

Collection and Authentication of Plant Material:

Salvia Splendens were collected from the Nurserylive, City Vista, Kharadi, Pune, Maharashtra - 411014 India. The plant materials were identified, verified, and authenticated by Prof. Kajal Apale, Botanist, Vidyabharti Mahavidyalaya, Amravati, Maharashtra, India.

Experimental Animals:

The experiment was performed on Wistar rats, 150-160 g, which were obtained from the animal house of the Department of Pharmacology, Vidyabharati College of Pharmacy, Amravati. All the animals were acclimatized to the animal house prior to use. They were kept in cages in the animal house with a 12 h light: 12 h dark cycle. Animals were fed on pellets and tap water ad libitum. The care and handling of rats were in accordance with the internationally accepted standard guidelines for the use of animals (CPCSEA). Permission (Registration number - 1504/PO/RE/S/11/CPCSEA) and approval for animal studies were obtained from the Institutional Animal Committee (IAEC) of Vidyabharati College of Pharmacy, Amravati, SGB Amravati University.

Drugs and Chemicals:

Test Drug: *Salvia Splendens* were obtained from the Nurserylive, City Vista, Kharadi, Pune, Maharashtra - 411014 India.

Standard Drug: Simvastatin (SVS) tablet manufactured in India by Sun Pharmaceutical Ltd. and brought from a pharmacy store in Amravati.

Other Chemicals: Saline Water, Chloroform, Formalin.

Preparation of Plant Extract:

The leaves of *Salvia Splendens* were washed thoroughly with distilled water to remove any dirt or debris. The leaves were then dried under shade or in a drying oven at a temperature not exceeding 40°C. The dried leaves were ground into a fine powder using a blender or a mortar and pestle.

The powdered leaf sample (20 g) was placed in a cellulose or paper thimble and inserted into the Soxhlet extractor. Methanol (100-200 mL) was poured into the round-bottom flask of the Soxhlet apparatus. The Soxhlet apparatus was assembled, ensuring all connections were secure. The round-bottom flask was heated using a heating mantle or a hot plate, ensuring the methanol was boiling and the Soxhlet extraction cycle was maintained. The extraction was continued for 4-8 hours, or until the solvent in the siphon tube appeared colorless.

Solubility Analysis:

The solubility analysis of MESS of the leaves was carried out using different solvents. The extract was found to be soluble in water.

Phytochemical Screening Tests:

Standard phytochemical screening tests were performed to identify the presence of various classes of secondary metabolites in *Salvia Splendens*, including alkaloids, flavonoids, terpenoids, tannins, saponins, and cardiac glycosides.

Selection of Animal Species and Housing:

A total of 30 Wistar rats, weighing 150-160 g, were used in the study. The animals were divided into five groups, with 6 rats in each group. All the rats were trained before starting the dosing protocol for easy handling and dosing. The animals were maintained at $22 \pm 3^\circ\text{C}$ in a 12 h light: 12 h dark cycle with free access to food and water.

Treatment Protocol:

The treatment protocol is summarized in the table below:

Sr. no.	Group	No. of Animals	Treatment and Dose	Route of Administration
1	I (Positive Control)	6	Induced High fat diet.	Oral
2	II (Std)	6	Simvastatin	Oral
3	III (Treatment 1)	6	Induced High fat diet + Low dose of MESS (200 mg/kg)	Oral
4	IV (Treatment 2)	6	Induced High fat diet + Moderate dose of MESS (300 mg/kg)	Oral
5	V (Treatment 3)	6	Induced High fat diet + High dose of MESS (400 mg/kg)	Oral

Table No.01: Treatment Protocol

Determination of Acute Toxicity:

The acute oral toxicity study of *Salvia Splendens* was carried out according to the fixed-dose procedure of the OECD Guideline 423. The safe dosage range of the methanolic extract of *Salvia Splendens* (MESS) for oral administration was found to be 50 – 2000 mg/kg body weight per day.

Methodology:

The high-fat diet (HFD) induced obesity in rats is considered a reliable tool for the evaluation of antiobesity activity. The study comprised 5 groups with 6 animals in each group. Group 1 represented the positive control, where the rats were fed a high-fat diet (HFD) for a period of 45 days. Group 2 represented the standard control, where rats were treated with simvastatin (3 mg/kg). Groups 3, 4, and 5 represented the test treatment groups, where rats were treated with the low dose of MESS (200 mg/kg), moderate dose of MESS (300 mg/kg), and high dose of MESS (400 mg/kg), respectively, along with the high-fat diet.

The HFD consisted of 58% fat, 25% protein, and 17% carbohydrate, with the addition of lard (13%), cholesterol (1%), and vitamins and minerals (0.6%).

Pharmacological Evaluation Parameters:➤ **Measurement of Body Weight:**

The body weight of the individual rat was recorded initially and at one-week intervals until the end of the experiment or the death of the rat.

➤ **Biochemical Estimations:**

On the 45th day of the experiment, all the animals were sacrificed by euthanasia, and blood samples were collected for the analysis of high-density lipoproteins (HDL-C), low-density lipoproteins (LDL-C), and very-low-density lipoproteins (VLDL-C).

➤ **Liver Histopathology:**

The animals were sacrificed by euthanasia, and the liver was removed for histopathological evaluation.

Statistical Analysis:

The data obtained from the screenings were subjected to statistical analysis using one-way ANOVA followed by Dunnett's and Tukey's multiple comparison tests to assess the statistical significance of the results using GraphPad Prism-5 software. P-values less than 0.05 were considered statistically significant.

III. Results:**Phytochemical Screening:**

The phytochemical analysis of MESS revealed the presence of various bioactive compounds, including alkaloids, flavonoids, terpenoids, tannins, saponins, and cardiac glycosides.

Phytochemical Class	Test	Result
Alkaloids	Dragendorff's test	+
	Mayer's test	+
	Wagner's test	+
Flavonoids	Shinoda test	+
	Alkaline reagent test	+
Terpenoids	Salkowski test	+
	Liebermann-Burchard test	+
Tannins	Ferric chloride test	+
	Gelatin test	+
Saponins	Foam test	+
	Haemolysis test	+
Cardiac Glycosides	Keller-Killiani test	+
	Legal's test	+
Proteins	Biuret test	+

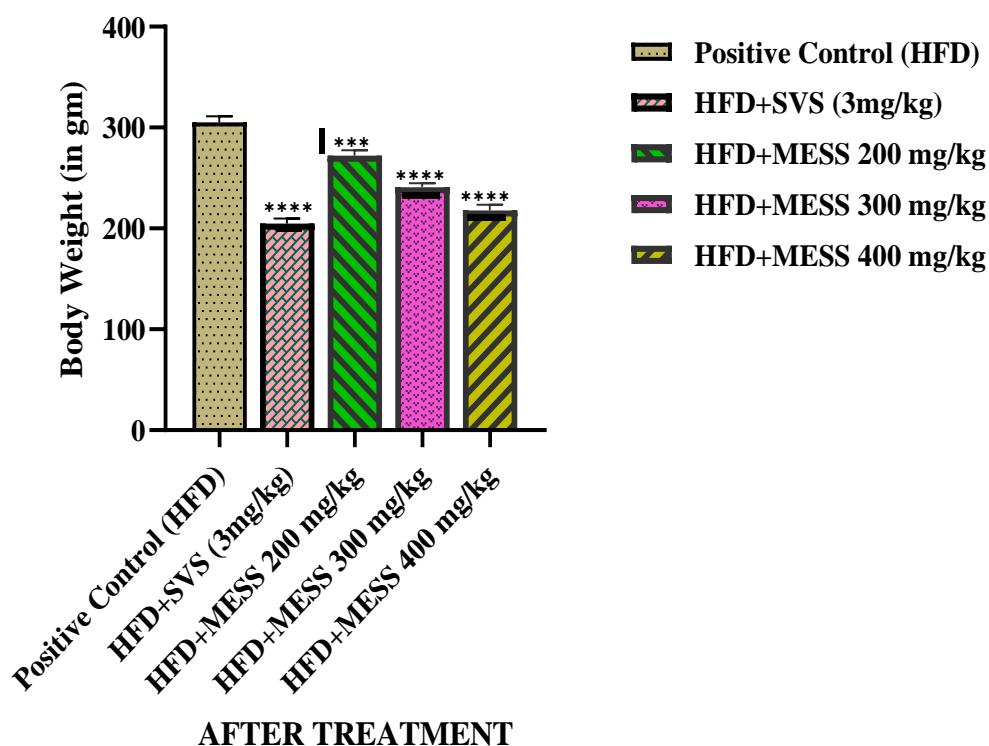
Table No. 02: Phytochemical Screening

Effect on Body Weight:

The effect of MESS on body weight in the high-fat diet (HFD) induced obese rats is shown in Figure 1. The positive control group (HFD) exhibited a significant increase in body weight compared to the initial body weight.

In contrast, the MESS treatment groups showed a dose-dependent reduction in body weight gain. The groups receiving 300 mg/kg and 400 mg/kg of MESS exhibited the most pronounced decrease in body weight, which was statistically significant compared to the positive control group ($p < 0.05$).

**Effect of MESS on Body Weight
At 7th Week**



AFTER TREATMENT

Figure 1. Effect of MESS on Body Weight in HFD-Induced Obese Rats

All data are expressed as mean \pm SEM for group of 6 rats in each. One-way Anova followed by Dunnett's multiple comparisons. Values are statistically Significant ***p = 0.0008 and ****P < 0.0001 for Final body weight as compared with Positive control.

Lipid Profile Analysis

The assessment of the lipid profile parameters revealed that the MESS treatment groups had a favorable impact on the lipid levels (Table 3).

Table 3. Effect of MESS on Lipid Profile in HFD-Induced Obese Rats

Parameters \rightarrow Groups \downarrow	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
HFD (Positive Control)	41.00 \pm 1.949	44.72 \pm 1.442	48.00 \pm 1.414
SVS 3 mg/kg	17.33 \pm 0.8819****	28.50 \pm 1.727****	26.50 \pm 1.118****
MESS 200 mg/kg	34.83 \pm 1.014**	37.93 \pm 0.5812*	40.15 \pm 1.250***
MESS 300 mg/kg	29.78 \pm 1.353****	33.22 \pm 1.117****	34.17 \pm 1.078****
MESS 400 mg/kg	20.27 \pm 0.5149****	30.00 \pm 1.236****	29.10 \pm 0.7280****

All data are expressed as mean \pm SEM for group of 6 rats in each. One-way Anova followed by Dunnett's multiple comparisons. Values are statistically Significant **P = 0.0061, *P = 0.0034, ***P = 0.0002 and ****P < 0.0001 for Blood parameter as compared with Positive control.

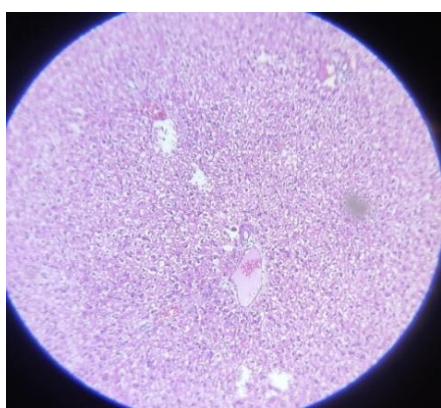
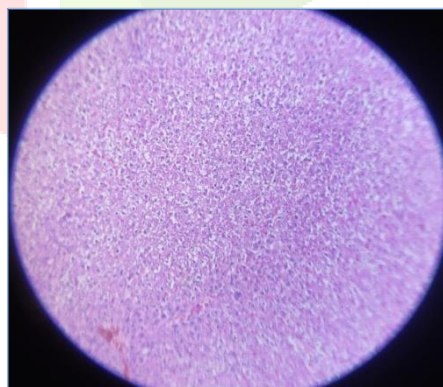
The MESS treatment groups exhibited a significant increase in HDL-cholesterol (HDL-C) levels and a significant decrease in LDL-cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) levels compared to the positive control group (p < 0.05). The effects were more pronounced in the higher dose groups (300 mg/kg and 400 mg/kg).

Histopathological Evaluation of the Liver:

The histopathological analysis of the liver samples revealed marked improvements in the MESS treatment groups compared to the positive control group.

The positive control group showed signs of fatty degeneration, cellular necrosis, and vascular congestion, indicating liver damage associated with the HFD. In contrast, the liver sections from the MESS treatment groups exhibited a recovery of normal hepatocyte architecture, reduced lipid accumulation, and decreased markers of liver injury.

The higher doses of MESS (300 mg/kg and 400 mg/kg) showed more pronounced protective effects on the liver, with a greater restoration of the normal liver histology.

**a. HFD****b. Standard (SVS)****c. HFD + MESS (200 mg/kg)****d. HFD + MESS (300 mg/kg)****e. HFD + MESS (400 mg/kg)****Fig No. 02 - Study of effect of *Salvia Splendens*, on liver histopathology.**

(CD: cellular degeneration, VCD: vascular congestion fatty deposition, FDH: foamy degeneration of hepatocytes, CV: central vein, and N: necrosis).

Discussion:

The phytochemical screening of *Salvia Splendens* revealed the presence of several important classes of secondary metabolites, including alkaloids, flavonoids, terpenoids, tannins, saponins, and cardiac glycosides. These phytochemicals have been associated with various health benefits, particularly in the context of obesity and related metabolic disorders.

The pharmacological evaluation of *Salvia Splendens* demonstrated its potent antiobesity activity in the high-fat diet induced obesity rat model:

Body weight: The *Salvia Splendens* treatment groups showed a significant reduction in body weight gain compared to the positive control group, with the higher doses (300 and 400 mg/kg) exhibiting the most pronounced effects.

Lipid profile: The *Salvia Splendens* treatment improved the lipid profile, significantly increasing HDL-cholesterol and decreasing LDL-cholesterol and VLDL-cholesterol levels, in a dose-dependent manner.

Liver histopathology: The liver sections from the *Salvia Splendens* treatment groups exhibited a recovery of normal hepatocyte architecture and reduced markers of liver injury, such as fatty degeneration and vascular congestion, indicating a protective effect on the liver.

These comprehensive results provide compelling evidence for the antiobesity potential of *Salvia Splendens*, which can be attributed to the presence of the diverse phytochemical constituents, including alkaloids, flavonoids, terpenoids, tannins, and saponins. Further research is warranted to elucidate the underlying mechanisms and translate these findings into viable therapeutic applications for the management of obesity and related metabolic disorders.

Conclusion:

The comprehensive phytochemical screening and pharmacological evaluation of *Salvia Splendens* provide compelling evidence for its potential as an effective antiobesity agent. The presence of diverse bioactive compounds, coupled with the observed improvements in body weight, lipid profile, and liver histopathology, underscores the therapeutic promise of this plant. Further research, including mechanistic studies and clinical trials, would be necessary to fully elucidate the underlying mechanisms and translate these findings into viable therapeutic applications.

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