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EVALUATION OF ANTIDIABETIC ACTIVITY OF HYPTIS SUAVEOLENS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

This study examines the antidiabetic potential of Hyptis suaveolens seed extract in Streptozocin (STZ)-induced diabetic rats. The experimental setup consisted of five groups to evaluate the efficacy of the extract. Wistar albino rats were categorized into five groups: Group I (normal control), Group II (diabetic control), Group III (diabetic + glibenclamide at 5 mg/kg b.wt./day orally), Group IV (diabetic + Hyptis suaveolens seed extract at 250 mg/kg) and Group V (diabetic + Hyptis suaveolens seed extract at 500 mg/kg). The extract was administered orally for a duration of 28 days. Various parameters were assessed, including fasting blood glucose levels, random blood glucose levels, serum insulin, HbA1c, C-peptide levels, and histopathological alterations in pancreatic tissues. The findings indicated a significant decrease in fasting blood glucose levels in Groups III, IV, and V in comparison to the diabetic control group. Notably, serum insulin and C-peptide levels were significantly elevated in Groups III, IV, and V, suggesting enhanced pancreatic beta-cell functionality. The treatment with Hyptis suaveolens seed extract in diabetic rats resulted in a marked reduction in HbA1c levels, implying an improvement in glucose metabolism and overall glycemic control. Histopathological examination demonstrated regeneration of pancreatic beta-cells and enhanced islet architecture in the treated groups. In conclusion, Hyptis suaveolens seed extract exhibited promising antidiabetic activity, underscoring its potential as a natural therapeutic option for diabetes management.

Key Words: Hyptis suveolensis seeds, Medicinal plants, Diabetic rats, Antidiabetic activity

Introduction

Hyptis suaveolens (L.) Piot. Commonly known as bush mint or pignut, is a perennial herbaceous plant belonging to the Lamiaceae family. This plant is native to tropical and subtropical regions and is widely distributed across Asia, Africa, and the Americas. Hyptis suaveolens has garnered significant attention in recent years due to its diverse pharmacological properties and potential therapeutic applications.

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The ethnomedicinal use of Hyptis suaveolens spans various cultures and traditions. It has been employed in traditional medicine to treat a wide array of ailments, including gastrointestinal disorders, respiratory conditions, and skin diseases. The leaves, stems, and seeds of Hyptis suaveolens are rich in essential oils, flavonoids, terpenoids, and other bioactive compounds, which contribute to its medicinal properties (1).

Phytochemical studies have revealed that Hyptis suaveolens contains significant amounts of 1, 8-cineole, β-caryophyllene, and sabinene, which are known for their antimicrobial, anti-inflammatory, and antioxidant activities (2). These compounds have been extensively studied for their role in combating various pathogens and mitigating oxidative stress-related conditions.

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels resulting from defects in insulin secretion, insulin action, or both. This condition affects millions of people worldwide and is associated with significant morbidity and mortality due to its complications, including cardiovascular disease, neuropathy, nephropathy, and retinopathy. Traditional medicine has long been a cornerstone in the management of diabetes, especially in developing countries where access to conventional medical treatment may be limited (3).

For centuries, various cultures have relied on traditional herbal remedies to manage diabetes and its symptoms. These remedies often involve the use of plants and natural products with hypoglycemic properties. Ethnobotanical studies have documented numerous plant species used in different traditional medicinal systems, including Ayurveda, Traditional Chinese Medicine (TCM), and traditional African medicine, to treat diabetes. These plants are rich in bioactive compounds such as alkaloids, flavonoids, terpenoids, and glycosides, which have been shown to modulate blood glucose levels through various mechanisms (4).

One of the key advantages of traditional medicine in diabetes management is its holistic approach. Unlike conventional treatments that primarily focus on lowering blood glucose levels, traditional medicine often emphasizes the overall well-being of the patient. This includes dietary modifications, lifestyle changes, and the use of medicinal plants that support overall health and help prevent the complications associated with diabetes (5).

Despite the widespread use and potential benefits of traditional medicine in diabetes management, it is crucial to scientifically validate these remedies. Rigorous clinical trials and pharmacological studies are necessary to establish the safety, efficacy, and mechanisms of action of these traditional treatments. Such research can bridge the gap between traditional knowledge and modern medicine, potentially leading to the development of novel antidiabetic therapies. Hence, the present study is undertaken to evaluate the antidiabetic activity of Hyptis suaveolens seeds in wistar albino rats.

In summary, Hyptis suaveolens is a versatile medicinal plant with a rich history of traditional use and a promising future in pharmacological research. Its diverse array of bioactive compounds and demonstrated therapeutic properties underscore its potential as a valuable resource in modern medicine.

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Animal Studies

Animals

This study adhered to the guidelines set forth by the Organisation for Economic Co-operation and Development (OECD) for animal testing. Ethical approval was granted by the Institutional Animal Ethical Committee at SVSMC, Mahabubnagar, under the specified project number SVSMC/IAEC no.2/2020 /648/A. Prior to commencing the experiments, all animals were thoroughly examined and allowed a period of acclimatization to reduce stress and establish a consistent baseline. The study used albino rats weighing between 150-190 grams, housed in controlled conditions with a temperature of 22 ± 3°C and relative humidity between 30% and 70%. The rats were kept on a 12-hour light/dark cycle to simulate natural environmental conditions.

Plant Material

The seeds of *Hyptis Suveolensis* were sourced from the local market for this research. A botanist, who is also an Assistant Professor in the Department of Botany at S.V. University, Tirupati, authenticated the plant material. The authenticated sample was assigned a voucher number 0709 for future reference.

The seeds were initially shade-dried, then coarsely powdered and sieved through a 40-mesh sieve to obtain a fine powder, which was stored in an airtight container. To extract active compounds, 100 grams of the dried powder was macerated in a 60% hydro-alcoholic solution for 7 days. After maceration, the mixture was filtered, and the solvent was evaporated from the filtrate to yield the concentrated extract of *Hyptis Suveolensis* (6).

Induction of Diabetes:

Diabetes was induced in the rats via a single intraperitoneal injection of streptozotocin (STZ) at a dose of 45 mg/kg body weight, administered after an overnight fast. To prevent hypoglycemia, the STZ-treated rats were given a 5% glucose solution for 24 hours post-injection. Rats with fasting blood glucose levels exceeding 200 mg/dl were considered diabetic (7, 8).

Experimental Design :

Group	Treatment	Description
Group I	Normal control	Normal rats receiving isotonic saline without induction of STZ
Group II	Diabetic Control	Diabetic rats receiving streptozotocin (STZ)
Group III	Standard	Diabetic rats treated with glibenclamide (5 mg/kg body weight/day orally)

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Group IV	HAHS 250mg/kgbw	Diabetic rats treated with hydro-alcoholic extracts of *Hyptis Suveolensis* seed extract (250 mg/kg once daily)
Group V	HAHS 500mg/kgbw	Diabetic rats treated with hydro-alcoholic extracts of Hyptis Suveolensis seed extract (250 mg/kg once daily)

Biochemical estimations:

We monitored the effects of the therapies on body weight by maintaining weekly records using an automated weighing scale. On day 1, one week after diabetes induction, and on day 29 of the therapy period, measurements of fasting blood glucose levels were taken. We conducted random blood glucose measurements on days 1, 7, 14, and 28 during the therapy period. Blood samples were obtained from the distal end of the rat's tail and examined using an Accu-Check Glucometer (9).

Following a treatment period of 28 days, Blood samples were obtained from rats that had fasted overnight using the retro-orbital venepuncture technique and then all the rats were sacrificed for tissue collection. The serum was separated from the blood using a centrifuge machine at a speed of 3000 rpm for 15 minutes. Before centrifugation, the blood was left to remain at room temperature for at least 30 minutes, following standard guidelines. The collected serum samples were subjected to the analysis HbA1C (Glycosylated haemoglobin), c-peptide and insulin levels immunosorbent assay kits (10).

Histopathological Analysis:

After blood collection, pancreas were excised, fixed in buffered formalin, sectioned, and prepared for histopathological examination. Pancreatic tissue blocks were embedded in paraffin, sectioned, and stained with haematoxylin and eosin for examination under a light microscope (11).

Statistical analysis:

The data are presented as mean ±SEM. The data were subjected to statistical analysis using one-way ANOVA, followed post-hoc multiple comparison test by using graph pad prism software. Significant differences in the means were determined at a significance level of $p \leq 0.05$.

Results:

The changes in body weight of the normal control, diabetic control, and experimental rats are illustrated in Table 1. Diabetic rats that received hydro alcoholic extract Hyptis suaveolens seed (HAHS) at various doses (250, and 500 mg/kg b.wt./day) exhibited a gradual increase in average body weight over time, in comparison to their beginning body weight. However, this gain was somewhat lower than that observed in normal control rats.

The alterations in fasting blood glucose levels in both the normal control and experimental rats

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are presented in Table 2. Group I, consisting of normal control rats, maintained a consistent blood glucose level throughout the whole duration of the trial. On the other hand, untreated diabetic control rats (group II) showed a consistent rise in fasting blood glucose levels. These levels increased when compared to the corresponding values on day1. The fasting blood glucose levels in diabetic rats treated with doses of 250 and 500 mg/kg b.wt./day of HAHS seed extract exhibited a significant and dose-dependent decrease after the treatment when compared to day1. In diabetic rats treated with glibenclamide the fasting blood glucose level decreased considerably after 29 days of treatment.

Table 3 displayed the alterations in plasma insulin, c-peptide, and HbA1C levels in both normal and experimental rats. The diabetic rats showed a significant elevation in HbA1C, and significant decrease in levels of plasma insulin and c-peptide. The injection of Hptyis seed extract of different concentration and glibenclamide to diabetic rats reversed the alterations in plasma insulin, c-peptide and HbA1C levels, bringing them close to normal.

Figure 1 displayed the histological findings of Hyptis suaveolens seed extract treatment (at doses of 250, and 500 mg/kg) for a duration of 28 days in the pancreas of diabetic rats induced by STZ, across various groups. Figure 1A displayed healthy islets normal architecture. Figure 1B displayed atrophied islets with vacuolation, the existence of pancreatic acini in diabetic rat and condensed fibres around a blood capillary. Figure 1C demonstrated a moderate increase in the size of islets and reduction of necrotic areas following treatment with glibenclamide. Figures 1D demonstrate the minimization of the vacuolated cells and a reduction of necrotic areas with enlargement and widening of islet cells in diabetic rats following the administration of HAHS at doses of 250 mg/kg. Figure 1E demonstrated a significant increase in the size of islets and the development of hyperplastic islets in diabetic rats treated with HAHS at a dose of 500 mg/kg.

Table 1: Body weight

DAY	Normal control	Diabetic control	Glibenclamide	HAHS 250mg/kg B.W	HAHS 500mg/kg B.W
0	159 ± 1.79	158 ± 2.90	158 ± 1.01	158 ± 2.26	158 ± 1.66
7	166 ± 0.57	152 ± 2.68	153 ± 1.31	151 ± 2.38	152 ± 0.88
14	170 ± 0.57	143 ± 2.45	150 ± 0.45**	148 ± 2.18*	151 ± 0.38**
21	175 ± 0.93	135 ± 2.37	153 ± 0.72**	145 ± 1.92*	150 ± 0.59**
29	181 ± 0.90	131 ± 2.06	157 ± 0.27**	149 ± 1.40*	151 ± 1.31**

Mean body weight (gms) ± SEM; ** $p<0.05$, ** $p<0.01$, when compared to rats compared with Diabetic control. HAHS = Hydroalcoholic extracts of Hyptis suaveolens seeds;

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Table 2 Fasting blood sugar

DAY	Normal control	Diabetic control	Glibenclamide	HAHS 250mg/kg B.W	HAHS 500mg/kg B.W
1	85.9 ± 0.4	255.8 ± 2.64	256.6 ± 2.27	254.2 ± 1.85	256.3 ± 1.09
29	87 ± 0.68	271.8 ± 2.77	108.2 ± 0.69**	141.6 ± 1.07*	128.6 ± 2.12**

Mean body weight (mg/dL) ± SEM; $p<0.05$*, p<0.01** when compared to Diabetic control. Test drugs at high dose levels were capable to reduce fasting blood sugar significantly.
HAHS = Hydroalcoholic extracts of Hyptis suaveolens seeds

Table 3 Random blood sugar (mg/dL)

DAY	Normal control	Diabetic control	Glibenclamide	HAHS 250mg/kg B.W	HAHS 500mg/kg B.W
1	90 ± 1.79	262 ± 2.36	260 ± 0.99	262 ± 2.44	261 ± 1.75
7	91 ± 1.09	285 ± 4.08	219 ± 1.66*	256 ± 1.31*	243 ± 2.59*
14	90 ± 1.51	305 ± 3.25	178 ± 2.29**	248 ± 1.19*	214 ± 1.79**
21	91 ± 1.29	320 ± 2.6	131 ± 3.02**	228 ± 2.10*	183 ± 2.89**
28	91 ± 1.23	331 ± 1.28	106 ± 1.27**	177 ± 2.36**	134 ± 1.68**

Mean body weight (mg/dL) ± SEM; **$p<0.05$, **$p<0.01$ when compare with Diabetic control group.
HAHS = Hydroalcoholic extracts of Hyptis suaveolens seeds;

Table 4 Biochemical parameters

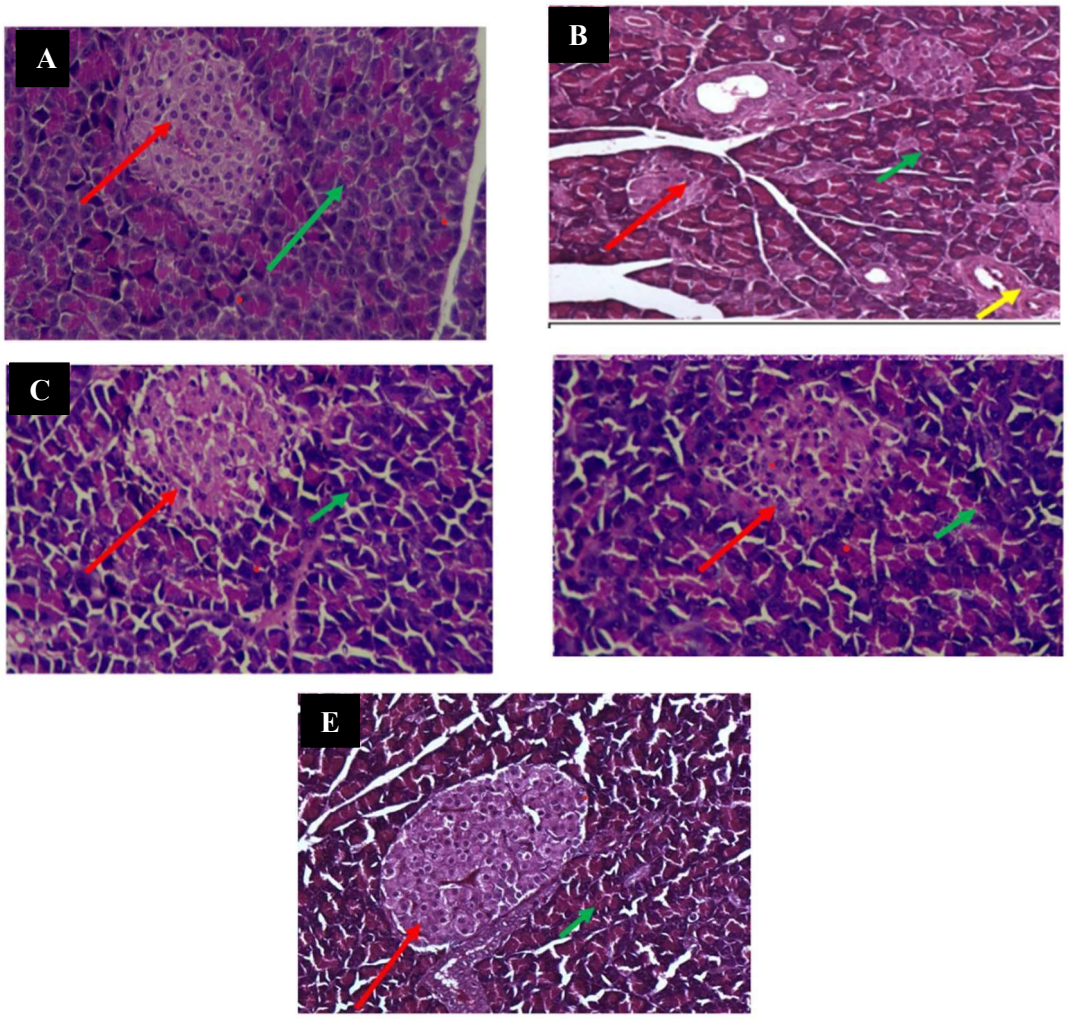
Parameter	Normal control	Diabetic control	Glibenclamide	HAHS 250mg/kg B.W	HAHS 500mg/kg B.W
Insulin µU/mL	17.57±0. 22	3.68 ± 0.17	14.97 ± 0.14**	8.59 ± 0.37*	11.39 ± 0.46**
C-Peptide ng/mL	1.68 ± 0.07	0.28 ± 0.05	1.35 ± 0.06**	0.58 ± 0.04*	1.13 ± 0.06**
HbA1C %	3.37 ± 0.09	9.07 ± 0.1	4.27 ± 0.14**	6.67 ± 0.11*	5.63 ± 0.10**

Mean ± SEM *$p<0.05$; **$p<0.01$ when compared to Diabetic control group.
HAHS = Hydroalcoholic extracts of Hyptis suaveolens seeds;

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Figure 1: Histopathological observations of the pancreas of streptozotocin-induced diabetic rats treated with hydro alcoholic extract of Hyptis Suveolens seed (HAHS) and glibenclamide



A: Normal control showing normal architecture of acini (AC) - green arrow, beta cells of Islets of Langerhans (IL)- red arrow; **B:** Diabetic control displayed atrophied islets with vacuolation (Red arrows) in islet of Langerhans (IL) ; (green arrows) acinar cells (AC) and condensed fibers around a blood capillary (yellow arrows); **C:** Diabetic + glibenclamide showing demonstrated a moderate increase in the size of islets and reduction of necrotic areas in beta cells with moderate hyperplastic of islets of acini (AC) -green arrow,beta cells of islets of Langerhans (IL) - red arrow; **D:** Diabetic + HAHS (250 mg/kg.body weight) showing the minimization of the vacuolated cells and a reduction of necrotic areas with enlargement and widening of islet cell in beta cells of islet of Langerhans (IL) and with moderate hyperplasia - red arrow and recovery of most normal acinar cells (AC) - green arrow; **E:** Diabetic + HAHS (500mg/kg body weight) with showing increase in the size of islets and the development of

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hyperplastic islets (mass restoration) of β cell of pancreases (IL)- **red arrow** and acinar cells (AC) - **green arrow**- positive sign in improvement

Discussion

The antidiabetic activity of *Hyptis suaveolens* seed extract in Wistar rats was investigated in this study, revealing promising results that support the traditional use of this plant in diabetes management. The administration of *Hyptis suaveolens* seed extract to diabetic Wistar rats resulted in a significant reduction in blood glucose levels compared to the diabetic control group. This hypoglycemic effect aligns with previous studies that have reported the antidiabetic potential of various plant extracts rich in bioactive compounds (9). The ability of *Hyptis suaveolens* to lower blood glucose levels suggests its potential as a natural alternative to conventional antidiabetic drugs.

Diabetic rats typically exhibit significant weight loss due to the inability to utilize glucose efficiently, leading to muscle wasting and fat depletion (10). In this study, the *Hyptis suaveolens* treated groups showed a marked improvement in body weight, indicating a reversal of the catabolic state induced by diabetes. This weight gain could be attributed to the improved metabolic profile and enhanced utilization of glucose facilitated by the seed extract (11).

The treatment with *Hyptis suaveolens* seed extract also resulted in significant improvements in key biochemical parameters. Plasma insulin levels were notably higher in the treated groups, suggesting that the extract may enhance insulin secretion or increase pancreatic β-cell function (12). Moreover, the reduction in glycosylated hemoglobin (HbA1c) levels in the treated rats indicates better long-term glycemic control, which is crucial in preventing diabetes-related complications (13).

Histopathological examination of the pancreas revealed that *Hyptis suaveolens* seed extract has a protective effect on pancreatic tissue. The treated groups exhibited fewer degenerative changes and preserved islet architecture compared to the diabetic control group. This finding is significant as it highlights the potential of *Hyptis suaveolens* to mitigate the histopathological damage caused by diabetes, thereby supporting better pancreatic function and insulin secretion (14).

The exact mechanisms through which *Hyptis suaveolens* exerts its antidiabetic effects are not entirely understood, but several hypotheses can be proposed. The presence of phytochemicals such as flavonoids, terpenoids, and alkaloids in *Hyptis suaveolens* may contribute to its hypoglycemic activity by enhancing insulin secretion, improving insulin sensitivity, or inhibiting carbohydrate-digesting enzymes. These compounds may also exert antioxidant effects, reducing oxidative stress, which is a known contributor to diabetes pathogenesis (15).

When compared to standard antidiabetic drugs like glibenclamide, *Hyptis suaveolens* seed extract demonstrated comparable efficacy in reducing blood glucose levels and improving biochemical parameters. This comparison underscores the potential of *Hyptis suaveolens* as a complementary or

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alternative treatment for diabetes, especially in resource-limited settings where access to conventional medications may be restricted (16).

In conclusion, *Hyptis suaveolens* seed extract exhibits significant antidiabetic activity in STZ-induced diabetic Wistar rats. The extract not only lowers blood glucose levels but also improves body weight, enhances plasma insulin levels, and protects pancreatic tissue from histopathological damage. These findings validate the traditional use of *Hyptis suaveolens* in diabetes management and highlight its potential for developing novel antidiabetic therapies. Further research is warranted to isolate and characterize the active compounds responsible for these effects and to elucidate their mechanisms of action more clearly.

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