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ANTIDIABETIC POTENTIAL OF ETHANOLIC EXTRACT OF FLOWER OF *PETUNIA HYBRIDA* IN RODENT

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ABSTRACT: The present work focused on preparing ethanolic extract of *Petunia hybrida* and evaluating its anti-diabetic potential in rats. The extraction of flowers was carried out using ethanol and the extraction yield was found to be 21.7% w/w. The total phenolic content of ethanolic extract of *Petunia hybrida* was found to be 39.18±1.46 GAE mg/g. The antidiabetic action of the extract was evaluated in terms of the effect of extract on body weight and serum glucose level. Other parameters like cholesterol and serum urea were also evaluated. The results revealed that the serum glucose levels were highly elevated by the administration of STZ in all the groups by day 7. In treatment groups III to V, the glucose levels dropped in comparison to group II and by the day 21, the levels reached almost the level of control group in group III and V. This indicates that the extract exhibits significant glucose lowering potential. Both SGOT and SGPT enzyme levels get elevated during liver damage which is more in diabetic rats. The results reveal a reduced levels of these enzymes in the EEPH treated rats. An increase in serum creatinine is associated with renal impairment in diabetes. The EEPH treatment was able to restore the levels of creatinine, suggesting a potential antidiabetic action.

INTRODUCTION: A range of metabolic illnesses, including diabetes, have grown to be a serious global health concern [1]. In Asia, especially in India, the tremendous economic growth and fast urbanization have caused a shift in health issues from communicable to non-communicable illnesses. More than 85% of diabetes people worldwide will reside in underdeveloped nations by 2030. It is projected that the number of people with diabetes in India alone would rise from 31 million in 2000 to 643 million in 2030 [2].

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Plants and their compounds have been used to cure illness for thousands of years [3]. Some animal experiments conducted to evaluate the purported activity have confirmed that some plants contain anti-hyperglycemic properties [4–10].

Furthermore, certain plants have been shown to be effective anti-diabetic medicines in clinical trials; however, the pure chemical compounds that were extracted from the crude extracts of these plants did not have any structural similarities nor comparable modes of action with the anti-diabetic medications that are now being used in clinical practice. However, the ongoing quest for novel anti-diabetic medication continues to support the use of plants as a possible source, which may be accomplished *via* the use of contemporary scientific technology and the most recent understanding of the physiological

alterations associated with diabetes [11]. *Petunia hybrida* is a flowering plant that has been associated with constituents like anthocyanins, flavonoids, phenolics and sterols [12]. The presence of these constituents offers several pharmacological properties to the flowers of this plant [13-20]. Literature also suggests that very less work has been reported for the pharmacological potential of *Petunia hybrida*. Hence it was envisioned to study the antidiabetic potential of the extract of the *Petunia hybrida* flowers in experimentally induced diabetes.

MATERIAL AND METHODS:

Extraction of Phytoconstituents [21]: The powdered authenticated flowers (213/Bot./Saifia Sci. College, dated 11/01/2023) were used for the extraction process by hot continuous extraction method using Soxhlet apparatus. 97 g of flower powder was evenly placed in the extractor of the apparatus and 350 mL of ethanol was poured over it and was allowed to collect in the attached flask. Extraction was achieved by heating the solvent at 65°C for 9 h till a colorless solution was collected in the siphon tube of the apparatus. The extract was concentrated using a rotary vacuum evaporator after being passed via a Whatman filter. After being gathered, the resinous extract was kept in a desiccator to eliminate excess moisture. For additional processing, the desiccator was used to preserve the dried extract.

Preliminary Phytochemical Screening [22]: The kind of plant secondary metabolites included in the extract was determined by qualitative phytochemical screening. Triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolic acids were all screened for. The experiments were conducted using the color intensity or the precipitate formation as analytical responses.

Total Phenolic Content [23]: 0.5 g of dry powder and 5 mL of methanol were combined, and the mixture was left overnight to ascertain the total phenolic content. After passing the suspension through a qualitative cellulose filter paper, the filtrate was diluted with methanol to a volume of 10 milliliters. The solution was used as the stock solution for further studies and was kept in amber bottles at 4°C.

To determine the total phenolic content, 200 µL of the sample was combined with 100 µL of Folin-Ciocalteu reagent and 1.4 mL of filtered water. 300 µL of 20% aqueous Na_2CO_3 solution was added after three minutes, and the mixture was left to settle for two hours. The absorbance was determined using a UV-Vis spectrophotometer at 760 nm. To create the calibration curve, standard solutions of gallic acid (20–100 ppm) underwent comparable treatment. The control solution was produced and incubated under the same conditions as the other samples, using 200 µL of water and appropriate reagents. Gallic acid equivalent (GAE) in milligrams per 100 grams of dry material was used to express the results.

Pharmacological Study:

Animal Used: Male rats weighing 180–230 g was utilized, and they were obtained from Bhopal-approved vendors. The rats had unlimited access to water and a pellet meal (Lipton India Ltd., Mumbai, Ind.). Throughout the trials, all laboratory setups and animal care were conducted in accordance with CPCSEA rules (194/PQ/a64/2023/CPCSEA).

Acute Toxicity Study: Both medications' short- and long-term harmful effects, as well as the extracts from them, were evaluated in accordance with OECD guideline no. 423.

The 150–200 g albino rats used in the study were housed in a 12-hour day–night cycle with unlimited access to water. The extract was dissolved in one percent Tween 80, which was made using purified water. The animals were given a 12-hour fast before the extract was given to them orally at dosages up to 2000 mg/kg, which was the highest weight that was considered. Any strange behavior, such as changes in skin and fur, eyes, hyperactivity, grooming, convulsions, sedation, hypothermia, salivation, tremor, coma, lethargy, body weight, and death, should be observed during the first four hours. Based on research and observations, therapeutic doses of one-tenth and one-fifth of the fatal dosage were employed, with 200 and 400 mg/kg as cut-off values to test dose-dependent effect and nootropic activity [24].

Induction of Diabetes: After a 12-hour fast, the animals received intraperitoneally (i.p.) doses of

freshly manufactured streptozotocin (STZ) at a concentration of 55 mg/kg bodyweight in 0.1 mol/L cold citrate buffer, pH 4.5. To recover from the drug-induced hypoglycemia, the STZ-treated mice were given a 5% glucose solution to consume throughout the night. Rats exhibiting prolonged glycosuria and hyperglycemia, defined as fasting blood glucose levels more than 250 mg/dL on the third day following the STZ injection, were classified as diabetic and utilized for subsequent studies [25].

Experimental Design: The rats were split up into five groups, each with six members. Group 2 represented STZ (55 mg/kg b.w., i.p.)-induced diabetic rats acting as the diabetic control group, whereas Group 1 served as the normal group and received water. For a duration of 21 days, diabetic rats induced by STZ (55 mg/kg b.w., i.p.) were treated with Glibenclamide (5 mg/kg b.w./p.o.); rats induced by STZ (55 mg/kg b.w., i.p.) were treated with EEPH 200 mg/kg b.w./p.o.; and rats induced by STZ (55 mg/kg b.w., i.p.) were treated with EEPH 400 mg/kg b.w./p.o [26].

Before extracts were administered, blood glucose levels were assessed after a fast. On the first, seventh, fourteenth, and twenty-first days of the therapy period, blood glucose levels were measured. After the rat's tail was chopped off, blood was extracted. Glucose oxidase peroxidase reactive strips and a glucometer were used to assess blood glucose levels.

Biochemical Study: Blood samples were taken, serum was extracted using a centrifuge, and the animal was slaughtered by beheading on the last day in order to examine the biochemical parameters. The Lowry technique was used to estimate the amount of protein [27]. The Folch [28] technique was utilized to extract blood lipids, and the Zlatkis method was employed to estimate serum cholesterol [29]. Burstein's approach was used to estimate HDL cholesterol while Foster and Dunn's method was used to assess serum triglycerides [30].

The TG/5 mg/dl method was used to calculate the VLDL cholesterol. The Friedwald technique was used to estimate the serum LDL cholesterol. The diacetyl monoxime technique was used to detect serum urea, Jaffe's method was used to assess plasma creatinine, and the Reitman and Frankel method (a colorimetric approach) was used to evaluate SGOT and SGPT [34].

RESULTS AND DISCUSSION: It was discovered that the *Petunia hybrida* flower's extraction in ethanol by hot continuous extraction was 21.7% w/w. The extract had a dark appearance and was resinous. The results of the phytochemical research indicate that the ethanolic extract of the flowers contains proteins, flavonoids, phenolics and tannins.

Total Phenolic Content: The total phenolic content of *Petunia hybrida* flowers was assessed using their ethanolic extract. A standard gallic acid curve was drawn in distilled water. The outcome of the Folin-Ciocalteu method's analysis of the extract's total phenolic content. It was discovered that the Petunia hybrida ethanolic extract has a total phenolic concentration of 39.18±1.46 GAE mg/g.

Acute Toxicity Study: In rats, neither plant exhibited any hazardous consequences or signs or symptoms, even at greater doses of 2000 mg/kg body weight. Thus, the effective dosage was determined to be 1/10th of the maximal dose. The 200 and 1/5th dosage cutoff values, or 400 mg/kg, were used to assess memory-enhancing activity.

Antidiabetic Activity of EEPH: The antidiabetic action of the extract was evaluated in terms of the effect of extract on body weight **Table 1** and serum glucose level **Table 2**. Other parameters like cholesterol and serum urea were also evaluated.

TABLE 1: EFFECT OF EEPH ON BODY WEIGHT

Group	Body weight (g)			
	Day 0	Day 7	Day 14	Day 21
1	195.3±1.910	194.5±2.257	205.1±1.033	213.4±1.161
2	197.4±2.010	157.1±1.042	141.3±1.266	132.2±0.964
3	192.3±1.930	181.2±2.010	182.4±1.033	190.6±1.780
4	191.4±3.165	171.3±2.026	172.5±0.933	177.1±1.033
5	194.6±2.186	177.4±1.865	179.3±1.166	183.8±1.303

Because structural proteins are known to contribute to body weight, experimental induction of hyperglycemia with STZ is linked to the characteristic loss of body weight. This loss is caused by the loss or degradation of structural proteins, which increases muscle wasting and tissue protein loss.

It was found that STZ administration reduced the body weight of animals significantly in comparison with the normal control animals (Group 1). On the other hand, the administration of glibenclamide or EEPH was able to increase the body weight in comparison to Group 2.

TABLE 2: EFFECT OF EEPH ON BLOOD GLUCOSE LEVELS

Group	Blood Glucose (mg/dl)			
	Day 0	Day 7	Day 14	Day 21
1	91.56±3.030	93.73±3.636	95.97±1.650	91.42±2.066
2	97.43±3.365	241.12±1.822	275.25±2.010	284.71±1.495
3	102.51±4.875	165.72±1.529	137.95±1.541	124.18±1.757
4	99.26±2.257	223.57±1.487	205.53±2.088	165.13±1.533
5	94.61±3.010	184.68±1.445	166.74±1.341	127.28±1.468

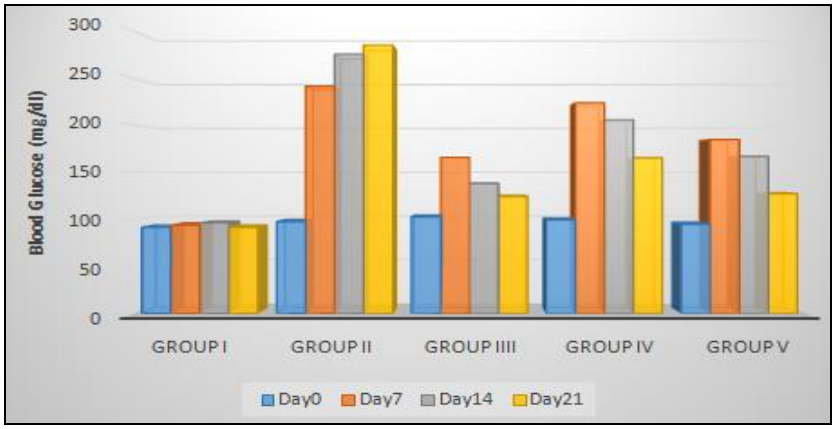

FIG. 1: COMPARISON OF BLOOD GLUCOSE OF TEST ANIMAL IN VARIOUS GROUPS

The result revealed that the serum glucose levels were highly elevated by the administration of STZ in all the groups by day 7. In the treatment groups 3 to 5, the glucose levels dropped in comparison to group 2 and by the day 21, the levels reached almost the level of control group in group 3 and 5. This indicates that the extract exhibits significant glucose lowering potential **Fig. 1.** One of the potential mechanisms lowering blood glucose levels could be decreased glucose transport or absorption from the gut, increased pancreatic action likely through stimulation of glucose utilization in peripheral tissues, increased activity of glycogenic or glycolytic enzymes in peripheral tissues, decreased secretion of growth hormones and other counter-regulatory hormones like glucagon.

TABLE 3: EFFECT ON EEPH ON LIPID PROFILE

Group	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
1	103.2±0.357	83.7±0.325	58.27±0.338	38.4±0.443	16.96±0.46
2	20.36±0.319	167.2±0.44	31.65±0.56	136.43±0.71	38.71±0.64
3	117.71±0.567	93.16±0.51	53.71±0.96	83.95±0.421	23.63±0.382
4	161.38±0.921	131.42±0.287	44.36±0.733	116.12±0.49	33.64±0.295
5	140.73±0.931	103.3±0.817	56.12±0.355	95.48±0.157	27.48±0.428

Under normal circumstances, hyperglycemia is accompanied by dyslipidemia; insulin causes the triglyceride-hydrolyzing enzyme lipoprotein lipase to be activated. However, in diabetic conditions, insulin shortage prevents lipoprotein lipase from being activated, which leads to hyper-triglyceridemia. Additionally, because of metabolic irregularities, insulin insufficiency is also linked to hypercholesterolemia. The results indicate that the treatment with EEPH was able to regulate the levels of the lipid (Cholesterol, triglycerides and others) suggesting its significant impact on improved metabolism rate **Table 3.**

TABLE 4: EFFECT ON EEPH ON SGOT, SGPT, CREATININE AND UREA

Group	Serum Urea (mg/dl)	Creatinine (mg/dl)	SGOT(U/L)	SGPT (U/L)
1	6.83±0.167	0.45±0.018	46.87±0.21	22.08±0.21
2	5.42±0.143	1.64±0.037	113.30±0.46	47.18±0.45
3	6.84±0.182	0.60±0.015	44.68±0.85	25.68±0.54
4	6.32±0.217	1.31±0.026	64.38±0.91	38.31±0.69
5	6.28±0.138	0.86±0.018	51.68±0.63	30.66±0.33

When liver damage occurs, both SGOT and SGPT enzyme levels rise, and this is especially pronounced in diabetic rats. The rats treated with EEPH had lower levels of these enzymes, according to the data. Renal impairment in diabetes is linked to an increase in serum creatinine.

The EEPH treatment was able to restore the levels of creatinine suggesting a potential antidiabetic action **Table 4.**

CONCLUSION: In this work, Petunia hybrida flower extract was prepared using ethanol as the solvent; secondary metabolites were identified; total phenolics and flavonoids were calculated; and the extract's anti-diabetic properties were assessed in rats and mice.

The results obtained from the study led to the conclusion that the flowers of the plant possess phenolics and flavonoids that are beneficial in maintaining the lipid profile as well as the glucose levels in STZ induced diabetes in rats. The body weight of the animals was also regulated and the protein, creatinine, SGOT and SGPT levels were also regulated by the extract. Hence it could be concluded from the study that *Petunia hybrida* flowers possess significant anti-diabetic activity and could be explored for the particular constituents responsible for their action.

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