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EVALUATION OF ANTIDIABETIC ACTIVITY OF *ANANAS COMOSUS*

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Ananas comosus (Pineapple), Diabetes mellitus, STZ, Insulin

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ABSTRACT: The present study evaluates the antidiabetic potential of hydroalcoholic extracts derived from the fruit and peel of *Ananas comosus* (pineapple). Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from impaired insulin secretion or action. Natural plant-based remedies have gained attention for their efficacy and minimal side effects. The hydroalcoholic extracts were prepared using standardized extraction methods and subjected to phytochemical screening to identify bioactive constituents. The antidiabetic activity was assessed using *in-vivo* models of diabetes induced in experimental animals. Blood glucose levels and body weight were monitored throughout the treatment period. Results demonstrated a significant reduction in fasting blood glucose levels and improvement in lipid profile in animals treated with both fruit and peel extracts, with peel extract exhibiting comparatively higher efficacy. The findings suggest that *Ananas comosus* hydroalcoholic extracts possess promising antidiabetic properties, potentially attributable to their rich phytochemical content, including flavonoids, phenolics, and enzymes. These results warrant further studies to isolate specific active compounds and elucidate underlying mechanisms for clinical application in diabetes management.

INTRODUCTION: Diabetes mellitus (DM) is a long-term, complex metabolic disease marked by elevated blood glucose levels brought on by deficiencies in either insulin action or production, or both. One of the main factors thought to contribute to diabetes complications is hyperglycemia [1]. In the upcoming years, the prevalence of DM will rise at an alarming rate worldwide. The International Diabetic Federation's diabetic atlas states that 382 million people globally had diabetes in 2013, and that number is predicted to rise to 592 million by 2035 [2].

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According to World Health Organization projections, diabetes would rank as the seventh most common cause of death by 2030 [3]. Diabetes management is a worldwide health issue for which there is now no effective solution [4]. Insulin and a number of oral antidiabetic medications, including glinides, biguanides, and sulfonylureas, are now accessible treatments for diabetes. Finding safer and more efficient hypoglycemic medications is a crucial field of research as many of them have a variety of harmful side effects [5].

It is often believed that natural medicine can strengthen the body's defences against sickness. Even in places where access to modern medicine is available, interest in herbal remedies and their use has been growing quickly. Herbal plants are always an important source of bioactive components, including an integrated approach to studying plants as a means of treating diseases that are getting

worse. Its phytochemical analysis might lead to novel synthetic compound discoveries [6]. The use of *A. comosus* is often more beneficial for chronic illnesses. The fruits are pendulous, linear, three-sided pods with nine longitudinal ridges that are 2.0 to 2.5 cm broad and typically 20 to 50 cm long, however they can infrequently reach a length of one metre [7]. Up to 26 seeds, which develop three months after flowering, are often found in each pod. When they mature, they become brown and crack apart lengthwise along the three angles, releasing the 1 cm-diameter seeds [8, 9]. Phenolics are abundant in pineapple fruit. Despite the fact that many flavonoids and phenolics have been found in several pineapple cultivars, nothing is known about the phenolics' antioxidant action [10]. Recent research has demonstrated the potent antioxidant action of phenolics found in edible fruits [11, 12]. In view of these aspects, the present work is carried out to evaluate the antidiabetic activity of *A. comosus* fruit extract.

MATERIALS AND METHODS:

Collection or Procurement of Pine Apple Fruit: Pineapples (*Ananas comosus* (L.) Merr.) were procured from a local supermarket, selected based on uniform size, consistent ripening stage, and absence of visible defects. The fruits were thoroughly washed with clean water, manually peeled, and the core was mechanically separated from the pulp to prepare for further processing [13].

Extraction: The extraction of pineapple fruit peel was carried out using the maceration method. Dried pineapple peels were first ground into a coarse powder to increase the surface area for solvent interaction. A known quantity of the powdered peel was placed in a clean glass container and soaked in a suitable solvent such as methanol, or water at a specific ratio (e.g., 1:10 w/v). The mixture was covered and allowed to stand at room temperature for 24 to 72 hours with occasional stirring to facilitate the diffusion of bioactive compounds into the solvent. After the maceration period, the mixture was filtered using muslin cloth followed by Whatman No.1 filter paper to separate the extract from the solid residue. The filtrate (crude extract) was then concentrated under reduced pressure using a rotary evaporator or allowed to evaporate at room temperature, depending on the nature of the solvent, to obtain the final extract. The extract was stored in airtight containers at low temperature (4°C) until further use [14].

α-Amylase and α-Glucosidase Inhibition Assays:

α-Amylase: After mixing the sample with α-amylase (0.5 mg/ml) at different concentrations (100–500 µg/ml), 100 µl of 0.2 mm phosphate buffer (pH -6.9) and 1% starch solution were added. After five minutes at 37°C, the reaction was stopped by adding two millilitres of the 3, 5-dinitrosalicylic acid reagent. After being heated to 100°C for 15 minutes, the reaction mixture was diluted in an ice bath with 10 millilitres of distilled water. By using a spectrophotometer to measure colour intensity at 540 nm, α-amylase activity was ascertained [15-17].

α-glucosidases Enzyme: By combining 0.2 M tris buffer (pH 8) with 1 ml of starch solution (2% w/v maltose) and varying sample concentrations (100–500 mg/ml), the inhibitory action was ascertained. For ten minutes, the reaction mixture was incubated at 37°C. One millilitre of α-glucosidase enzyme (1 U/ml) was added to start the reaction, and it was then incubated for forty minutes at 35°C. Two millilitres of 6 N HCl were then added to stop the reaction. A spectrophotometer was used to measure the color's intensity at 540 nm [18]. The results were expressed as % inhibition using the formula:

$$\% \text{ inhibitory activity} = (Ac\text{-}As) / Ac \times 100$$

Where, Ac is the absorbance of the control and As is the absorbance of the sample.

***In-vivo* Studies:**

Animals: The present study was conducted using healthy adult male Wistar albino rats, weighing between 150–200 grams, obtained from the in-house breeding colony maintained at the Animal House Facility of Shri Ramnath Singh Mahavidyalaya (Pharmacy), Gormi, Bhind, Madhya Pradesh, India. The experimental procedures were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Shri Ramnath Singh Mahavidyalaya (Pharmacy), Gormi, Bhind, in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute Toxicity Studies: The acute oral toxicity study of the hydroalcoholic extract of *Ananas comosus* (HEAC) was carried out following the fixed-dose procedure outlined in OECD Guideline No. 420 and in compliance with the recommendations of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Thirty healthy adult male Wistar albino rats, aged 10 weeks and weighing 150–200 g, were procured from the in-house breeding facility of Shri Ramnath Singh Mahavidyalaya (Pharmacy), Gormi, Bhind, M.P., India. Before dosing, the rats were fasted overnight for 12–16 hours with free access to water to ensure consistent absorption of the test material. The animals were randomly assigned into five groups (A, B, C, D, and E) with six rats per group (n = 6) [19].

The test substance was administered orally using a sterile orogastric tube to ensure precise and safe delivery of the extract directly into the stomach. Following administration, all animals were carefully observed individually at 2 hours, 6 hours, 24 hours, and 48 hours post-dosing for any signs of toxicity, behavioral changes, or autonomic responses. Observations were made at regular intervals during the first 24 hours, with special attention given during the first 4 hours post-dosing, a critical period for detecting acute toxic effects.

Evaluation of Antidiabetic Activity:

Induction of Diabetes: Diabetes was induced in rats by single intra peritoneal (i.p.) injection of streptozotocin (STZ, Sigma chemical Co. USA) at a dose 60 mg/kg b.w. freshly dissolved in 0.1 M cold citrate buffer of pH 4.5; 48 hr later blood samples were collected and blood glucose levels were determined to confirm the development of diabetes. Those animals which showed hyperglycemia (blood glucose levels >240 mg/dl) were used in experiment 90. The rats were divided into five groups of 6 animals (n = 6) each as below:

- **Group I:** Normal control (received distilled water 10 ml/kg b.w., p.o.).
- **Group II:** Diabetic control untreated (received distilled water 10 ml/kg b.w., p.o.).
- **Group III:** Diabetic treated with standard drug glibenclamide (0.25 mg/kg/day, p.o.).
- **Group IV:** Diabetic treated with HEAC (200 mg/kg/day, p.o.).
- **Group V:** Diabetic treated with HEAC (400 mg/kg/day, p.o.).

For 30 days blood glucose levels and body weights were measured on 1st, 10th, 20th and 30th day of the study. Finally on day 30, blood was collected to estimate various parameters [20].

Estimation of Plasma Glucose, Body Weight and Lipid Profile: Blood samples were collected weekly from all experimental animals to assess biochemical parameters related to glucose and lipid metabolism. Before each collection, animals were fasted overnight for 16 hours with free access to water to maintain consistency and minimize variations due to recent feeding. Blood was obtained from the retro-orbital plexus using sterile capillary tubes under light ether anesthesia to ensure minimal discomfort and safe handling [21]. Samples were immediately transferred into fluoride-containing tubes to prevent glycolysis and centrifuged at 3000 rpm for 10 minutes at room temperature to separate plasma.

The plasma fraction was used for estimating glucose concentration using the Glucose Oxidase-Peroxidase (GOD-POD) enzymatic method with a commercial kit from Span Diagnostics, India. In addition to glucose estimation, the body weight of each animal was recorded weekly using a precision digital weighing scale to monitor treatment-related physiological changes [22, 23]. These biochemical evaluations were crucial for determining the potential antihyperglycemic effects of the hydroalcoholic extract of *Ananas comosus* and for observing any metabolic alterations during the treatment period.

Estimation of Serum Insulin: A spectro-photometric radioimmunoassay kit (RIA kit supplied by BRIT, BARC, India) was used to measure the serum insulin concentration. The kit contained 125I-labelled human insulin antibody, which crossreacts similarly with rat insulin, and human insulin as a reference [24].

Estimation of Glycated Hemoglobin: Following a 30-day trial period, the rats that had fasted for 12 hours were killed by cervical decapitation, their

blood was extracted *via* retroorbital puncture while under light ether anaesthesia, and the amount of glycated haemoglobin was calculated [25].

Statistical Analysis: The results were expressed as mean± S.E.M. Statistical difference was tested by using one-way analysis of variance (ANOVA) test. A difference in the mean p value <0.05 was considered as statistically significant [26-27].

RESULTS AND DISCUSSION:

Extraction And % Yield: The hydro-alcoholic extraction of the pineapple (*Ananas comosus*) fruit peel and/or pulp was carried out using the maceration technique. The thick extract was further dried in a desiccator to obtain a solid/semi-solid mass and percentage yield was calculated. Thus, the percentage yield of the hydroalcoholic extract of *Ananas comosus* fruit and peel was found to be 6.78% w/w.

In-vivo Study:

Acute Toxicity Study: In the LD50 value determination, we observed that the HEAC was safe to use in animals and showed no mortality on 2000 mg/kg b.w. Therefore 2000 mg/kg dose was considered as a safe dose, 1/5th (400 mg/kg b.w.) and 1/10th (200 mg/kg b. mg/kg b.w.) of that was selected for all *in-vivo* experiments as maximal dose.

Hypoglycemic Effect of the Hydroalcoholic Extract: The effect of the hydroalcoholic extract of *Ananas comosus* (HEAC) on serum glucose levels in STZ-induced diabetic rats over a 30-day period is presented in **Table 1.** The normal control group (Group I) maintained stable glucose levels throughout the study, with values ranging from 92.11 ± 2.32 to 93.62 ± 2.45 mg/dL, indicating normal glucose homeostasis. In contrast, the diabetic control group (Group II) showed a progressive increase in glucose levels from 253.43 ± 4.54 mg/dL on day 1 to 385.21 ± 2.35 mg/dL on day 30, indicating worsening hyperglycemia due to untreated diabetes. Treatment with glibenclamide (Group III) significantly reduced blood glucose levels beginning on day 10 ($p < 0.01$), with a marked decline to 113.65 ± 2.65 mg/dL by day 30 ($p < 0.01$), demonstrating the efficacy of the standard drug. Both HEAC-treated groups showed a dose-dependent reduction in blood glucose.

The 200 mg/kg dose (Group IV) showed significant reductions starting from day 10 ($p < 0.01$), with glucose levels reaching 136.43 ± 0.45 mg/dL by day 30. The 400 mg/kg dose (Group V) showed an even greater glucose-lowering effect, with significant reductions on all measured days after day 1 and a final glucose level of 125.32 ± 2.04 mg/dL by day 30 ($p < 0.01$).

The results from the study clearly indicated that the aqueous extract exhibited significant hypoglycemic activity in STZ-induced diabetic rats, whilst there was no significant effect observed on normoglycemic rats. However, at the end of 30 days of treatment, there was a 70.12%, 64.96% and 68.09% ($p < 0.01$) decrease of serum glucose levels with the glibenclamide and aqueous extract (250 and 500 mg/kg) respectively when compared with diabetic control after 30 days **Table 1**.

The results clearly demonstrate that the hydroalcoholic extract of *Ananas comosus* (HEAC) significantly reduces serum glucose levels in STZ-induced diabetic rats over a 30-day period. The progressive decrease in glucose levels observed in HEAC-treated groups suggests a sustained antihyperglycemic effect, with the 400 mg/kg dose being more effective than the 200 mg/kg dose.

TABLE 1: EFFECT OF HYDROALCOHOLIC EXTRACT OF *A. COMOSUS* ON SERUM GLUCOSE LEVEL

Group/ Treatment	1st day	10th day	20th day	30th day
I. Normal Control	92.11 ± 2.32	93.43 ± 2.54	94.53 ± 1.62	93.62 ± 2.45
II. Diabetic Control	253.43 ± 4.54	282.11 ± 3.62	310.11 ± 5.43	385.21 ± 2.35
III. Diabetic + Glibenclamide (0.25 mg/kg)	253.67 ± 3.64	263.11 ± 3.76 **	208.29 ± 2.72**	113.65 ± 2.65 **
IV. Diabetic + HEAC (200 mg/kg)	253.11 ± 2.97	272.11 ± 2.65**	233.12 ± 2.65**	136.43 ± 0.45**
V. Diabetic + HEAC (400 mg/kg)	254.21 ± 3.54	266.11 ± 2.65 **	222.82 ± 3.65**	125.32 ± 2.04 **

*p < 0.05; **p < 0.01 Values are mean ± SEM, n = 6, when compared with diabetic control by using one way ANOVA followed by Dunnette's multiple comparison test

The comparison with glibenclamide a well-known sulfonylurea shows that while HEAC is not as potent, its glucose-lowering effect is still significant and potentially clinically relevant. The delayed onset but sustained effect observed with HEAC could indicate that it works via different mechanisms, possibly involving improved insulin sensitivity, β-cell protection, or modulation of hepatic glucose production. These findings support the traditional use of *A. comosus* in managing diabetes and highlight its potential as a natural adjunct therapy. The presence of phytoconstituents such as flavonoids, bromelain, and polyphenols may contribute to the observed antidiabetic activity by exhibiting antioxidant and insulin-enhancing effects.

Changes in Body Weight: The normal control group (Group I) exhibited a steady increase in body weight from 160.45 ± 2.18 g on day 0 to 190.11 ± 2.14 g on day 30, indicating normal growth and metabolic health. The diabetic control group (Group II) showed a significant and progressive decrease in body weight, dropping from 163.23 ± 2.57 g at day 0 to 144.18 ± 2.73 g by day 30, which is characteristic of uncontrolled diabetes and associated catabolic effects.

Treatment with glibenclamide (Group III) resulted in significant weight gain over the study period, reaching 173.12 ± 2.54 g on day 30 ($p < 0.01$ compared to diabetic control), suggesting a reversal of diabetic wasting. The HEAC-treated groups also demonstrated a dose-dependent improvement in body weight. The 200 mg/kg dose (Group IV) resulted in significant weight gain from day 10 onward, reaching 180.23 ± 2.76 g on day 30 ($p < 0.01$). Similarly, the 400 mg/kg dose (Group V) led to significant increases from day 10 and continued through day 30, ending at 178.11 ± 2.43 g ($p < 0.01$).

TABLE 2: EFFECT OF HYDROALCOHOLIC EXTRACT OF *A. COMOSUS* ON BODY WEIGHT IN STZ-INDUCED DIABETIC RATS

Group /Treatment	Day 0	Day 10	Day 20	Day 30
I. Normal	160.45 ± 2.18	171.11 ± 1.76	177.14 ± 3.14	190.11 ± 2.14
II. Diabetic Control	163.23 ± 2.57	158.12 ± 3.12	153.54 ± 2.63	144.18 ± 2.73
III. Positive Control (Glibenclamide)	162.23 ± 2.52	166.11 ± 1.32 **	168.54 ± 2.43 **	173.12 ± 2.54 **
IV. HEAC (200 mg/kg)	168.12 ± 3.44	172.52 ± 1.43 **	174.54 ± 2.54 **	180.23 ± 2.76 **
V. HEAC (400 mg/kg)	164.11 ± 2.65	170.26 ± 1.90 **	172.12 ± 1.49 **	178.11 ± 2.43 **

*$p < 0.05$; **$p < 0.01$. Values are mean ± SEM, n = 6, when compared with normal by using one way ANOVA followed by Dunnette's multiple comparison test

Changes of Serum Insulin, Liver Glycogen and Glycolsylated Hemoglobin: Table 3 summarizes the impact of the hydroalcoholic extract of *Ananas comosus* (HEAC) on key serum parameters serum insulin, glycosylated hemoglobin (HbA1c), and liver glycogen content in STZ-induced diabetic rats after 30 days of treatment.

In the normal control group (Group I), serum insulin was maintained at 17.54 ± 0.49 µU/mL, glycosylated hemoglobin at 0.23 ± 0.004 mg/g Hb, and liver glycogen at 15.11 ± 1.23 mg/g, reflecting normal metabolic status. The diabetic control group (Group II) exhibited significant metabolic derangements: serum insulin levels were markedly reduced (6.43 ± 0.19 µU/mL), glycosylated hemoglobin was elevated (0.60 ± 0.004 mg/g Hb), and liver glycogen stores were depleted (8.23 ± 0.23 mg/g), confirming the hyperglycemic and catabolic state associated with diabetes.

Treatment with glibenclamide (Group III) significantly improved all three parameters compared to the diabetic control ($p < 0.01$), with serum insulin at 15.12 ± 0.24 µU/mL, HbA1c at 0.26 ± 0.005 mg/g, and liver glycogen at 14.68 ± 0.87 mg/g.

The HEAC-treated groups also showed significant improvements:

- 200 mg/kg dose (Group IV): serum insulin increased to 12.43 ± 0.65 µU/mL, HbA1c reduced to 0.32 ± 0.004 mg/g, and liver glycogen rose to 12.31 ± 0.53 mg/g ($p < 0.01$).
- 400 mg/kg dose (Group V): serum insulin increased further to 14.33 ± 0.51 µU/mL, HbA1c decreased to 0.28 ± 0.002 mg/g, and liver glycogen increased to 13.65 ± 0.62 mg/g ($p < 0.01$).

TABLE 3: EFFECT OF HYDROALCOHOLIC EXTRACT OF *A. COMOSUS* ON SERUM PARAMETERS AFTER 30 DAYS

Group /Treatment	Serum Insulin (µU/mL)	Glycosylated Hemoglobin (mg/g Hb)	Liver Glycogen (mg/g)
I. Normal Control	17.54 ± 0.49	0.23 ± 0.004	15.11 ± 1.23
II. Diabetic Control	6.43 ± 0.19	0.60 ± 0.004	8.23 ± 0.23
III. Diabetic + Glibenclamide (0.25 mg/kg)	15.12 ± 0.24**	0.26 ± 0.005**	14.68 ± 0.87**
IV. Diabetic + HEAC (200 mg/kg)	12.43± 0.65**	0.32 ± 0.004**	12.31 ± 0.53**
V. Diabetic + HEAC (400 mg/kg)	14.33 ± 0.51**	0.28 ± 0.002**	13.65 ± 0.62**

*p < 0.05; **p < 0.01. Values are Mean±SEM, n = 6, when compared with diabetic control by using one way ANOVA followed by Dunnette's multiple comparison test

CONCLUSION: In conclusion, the hydro-alcoholic extract of *A. comosus* has no impact on normal rats but is effective in lowering the high blood glucose level and lipid profile of rats with STZ-induced diabetes. Consequently, the assertion stated by Ayurvedic classics is justified.

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