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# Antidiabetic Activity of Hydroethanolic extract of Phoenix sylvestris Seeds On Streptozotocin Induced Wistar rats.

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## Introduction

### Diabetes Mellitus

Diabetes mellitus is common metabolic disorder or endocrinological disorder, which is recognized by high blood glucose concentration, term as hyperglycaemia. Diabetes mellitus is produced by insulin deficiency or insulin resistance. [1] Throughout the world millions of people endure from diabetes mellitus. The life anticipation may be split by diabetes mellitus. Adult blindness, nephropathy, gangrene neuropathy, heart attacks and strokes are the major cause of diabetes mellitus. [2] By the year 2025, the universal occurrence of diabetes mellitus is estimate to increase. It is predicted by WHO, the major affliction will occur in developing countries. In last decade the studies accompanied in India, emphasized that not only the prevalence of diabetes is high but also it is increasing in urban occupants. [3] Mortality and morbidity is a major cause of diabetes mellitus. Four clinical classifications of diabetes mellitus are identified by the American Diabetes Association (ADA). Type 1 diabetes mellitus or insulin dependent

diabetes mellitus (IDDM), Type 2 diabetes mellitus or non-insulin diabetes mellitus (NIDDM), gestational diabetes, and diabetes occurs due to other sources like genetic defects and medications. [4] The symptoms of diabetes mellitus arises when renal edge for reabsorption of glucose is exceeded, result in glucose falls in urine termed glycosuria, which causes an osmotic diuresis termed polyuria, result in dehydration due to dehydration thirst and increased drinking arises termed polydipsia. Insulin deficiency reduces protein synthesis and increases the breakdown. Diabetic ketoacidosis is also an acute metabolic hurdle. Diabetic ketoacidosis typically occurs in type 1 diabetes mellitus. Although, it may occur in type 2 diabetes mellitus. It arises in the absence of insulin because of the breakdown of fat to acetyl-CoA, is increased and due to lack of aerobic carbohydrate metabolism, transformed to acetoacetate, β-hydroxybutyrate and acetone. [4, 5]

## Treatment of Diabetes Mellitus [6]

### Oral Hypoglycaemic Drugs

These are the drugs, which reduces the blood glucose level in individuals. Insulin is also used for the treatment of diabetes mellitus but if insulin is given by oral route, gets degraded in the g.i.t. Oral hypoglycaemic drugs used as secretagogues to enhance the release of insulin.

### Material & Methods

### Plant Collection & Authentication

The fruits of *Phoenix sylvestris* were purchased from the local market of Lucknow. Seeds were separated from fruits and collected. It was authenticated as *Phoenix sylvestris* Roxb belonging to family: Arecaceae, from plant diversity, systemic and herbarium division of CSIR- National Botanical Research Institute (NBRI), Lucknow. The identification number for same is NBRI/CIF/582/2018. The specimen was preserved in institute for future reference.

### Hydro-Ethanolic Extraction of Phoenix sylvestris Seeds

The seeds were separated from fruits and washed with tap water and shed-dried. The dried seeds were crushed and made powder by kitchen mixture machine. The powder was passed through 24 # sieve and fine crude drug powder was collected. 250 ml of hydroalcoholic

solution (70% ethanol) was added to round bottom flask (RBF) and RBF was placed on heating mantle. 50 g powdered plant material was loaded into the thimble (made from what's man filter paper) and placed inside the soxhlet extractor. The soxhlet extractor was placed at the top of the round bottom flask and condenser at the top of soxhlet extractor. To the water inlet of condenser, running tap water was connected and water passed through water inlet continuously before switched on of the heating mantle. The solvent was heated and began to evaporate at 50-60 ºC and moved through condenser. The condensed liquid was dropped down into the thimble. Once the level of solvent was reached up to siphon tube and the solvent was dropped down into the round bottom flask and one cycle was completed. Again, same process was

begun for 6-8 hours. After, 6-8 hours of extraction process, the solvent was evaporated by using Rota evaporator (50-60º C) and viscous liquid was obtained. [7]

**Preliminary Phytochemical Screening**

The quantitative analysis of hydroalcoholic extract of plant revealed the presence of alkaloids, saponins, Carbohydrates, amino acids and flavonoids and phenolic compounds. The presence of phytochemicals was confirmed through quantitative analysis as given-

| **Test Name** | **Result** |
|---|---|
| • **Carbohydrate** | |
| Benedict test | + |
| Molish'test | + |
| • **Saponin (foam test)** | + |
| • **Fixed oil** | |
| Spot test | + |
| • **Phenolic compounds and tannins** | |
| Lead acetate &Alkaline reagent test | + |
| Gelatin test | + |
| Ferric chloride test | + |
| • **Protein and amino acids** | |
| • Biuret test | + |
| • Ninhydrin test | + |
| • **Alkaloid** | |
| Mayer's, Wagner's& Hager's test | + |
| Dragendorff test | - |

**Experimental Animals**

Adult wistar rats of either sex having body weight 150-200 g were used. The animals were purchased from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow. Animals were kept in clean cages (22.5cm× 37.5cm), five animals per cage to provide them appropriate space, under controlled temperature (24-27º C), and 12-h light and 12-h dark cycle. Animals were fed with standard rat pellets diet and water ad libitum [23]. Permission for the study was obtained by Institutional Animal Ethics committee (IAEC), Amity institute of pharmacy, Amity University, Uttar Pradesh, India (1492/PO/Re/S/003/CPCSEA).

**Induction of Diabetes**

Diabetes was induced by single Intraperitonial (i.p) injection of streptozotocin (60 mg/kg) in wistar rats. Freshly prepared citrate buffer, pH (4- 4.5) was used as vehicle for administration of streptozotocin (STZ) after overnight fasting. After 48 hours of STZ administration, blood glucose levels were determined by glucose oxidase method in surviving animals. Animals with fasting blood glucose level 200 – 500 mg/dl were considered as diabetic and used for further experiments [8, 9, 10].

**Table. 1 Animals grouping for STZ induced diabetic model**

| S. No. | Group | Treatment | No. of animals |
|---|---|---|---|
| 1 | Normal control | Normal saline + Distilled water | 5 |
| 2 | Diabetic control | STZ + Distilled Water | 5 |
| 3 | Standard group | Glibenclamide + Distilled water | 5 |
| 4 | Test group I | Plant extract (200 mg/kg) + Distilled water | 5 |
| 5 | Test group II | Plant extract (500 mg/kg) + Distilled water | 5 |

**Biochemical Parameters**

Fasting blood glucose level was measured on 0, 7 and 14 days by glucose oxidase method. After 14 days of treatment, blood was drawn from retro orbital sinus, collected in tubes (anticoagulant tubes: containing sodium fluoride and potassium oxalate) and analyzed for serum glutamic pyruvic acid (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and lipid profile [11, 12, 13].

**Data & Statistical Analysis**

All the values or data were expressed as mean ± S.D. Statistical analysis was carried out by one –way ANOVA followed by post dunnett's t-test using graph pad statistical software. The criterion between all the groups for significance was $P \leq 0.05$.

## Histopathology

All the animals of each group were sacrificed by using anaesthesia. Pancreas was removed and the entire tissue sample was kept in 10 % buffered neutral formalin solution. The tissue sample was send to pathology for histopathological study. Tissue sample were stained with haematoxylin and eosin dyes and section of pancreas was observed under light microscope [14,15].

## Results

Effect of Phoenix sylvestris seeds extract on serum triglycerides, cholesterol in diabetic rats. Serum triglycerides and cholesterol were increased, after the administration of streptozotocin. The administration of extract (200 and 500 mg/kg b.w) and glibenclamide (5mg/kg b.w) significantly decreased serum triglycerides and cholesterol, ** ($P \leq 0.01$), *** ($P \leq 0.001$) and * ($P \leq 0.05$) respectively. The extracts were also shown to significantly lower the LDL ($P \leq 0$level and enzymatic activity of liver marker enzymes serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT).

**Table. 2** Effect of *Phoenix sylvestris* seeds on lipid profile

| Group | Cholesterol | Triglycerides | LDL |
|---|---|---|---|
| **I. Control** | 50.59 ± 2.77 | 84.4 ± 7.53 | 83.35 ± 3.22 |
| **II. Diabetic control** | 149.33 ± 6.93 | 189.8 ± 8.19 | 189.38 ± 5.61 |
| **III. Test I** | 88.12 ± 3.79 | 165.4 ± 8.14*** | 142.77 ± 4.23 |
| **IV. Test II** | 74.35 ± 2.92** | 98.2 ± 6.97** | 132.54 ± 3.46** |
| **V. Standard** | 75.83 ± 2.73 ** | 98.8 ± 6.22* | 128.54 ± 3.46** |

The values are expressed as mean ± S.D; N = 5 in each group.

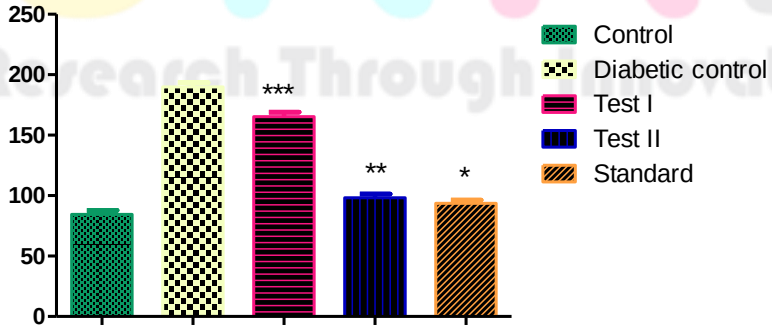



**Figure.1**

**Effect of Extract on Liver Marker Enzyme**

**Table.3** Effect of extract of *Phoenix sylvestris* seeds on liver marker enzyme

| Groups | SGPT (U/L) | SGOT (U/L) |
|---|---|---|
| **I.** Control | 92.23 ± 6.75 | 115.00 ± 5.14 |
| **II.** Diabetic control | 209 ± 6.35 | 243.00 ± 9.57 |
| **III.** Test I (200 mg/kg b.w) | 121.85 ± 6.69 | 138.53 ± 2.94 |
| **IV.** Test II (500 mg/kg b.w) | 112.83 ± 7.11** | 129.66 ± 8.08** |
| **V.** Standard | 103.73 ± 3.00** | 119.50± 4.43** |

The values are expressed as mean ± S.D; N = 5 in each group.

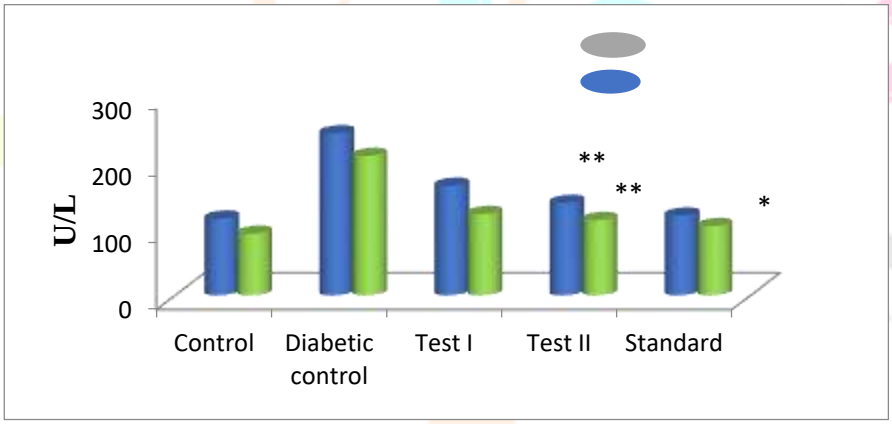



**Figure.2**

**Anti-Hyperglycaemic Effect of Extract & Glibenclamide**

After the induction of streptozotocin, the fasting blood glucose level was increased in diabetic groups (group II, III, IV and V) when compared with normal group at first day. Although, the comparison was continued until the end of 14 days. All groups of animals were treated with extract and glibenclamide (5mg/kg b.w) group III, IV and V respectively except group I. At the end of the 14 days the fasting blood glucose level were decreased in groups (III, IV and V;). The *($P < 0.05$) indicated the statistical significance between the groups.

**Table.4** Antihyperglycemic effect of extract and glibenclamide on fasting blood glucose level.

| Groups | 0 day | 7 day | 14 day |
|---|---|---|---|
| **I.** Control | 92 ± 2.12 | 100.5 ± 2.12 | 98 ± 1.41 |
| **II.** Diabetic control | 211 ± 4.24 | 246.5 ± 2.12 | 253.5±1.7 |
| **III.** Test I (200 mg/kg b.w) | 208 ± 2.82 | 197.5 ± 1.7 ** | 188± 1.41** |
| **IV.** Test II (500 mg/kg b.w) | 207.5 ± 3.53 | 174 ± 5.65 ** | 168.5±12.02** |
| **V.** Standard | 212 ± 4.24 | 191 ± 1.41** | 181.5 ± 7.77** |

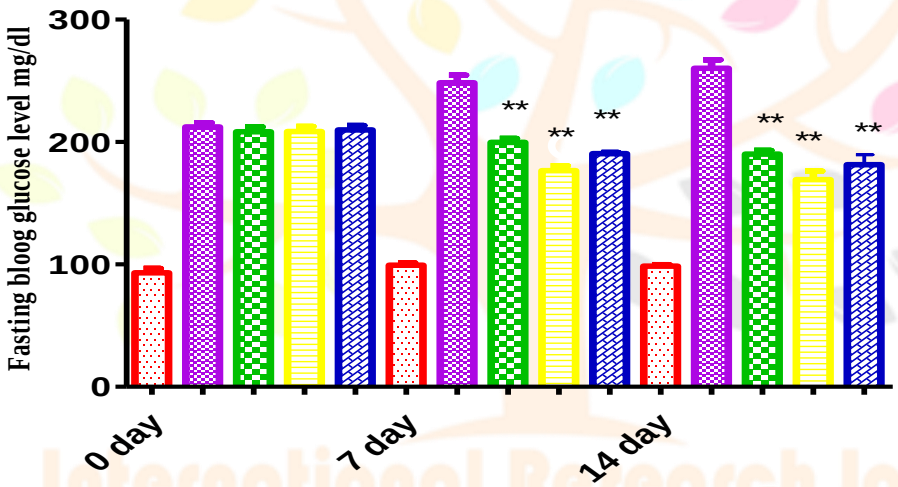



**Figure.3** Effect of extract and glibenclamide on fasting blood glucose level

The values are expressed as mean ± S.D; n = 5 in each group. ** represent statistical significance, ** $P \leq 0.01$ as compared with diabetic control at the same time (one-way ANOVA followed by Dunnett's multiple comparison tests).

**Histopathology**

**Figure.4 (a) Control**

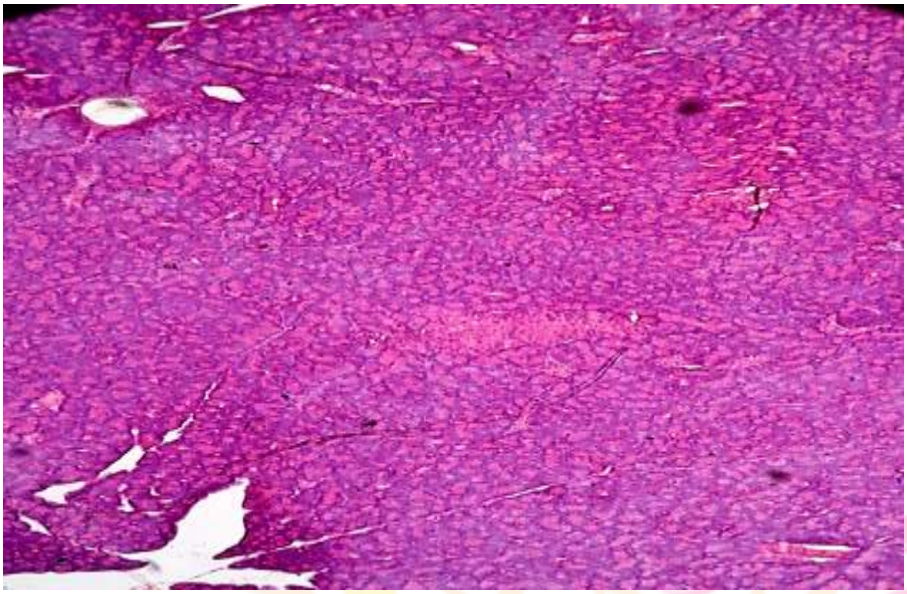


**Figure.5 (b) Diabetic Control**

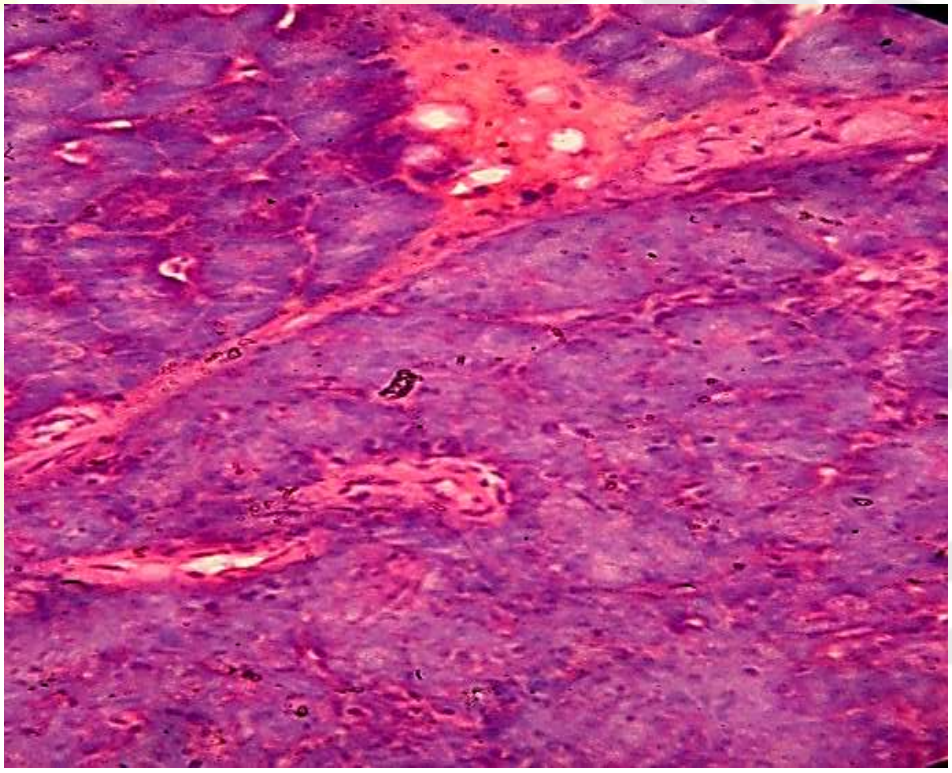


**Figure.6 (c) Test- I**

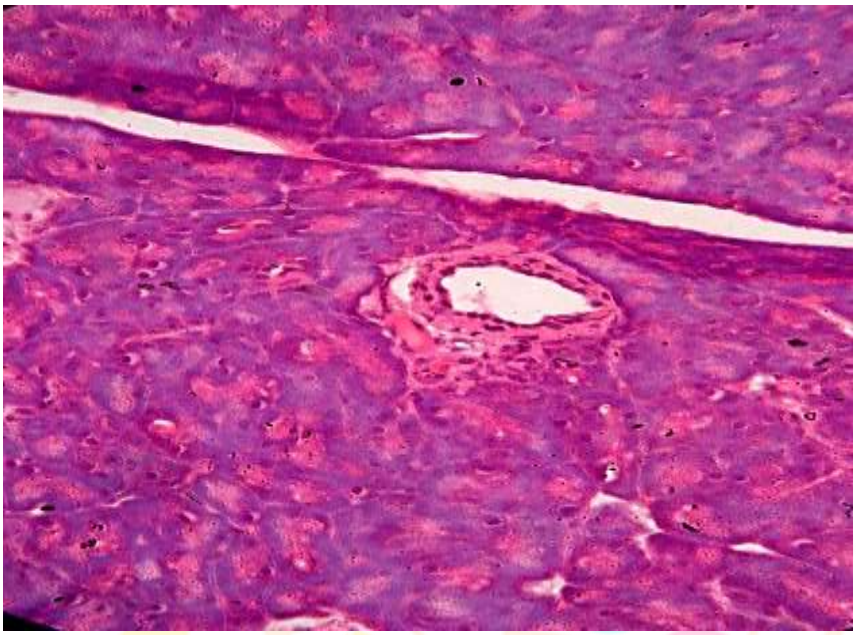


**Figure.6 (d) Test- II**

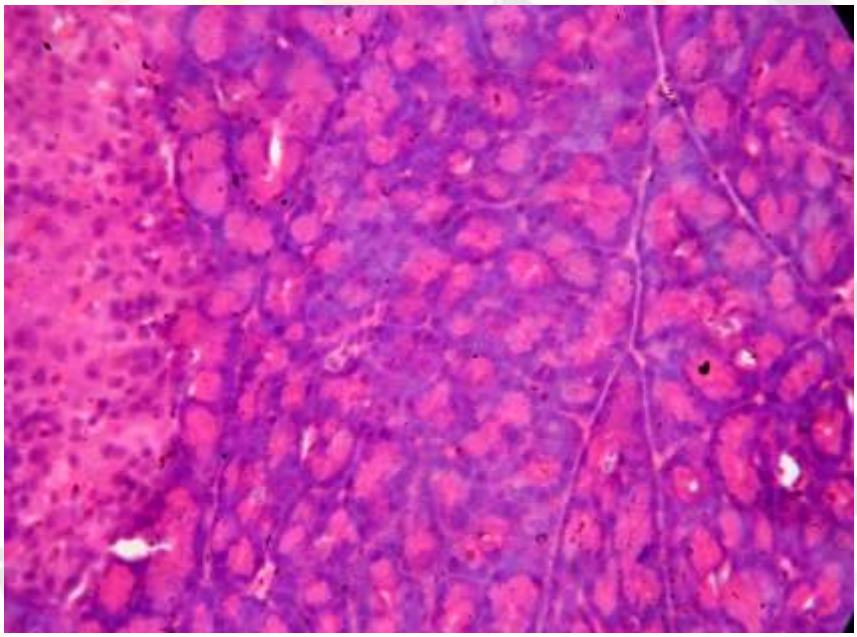
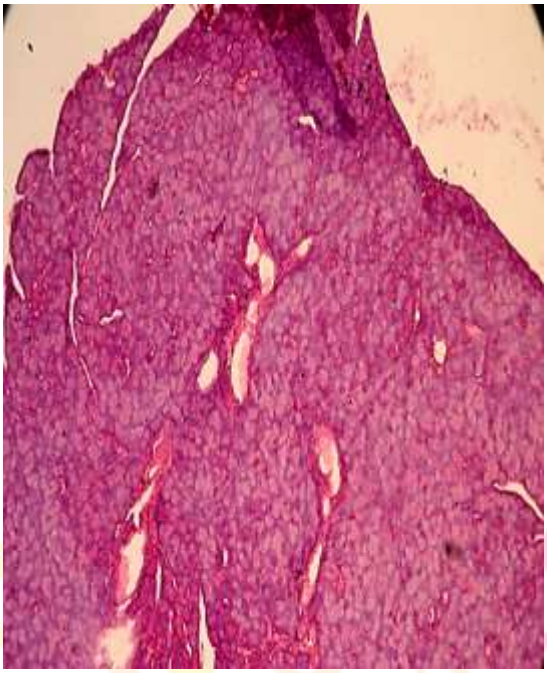


**Figure.7 (e) Standard**



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