# STUDY OF DIANTHUS CHINENSIS STEM AS POTENTIAL ANTIOXIDANT AND ANTI-DIABETIC ACTIVITY ON EXPERIMENTAL RATS

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## Abstract

Diabetes is a metabolic disorder worldwide which is also the cause for other associated complications like diabetic nephropathy So for the more exhaustive research on herbs this study aimed to evaluate the antioxidant and anti-diabetic potential of *Dianthus chinensis* stem using experimental rat models. The plant was selected based on its traditional medicinal usage and collected from authenticated sources. After proper botanical identification, the stems were shade-dried, powdered, and subjected to extraction using a suitable solvent. The percentage yield of the extract was recorded. Phytochemical screening was conducted to assess the presence of bioactive constituents, with both qualitative and quantitative estimations carried out. Quantitative analysis revealed significant amounts of total phenolic content (TPC) and total flavonoid content (TFC), both known for their strong antioxidant properties. An acute toxicity study was performed to determine the safety profile of the extract. For the pharmacological investigation, streptozotocin (STZ)-induced diabetic rats were used to evaluate the anti-diabetic efficacy of the plant extract. Parameters assessed included body weight, fasting blood glucose levels, and a range of biochemical markers such as serum insulin, lipid profile (total cholesterol, triglycerides, and HDL), and glycated hemoglobin (HbA1c). Histopathological examination of pancreatic tissues was also carried out to determine cellular damage and regeneration. The findings demonstrated that Dianthus chinensis leaf extract significantly improved glycemic control, reduced oxidative stress, and preserved pancreatic architecture in diabetic rats. These results suggest that the plant possesses notable antioxidant and anti-diabetic activities, supporting its traditional use and potential application as a natural therapeutic agent for managing diabetes and oxidative stress-related complications.

**Keywords: -**Dianthus chinensis, Antioxidant activity, Anti-diabetic activity, Streptozotocin (STZ), Phytochemical screening, Histopathology.

## 1. Introduction

Herbal remedies are becoming increasingly popular around the world. Because of the increased usage, safety concerns have become more relevant. Many unfavorable effects of herbal medications might be due to low quality raw materials or final goods. Different forms of herbal medications are related with various issues. According to the World Health Organization, trade in medicinal plants, herbal raw materials, and herbal pharmaceuticals is increasing at a pace of approximately 15% each year.[1] The growing popularity and acceptance of herbal therapy stems from the notion that all natural products are safe, inexpensive, and readily available.[2] However, there are certain problems about herbal medicine in terms of pharmacognosy and standardization when compared to conventional pharmaceuticals. For the last two decades, research efforts have been strengthened in both industrialized and developing countries to scientifically test and validate herbal medications through clinical trials (Khan and Ahmad 2019) [3,4] Many research have confirmed the efficacy of medicinal plants with hypoglycemic properties in the treatment of diabetes.[5] The benefits of these plants may delay the onset of diabetes problems and perhaps help to rectify metabolic imbalances.[6] In current allopathic medicine, however, their significance is confined to the use of natural polymers such as guar gum, gum acacia, gum Arabic, and others. Natural gums have the therapeutic benefit of lowering the caloric value of the consumed food by limiting carbohydrate absorption from the gastrointestinal system. [7] Diabetes mellitus (DM) was first identified as a disease some 3000 years ago by the ancient Egyptians and Indians, who demonstrated several clinical signs that are quite similar to what we now call diabetes. DM is a mixture of two words: "diabetes," a Greek term derivation that meaning siphon - to pass through,[8] and the Latin word "mellitus," which means honeyed or sweet. In 1776, the presence of excess sugar in blood and urine was first verified in Great Britain. With the passage of time, a comprehensive understanding of diabetes, including its etiology and pathophysiology, has been attained. [9,10] The term "DM" refers to "a metabolic disorder characterized by hyperglycemia resulting from either the deficiency in insulin secretion or the action of insulin." The poorly controlled DM can lead to damage various organs, especially the eyes, kidney, nerves, and cardiovascular system (Ahmed *et. al* 2002). [5,11,12] Diabetes mellitus is a diverse set of illnesses distinguished by hyperglycemia caused by an absolute or relative deficiency in insulin synthesis or function. Diabetes mellitus' persistent hyperglycemia causes damage, malfunction, and failure of end organs such as the retina, kidney, neurological system, heart, and blood vessels.[13] All macromolecule meals are countermined into aldohexose in the circulation. The endocrine system facilitates the transport of aldohexose into cells.[14] The endocrine system transports sugar from the bloodstream into your cells, where it is stored or used for energy. With polygenic disease, your body either does not generate enough endocrine or properly uses the endocrine that it does build. Untreated high blood glucose levels from polygenic disease can harm your nerves, eyes, kidneys, and other organs (Kumar and Clark 2002). [15,16,17] The term 'diabetes' is derived from the Greek word "Diab" (meaning to pass through, referring to the cycle of strong thirst and frequent urination);'mellitus' is the Latin word for "sweetened with honey" (relating to the presence of sugar in the urine). Greeks were aware of an illness associated with polyurea and body wasting, but Aretaeus of Cappadocia described a disease associated with thirst and polyurea. Later, the knowledge was transferred to Chinese, Iranians, and Arabians.

Diabetes mellitus was known in Spain as a disease characterized by polyurea and polydipsia with sugary-flavored urine after spreading from the Middle East. The discovery of sugar in urine and its identification by laboratory test spread throughout the 18th century. Different geographical regions. [18] Diabetes pathophysiology is associated with insulin levels in the body as well as the body's ability to use insulin. Type 1 diabetes has no insulin, whereas type 2 diabetes has peripheral tissues that resist insulin's actions. Normally, pancreatic beta cells produce insulin in response to elevated blood glucose levels. The brain requires glucose to perform normal functioning on a continuous basis. Hypoglycemia,[19] or low plasma glucose levels,[20] is most commonly caused by diabetic medications such as insulin and oral antihyperglycemics. Diabetes pathogenesis involves elevated plasma glucose levels telling the central nervous system to mobilize energy reserves.[21] Occult diabetes with late hypoglycemia may develop in some people with poor glucose tolerance or early type 1 or type 2 diabetes. Following a highcarbohydrate meal, the patient develops hypoglycemia. Studies on identical twins have demonstrated that genetically predisposed individuals must also be exposed to an environmental trigger, such as viral infection. Viral infection can destroy pancreatic B cells and expose antigens, triggering a self-perpetuating autoimmune response. The patient becomes obviously diabetic only when more than 90% of the B cells have been eliminated. [9,22] The study of Dianthus chinensis leaves as a potential antioxidant and anti-diabetic agent in experimental rats aimed to investigate the therapeutic potential of this plant, which had been primarily recognized for its ornamental value but was also believed to possess medicinal properties. [23] Dianthus chinensis, commonly known as the Chinese pink or Indian pink, was a member of the Caryophyllaceae family and had been traditionally used in folk medicine for its purported healing effects. This research sought to explore the pharmacological properties of the plant, particularly its ability to modulate oxidative stress and manage diabetes-related complications, both of which were prevalent and significant global health issues. [1,3,8,24] The primary focus of the study was to determine the antioxidant potential of Dianthus chinensis leaf extracts. Antioxidants were substances that could neutralize free radicals—unstable molecules that contributed to cellular damage, aging, and the development of various diseases, including diabetes. The leaves of Dianthus chinensis were found to be rich in various phytochemicals, including flavonoids, phenolic compounds, and alkaloids, which had been shown to possess strong antioxidant and anti-inflammatory activities. [25]

## 2. MATERIALS AND METHODS

### 2.1 Materials

The selection of reagents, chemicals, and glassware played a crucial role in ensuring the accuracy and reliability of the experimental procedures. All chemicals, including solvents like methanol, petroleum ether, and chloroform, as well as acids such as hydrochloric acid, nitric acid, and sulfuric acid, were of analytical grade and sourced from reputed suppliers like Merck, Rankem, and Tata Chemicals, ensuring high purity and consistency. Reagents like sodium hydroxide, copper sulfate, and nitroprusside were essential for various qualitative and quantitative analyses throughout the study. The glassware used, including pipettes, volumetric flasks, beakers, and test tubes, were all high-quality borosilicate, known for their chemical

resistance and durability, which ensured safety and precision during handling of reagents and sample preparation. The use of standardized materials and equipment contributed significantly to the reproducibility and validity of the experimental results.[26]

## 2. Methods

### 2.1 Plant Collection

The medicinal plant Dianthus chinensis (300 gm) was harvested. After cleaning, plant was dried in the shade at room temperature for three days, followed by oven drying at 45°C until completely dry. To prevent contamination and deterioration, dried plant components were stored in airtight glass containers in a dry, cool environment. Authentication of chosen traditional plant - medicinal plant a plant taxonomist authenticated Dianthus chinensis to ensure its identification and purity.

### 2.2 Extraction

In the current investigation, plant material was extracted utilizing the continuous hot percolation method with Soxhlet equipment. Dianthus Chinensis powder was placed in a Soxhlet apparatus thimble. Soxhlation was carried out at 60°C using petroleum ether as the nonpolar solvent. The exhausted plant material (marc) was dried and then extracted again with methanol solvent. For each solvent, soxhlation was continued until no visible color change was observed in the siphon tube, and extraction was confirmed by the absence of any residual solvent when evaporated. The obtained extracts were evaporated at 40°C in a rotary vacuum evaporator (Buchi type). The dried extract was weighed, and each extract's % yield was calculated using the following formula:

$$\%Yield = \frac{weight\ of\ extract}{weight\ of\ plant\ material\ used} \times 100$$

The prepared extracts were examined for organoleptic characteristics (percentage yield, color, and odor) and then packaged in an airtight container and labeled for future use.

### 2.3 Phytochemical Investigation

An experiment was carried out to determine the presence or absence of several phytoconstituents using thorough qualitative phytochemical analysis. Medical reactions to testing were based on colour intensity or precipitate formation. The following standard methods were used Alkaloids taste, Test for Carbohydrates, Test for Saponins, Test for Triterpenoids and Steroids, Test for Tannin and Phenolic Compounds, Test for protein and amino acids and Test for Glycosides.

### 2.4 Quantitative Phytochemical Estimation

#### 2.4.1 Total phenolic content (TPC)

Dianthus chinensis extract's total phenolic content was calculated using the Folin-Ciocalteu Assay. The Dianthus chinensis extracts (0.2 ml of stock solution) were combined with 2.5 ml of Folin-Ciocalteu Reagent and 2 ml of 7.5% sodium carbonate. This mixture was diluted with distilled water until it reached 7 ml. The resultant solutions were then allowed to rest at room temperature for two hours before being measured spectrophotometrically at 760 nm. Calibration curves were created with standard solutions of Gallic Acid Equivalent (GAE) mg/gm. Gallic Aid was produced at concentrations of 20, 40, 60, 80, and 100 µg/ml. The Folin-Ciocalteu reagent is sensitive to reducing substances, including polyphenols. They generate a blue color when reacting. The blue colour was assessed spectrophotometrically.

#### 2.4.2 Total flavonoid content (TFC)

The flavonoid content was measured using the aluminium chloride technique. 0.5 ml of Dianthus chinensis extract solution was combined with 2 ml of distilled water. Then, 0.15 ml of sodium nitrite (5%) was added and properly mixed. After that, wait 6 minutes before adding 0.15 ml of 10% aluminium chloride and letting it stand for 6 minutes. Then, 2 milliliters of 4% sodium hydroxide were added. The mixture was shook and well mixed. The absorbance of the mixture was measured at 510 nm with a UV spectrophotometer. Calibration curves were developed using standard solutions of Rutin Equivalent (RE) mg/g. Rutin was concentrated to 20, 40, 60, 80, and 100 µg/mL. The total flavonoid concentration was determined using the calibration curve and represented as mg Rutin equivalent per gram of dry extract weight.

### 2.5 Antioxidant activity test

The antioxidant activity of Dianthus chinensis extract was determined using the DPPH free radical scavenging test. A 1 mg/mL methanol solution of extracts/standard was prepared. Dianthus chinensis extracts/standards (20-100µg/ml) were produced from a 1mg/ml stock solution with 2ml of 0.1mm DPPH solution added. The resulting mixture was vortexes, incubated for 30 minutes at room temperature in a relatively dark environment, and then measured at 517 nm using a UV spectrophotometer (Shimadzu 1700). For the control, add 3 mL of 0.1mm DPPH solution and incubate in the dark at room temperature for 30 minutes. The absorbance of the control was measured against methanol (as a blank) at 517. The percentage antioxidant activity of the sample/standard was estimated using the following formula: [27]

**% Inhibition = [(Ab of control- Ab of sample)/ Ab of control x 100]**

### 2.6 Acute Toxicity Study

The acute toxic class approach outlined in the guideline is a step-by-step procedure that uses three animals of the same sex in each phase. Depending on the mortality and/or moribund stage

of the animals, 2-4 steps may be required to determine the acute toxicity of the test chemical. A set of experimental animals receives the medicine orally in one of the prescribed doses. The chemical is evaluated step by step, using three animals of the same sex in each phase. The lack or presence of compound-related mortality in the animals dosed at one stage defines the next phase, which includes no more testing, dosing three further animals with the same dose, and dosing three more animals at the next higher or lower dose level. Each step includes three creatures. The dose level to be used as the initial dose is selected from one of four established levels. 5, 50, 300, or 2000 mg per kg body weight.

### 2.7 In Vivo of Anti-diabetic Activity

#### 2.7.1 Collection of Blood Samples for Glucose Analysis

Blood samples for glucose measurement were taken from the tail veins of overnight fasting rats (12 h) three days following Alloxan administration (day 0), and again on days 1, 3, 5, 7, and 14. The first blood drop was wiped away, while the second was placed on a glucose strip in the Accu-Chek glucometer (Roche Diagnostics, Germany) to obtain a glucose reading. The tails were then rubbed with ethanol to avoid infection.

#### 2.7.2 Biochemical Parameters

On days 0 and 14, blood samples for serum lipid levels were taken from overnight fasting rats under diethyl ether anesthesia using the retro orbital plexus puncture procedure and placed aside for 30 minutes to clot. The serum was separated by centrifuging the sample at 2500 rounds per minute for 10 minutes at 25 degrees Celsius. It was then tested for TG, HDL, and TC using the Mission Cholesterol Meter (Acon Laboratories, Inc., San Diego, CA, and United States). Very Low-Density Lipoprotein (VLDL) was determined as Triglycerides (TG); TG/5 and LDL were estimated using the Friedewald formula as follows:

**LDL (mg/dl) =TC - (HDL+VLDL)**

## 3. Result

### 3.1 Percentage Yield

In phytochemical extraction, percentage yield is crucial in identifying the standard extraction efficiency for a certain plant, different sections of the same plant, or various solvents used. Table 3 shows the yield of extracts obtained from Dianthus chinensis.

**Table 1: Percentage Yield of crude extracts of Dianthus chinensis extract**

| S.No | Plant Name | Solvent | Theoretical weight | Yield (gm) | % Yield |
|---|---|---|---|---|---|
| **1** | | Pet ether | 295 | 1.20 | 0.40% |

| | | | | | |
|---|---|---|---|---|---|
| | *Dianthus Chinensis* | Methanol | 250 | 4.60 | 2.85 % |

**Figure 1**: Percentage Yield of crude extracts of Dianthus chinensis extract

### 3.2 Preliminary Phytochemical study

**Table 2: Phytochemical testing of extract**

| S. No | Experiment | Presence or absence of phytochemical test, petroleum ether extract |
|---|---|---|
| **1** | **Alkaloids** | |
| **1.1** | Dragendroff's test | Absent (-ve) |
| **1.2** | Mayer's reagent test | Absent (-ve) |
| **1.3** | Wagner's reagent test | Absent (-ve) |
| **1.4** | Hager's reagent test | Absent (-ve) |
| **2** | **Glycoside** | |
| **2.1** | Borntrager test | Absent (-ve) |
| **2.2** | Legal's test | Absent (-ve) |
| **2.3** | Killer-Killiani test | Absent (-ve) |
| **3** | **Carbohydrates** | |
| **3.1** | Molisch's test | Present (+ve) |
| **3.2** | Fehling's test | Present (+ve) |
| **3.3** | Bendict's test | Present (+ve) |
| **3.4** | Barfoed's test | Present (+ve) |

| | | |
|---|---|---|
| | | |
| **4** | **Proteins and Amino Acids** | |
| **4.1** | Biuret test | Present (+ve) |
| **4.2** | Ninhydrin test | Present (+ve) |
| **5** | **Flavonoids** | |
| **5.1** | Alkaline reagent test | Present (+ve) |
| **5.2** | Lead Acetate test | Present (+ve) |
| **6** | **Tannins and Phenolic Compounds** | |
| **6.1** | Ferric Chloride test | Present (+ve) |
| **7** | **Saponin** | |
| **7.1** | Foam test | Present (+ve) |
| **8** | **Test for Terpinoids and Steroids** | |
| **8.1** | Sakowski's test | Absent (-ve) |
| **8.2** | Libbermann-Burchard's test | Absent (-ve) |

**Table No. 3: Phytochemical testing of methonol**

| **S. No.** | **Experiment** | **Presence or absence of phytochemical test, petroleum ether extract** |
|---|---|---|
| | | **Methonal extract** |
| **1** | **Alkaloids** | |
| **1.1** | Dragendroff's test | Present (+ve) |
| **1.2** | Mayer's reagent test | Present (+ve) |
| **1.3** | Wagner's reagent test | Present (+ve) |
| **1.4** | Hager's reagent test | Present (+ve) |
| **2** | **Glycosides** | |
| **2.1** | Borntrager test | Present (+ve) |
| **2.2** | Legal test | Present (+ve) |
| **2.3** | Killer-Killiani test | Present (+ve) |

| | | |
|---|---|---|
| **3** | **Carbohydrates** | |
| **3.1** | Molisch's test | Present (+ve) |
| **3.2** | Fehling's test | Present (+ve) |
| **3.3** | Benedict's test | Present (+ve) |
| **3.4** | Barfoed's test | Present (+ve) |
| **4** | **Proteins and Amino Acid** | |
| **4.1** | Biuret test | Absent (-ve) |
| **4.2** | Ninhydrin test | Absent (-ve) |
| **5** | **Flavonoids** | |
| **5.1** | Alkaline reagent test | Present (+ve) |
| **5.2** | Lead Acetate test | Present (+ve) |
| **6** | **Tannin and Phenolic Compounds** | |
| **6.1** | FerricChloride test | Present (+ve) |
| **7** | **Test for Triterpenoids and Steroids** | |
| **7.1** | Salkowiski's test | Present (+ve) |
| **7.2** | Libbermann-Burchard's test | Present (+ve) |
| **8** | **Saponins** | |
| **8.1** | Foam test | Present (+ve) |

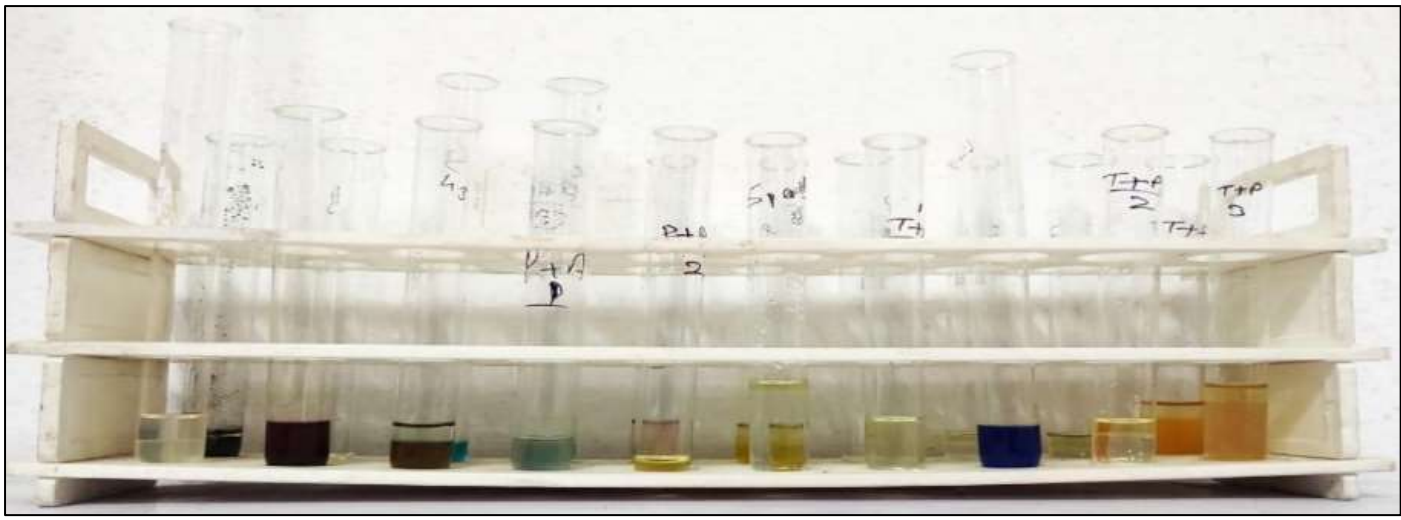


**Figure 2:** Phytochemical testing of petroleum ether extract

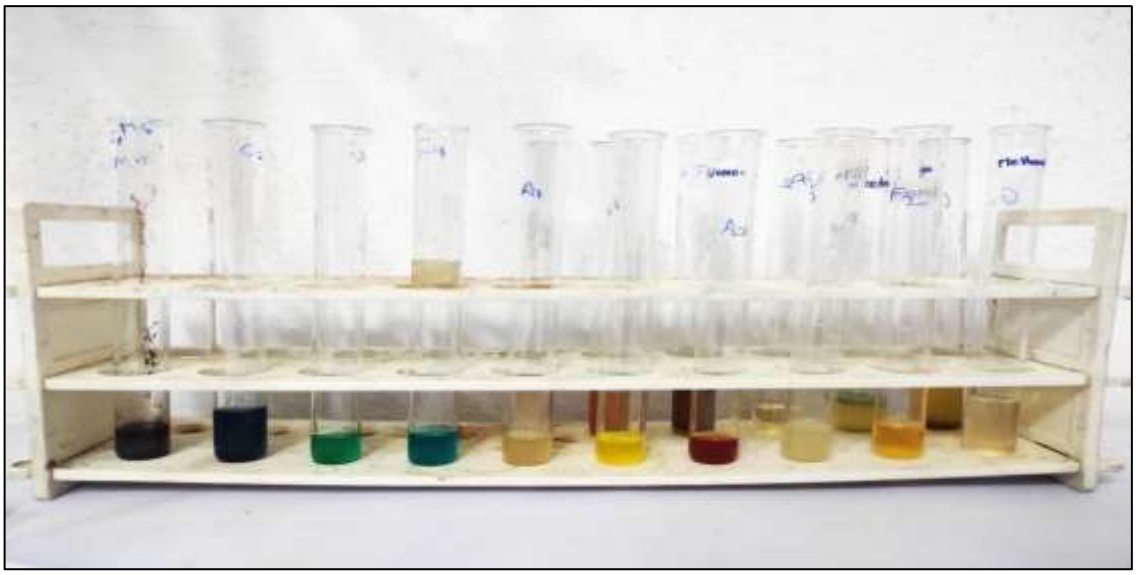


**Figure 3:** Phytochemical testing of Methanolic extract

### 3.4 Quantitative Analysis

A preliminary phytochemical study of crude extracts revealed the presence of phenols and flavonoids in plant material. Assays were performed to evaluate the amount of total phenolic (TPC) and total flavonoid content (TFC).

### 3.4.1 Total Phenolic content (TPC) estimation

**Table 4: Standard table for Gallic acid**

| S. No | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1 | 20 | 0.139 |
| 2 | 40 | 0.170 |
| 3 | 60 | 0.186 |
| 4 | 80 | 0.239 |

| | | |
|---|---|---|
| 5 | 100 | 0.275 |

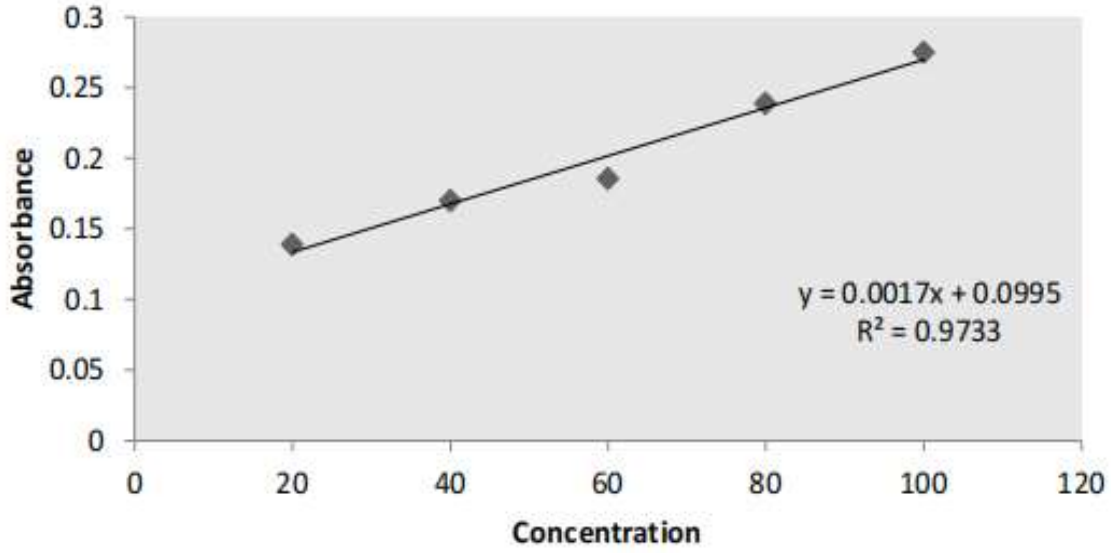



**Figure 4:** Represent standard curve of Gallic acid

### 3.4.2 Total Phenolic Content in extract

**Table 5: Total Phenolic Content**

| S.No | Absorbance | TPC in mg/gm equivalent of Gallic Acid |
|---|---|---|
| **1** | **0.142** | **67.5 mg/gm** |
| **2** | **0.175** | |
| **3** | **0.185** | |

**Table 8: Total Phenolic Content of extract *Dianthus chinensis***

| Extract | Total Phenolic content (mg/gm equivalent of Gallic acid) |
|---|---|
| **Methanol** | **67.5** |

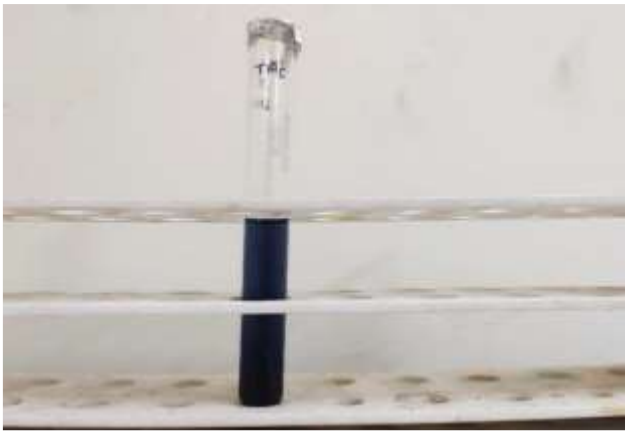


**Figure 5:** Total Phenolic Content

### 3.4.3 Total Flavonoids content (TFC) estimation

**Table 6: Standard table for Rutin**

| S.No | Concentration (µg/ml) | Absorbance |
|---|---|---|
| 1 | 20 | 0.175 |
| 2 | 40 | 0.199 |
| 3 | 60 | 0.276 |
| 4 | 80 | 0.314 |
| 5 | 100 | 0.330 |

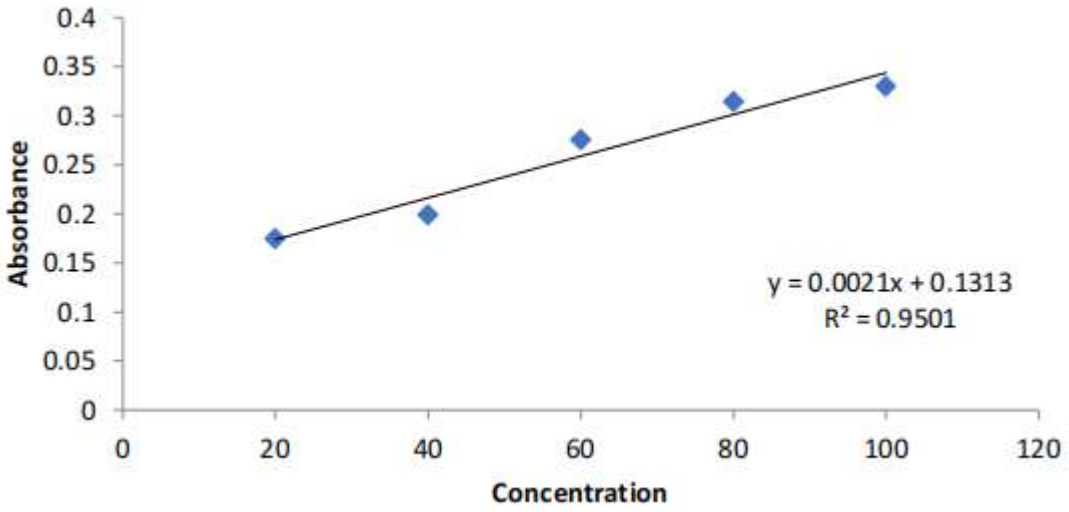



**Figure 6**: Represent standard curve of Rutin

### 3.4.4 Total Flavonoid Content in extract

**Table 7: Total Flavonoid Content**

| S.No | Absorbance | TFC in mg/gm equivalent of Rutin |
|---|---|---|
| 1 | 0.189 | |

| | | |
|---|---|---|
| **2** | **0.243** | **56.35 mg/gm** |
| **3** | **0.301** | |

**Table 8: Total Flavonoid Content of extract *Dianthus Chinensis.***

| Extract | Total Flavonoid content (mg/gm equivalentof rutin) |
|---|---|
| **Methanol** | **56.35** |

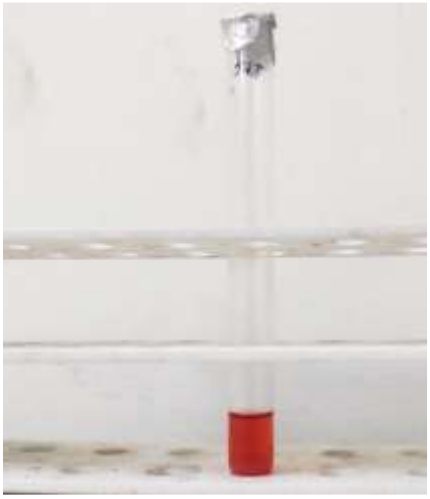


**Figure 7:** Total Flavonoid Content

### 3.5 *In vitro* Antioxidant Assays

The present study assessed the in vitro anti-oxidant potential of *Dianthus chinensis* extracts usingDPPH radical scavenging activity. The findings are given in tables.

### 3.5.1 DPPH 1, 1- diphenyl-2-picryl hydrazyl Assay

**Table 9: DPPH radical scavenging activity of Std. Ascorbic acid**

| Concentration (µg/ml) | Absorbance | % Inhibition |
|---|---|---|
| **20** | **0.475** | **52.02** |
| **40** | **0.420** | **57.75** |
| **60** | **0.335** | **66.12** |
| **80** | **0.277** | **72.02** |
| **100** | **0135** | **86.36** |
| **Control** | **0.990** | |
| **IC50** | | **19.54** |

**Table 10: DPPH radical scavenging activity of methanol extract of *Dianthus chinensis***

| Concentration (µg/ml) | Absorbance | % Inhibition |
|---|---|---|
| **20** | **0.519** | **43.586** |
| **40** | **0.461** | **49.891** |

| | | |
|---|---|---|
| **60** | **0.454** | **50.652** |
| **80** | **0.421** | **54.239** |
| **100** | **0.370** | **59.782** |
| **Control = 0.920**<br>**IC50= 51.32** | | |

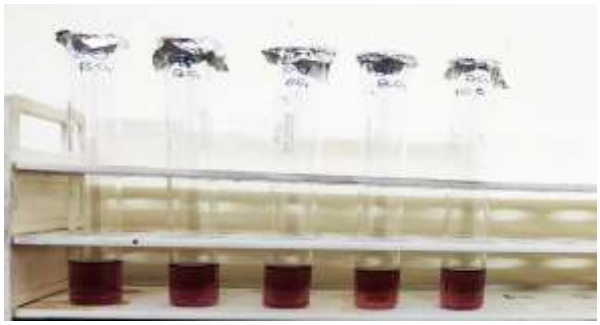


**Figure 8:** DPPH radical scavenging activity

### 3.5.2 Streptozotocin Induced Diabetes Model

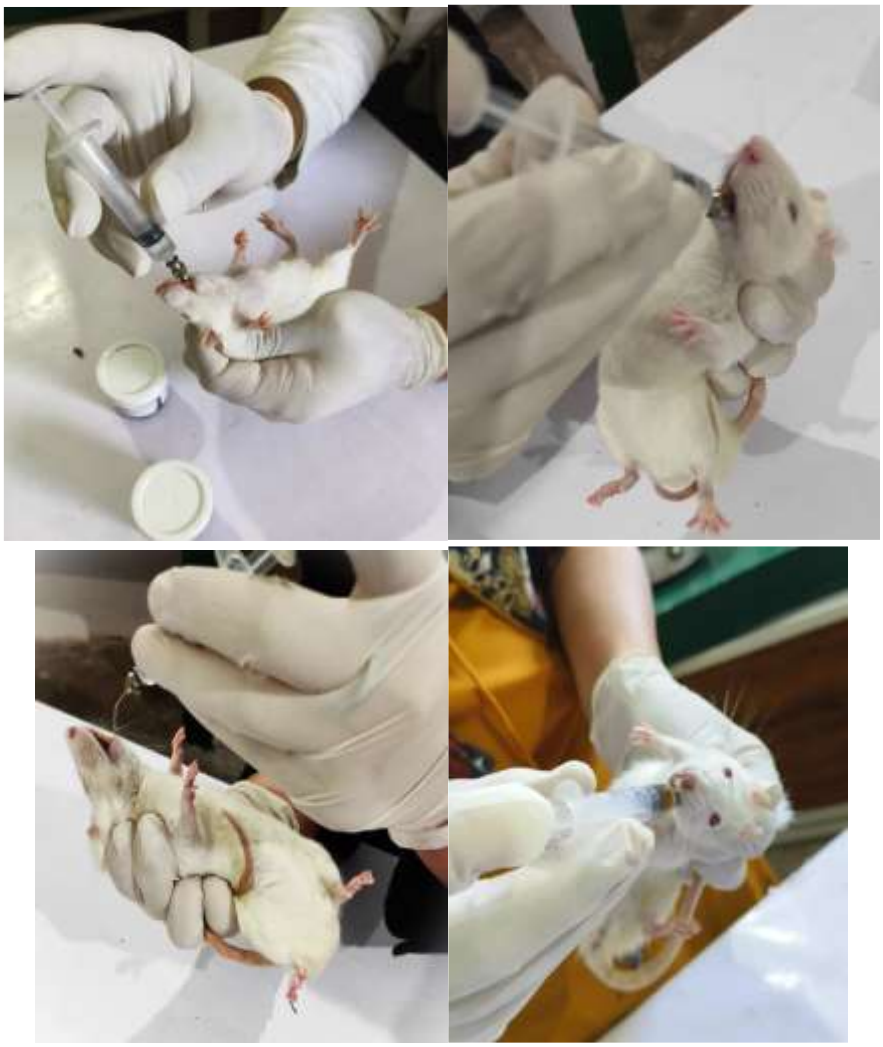
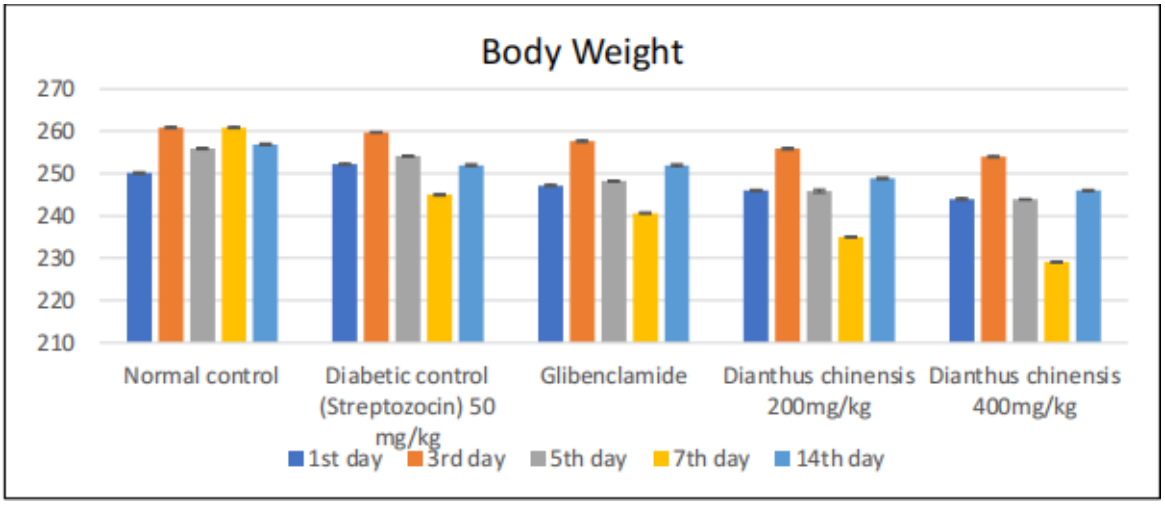


**Figure 9:** Streptozotocin induced diabetes Model

### 3.6 *In vivo* anti -diabetic study

**Table 11: Effect of *Dianthus chinensis* extract on Body weight of the rats**

| **Body weight(gms)** | | | | | | |
|---|---|---|---|---|---|---|
| **Groups** | **Treatment** | **1 day** | **3day** | **5day** | **7day** | **14day** |
| **Group 1** | Normal control | 250.11±0.261 | 252.22±0.233 | 247.18±0.220 | 245.99±0.211 | 243.98±0.251 |
| **Group II** | Diabetic control (Streptozocin) 50 mg/kg | 260.89±0.214 | 259.76±0.138 | 257.65±0.188 | 255.87±0.250 | 254±0.263 |
| **Group III** | Glibenclamide (10 mg/kg) | 255.93±0.249 | 254.10±0.271 | 248.203±0.187 | 245.87±0.204 | 243.89±0.272 |
| **Group IV** | *Dianthus chinensis* 200mg/kg | 260.90±0.223 | 244.99±0.263 | 240.65±0.394 | 235.02±0.157 | 229.05±0.241 |
| **Group V** | *Dianthus chinensis* 400mg/kg | 256.91±0.255 | 252.01±0.207 | 251.99±0.138 | 248.87±0.121 | 245.98±0.228 |



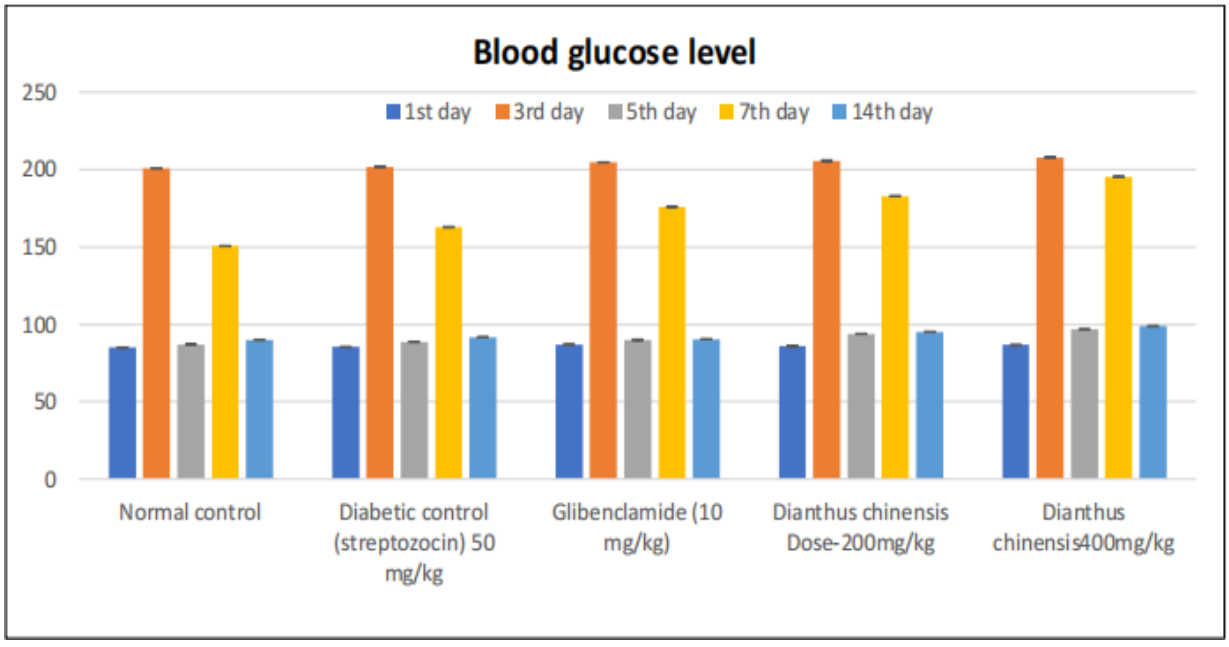

**Graph 1:** Graphical representation of effect of *Dianthus chinensis* extract on Bodyweight of the rats

### 3.7 Blood Glucose Level

**Table 12: Effect of test samples of extract on Blood Glucose Level in experimental rats**

| **Blood Glucose Level (gms)** | | | | | | |
|---|---|---|---|---|---|---|
| **Groups** | **Treatment** | **1days** | **3days** | **5days** | **7days** | **14days** |
| **Group 1** | Normal control | 85.07±0.192* | 85.45±0.208* | 86.98±0.334** | 85.99±0.194* | 86.76±0269** |
| **GroupII** | DiabeticControl(streptozocin)50 mg/kg | 200.89±0.179 | 201.87±0.260 | 204.76±0.238 | 205.65±0.36 | 207.89±0.228* |
| **GroupIII** | Glibenclamide(10 mg/kg) | 87.05±0.226* | 88.76±0.104* | 89.88±0.496* | 93.89±0.287*** | 96.78±0.093*** |
| **GroupIV** | *DianthusChinensis* Dose-200mg/kg | 150.67±0.095* | 162.89±0.601** | 175.98±0.201*** | 182.89±0.235*** | 195.45±0.208** |
| **GroupV** | *Dianthuschinensis*400mg/kg | 89.99±0.351* | 91.76±0.264** | 90.43±0.121* | 95.21±0.256** | 98.90±0.34*** |

The differences between treatment groups got statistically significant ($P < 0.001$), as determinedby the Bonferroni t-test, indicating that the observed effects got not due to chance.



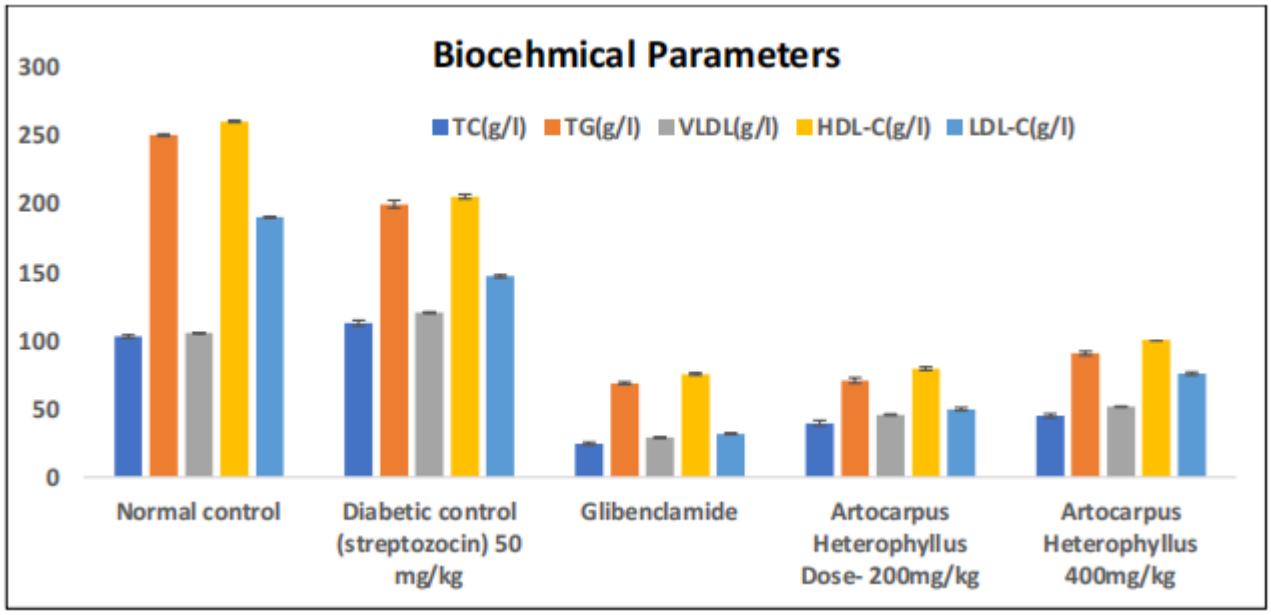

**Graph 2**: Graphical representation of effect of *Dianthus chinensis* extract on BloodGlucose Level of the rats

### 3.8 Biochemical Parameters

**Table 13: Effect of test samples of extract on Biochemical Parameters in experimental rats**

| Treatmentgroups | Biochemical parameters | | | | |
|---|---|---|---|---|---|
| | TC(g/l) | TG(g/l) | VLDL(g/l) | HDL-C(g/l) | LDL-C(g/l) |
| Normalcontrol | 103±1.25* | 113±0.90** | 24.54±0.25* | 39.67±0.81* | 45±0.45* |
| Diabeticcontrol(Streptozocin) 50mg/kg | 250.65±2.02 | 200.14±2.78 | 68.89±0.59 | 70.90±1.67 | 90.78±0.99* |
| Glibenclamide(10 mg/kg) | 105.54±1.10* | 120.54±0.99** | 28.98±0.26* | 45.78±0.89* | 51.67±0.66** |
| *Dianthus Chinensis*Dose-200mg/kg | 260.76±1.99** | 205.65±2.01** | 75.65±0.50* | 79.56±1.25* | 100.87±1.15*** |
| *Dianthuschinensis*400 mg/kg | 190.54±1.50** | 147.24±1.50*** | 31.87±0.34** | 49.89±0.99** | 75.87±1.16*** |

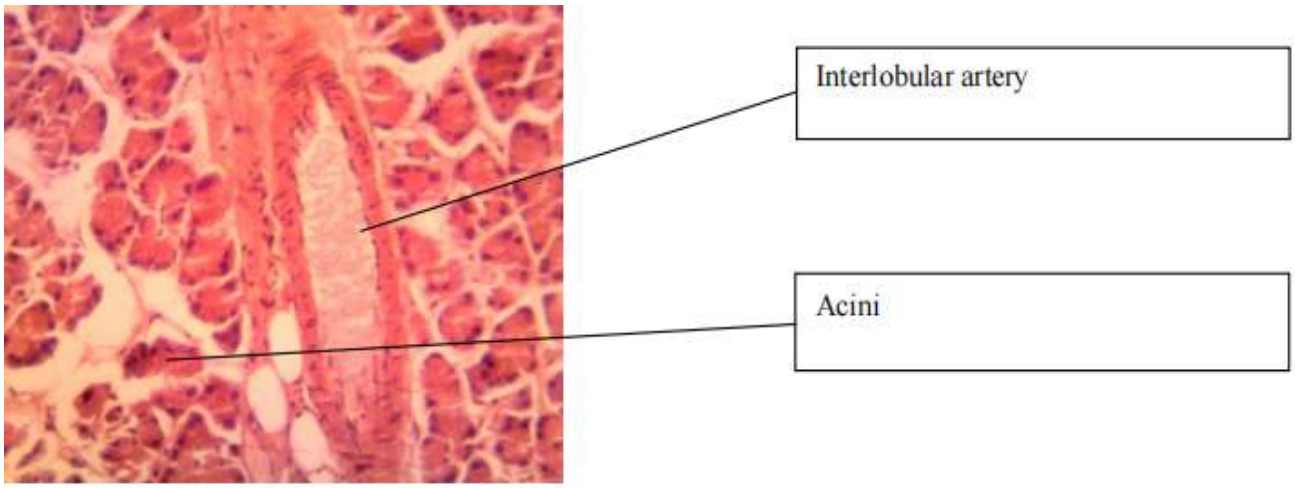
The differences between treatment groups got statistically significant ($P < 0.001$), as determinedby the Bonferroni t-test, indicating that the observed effects got not due to chance.



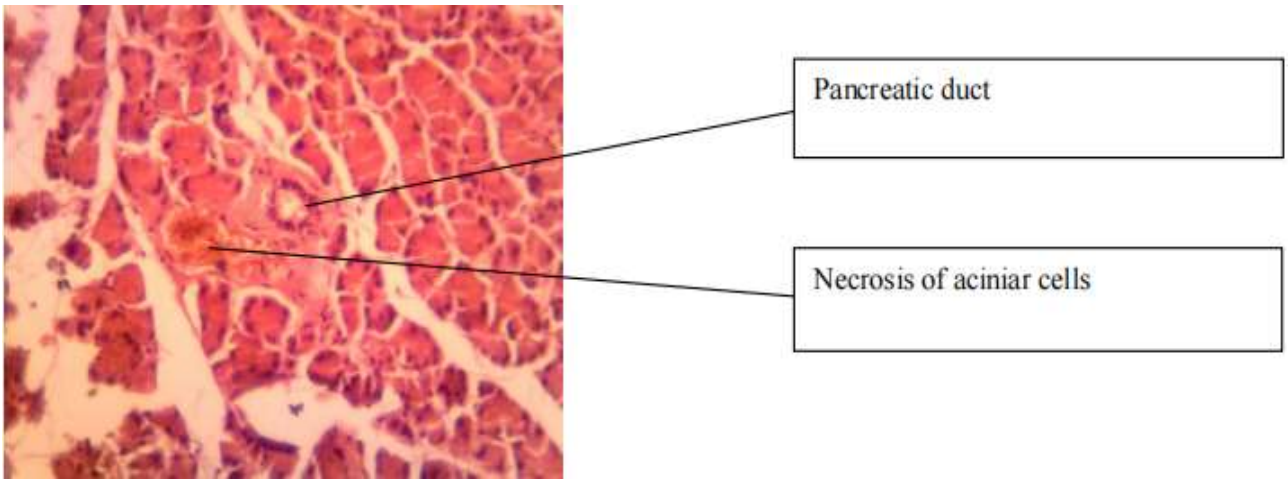

**Graph 3:** Graphical representation of effect of *Dianthus chinensis* extract onBiochemical Parameters of the rats.

### 3.9 Histopathological examination

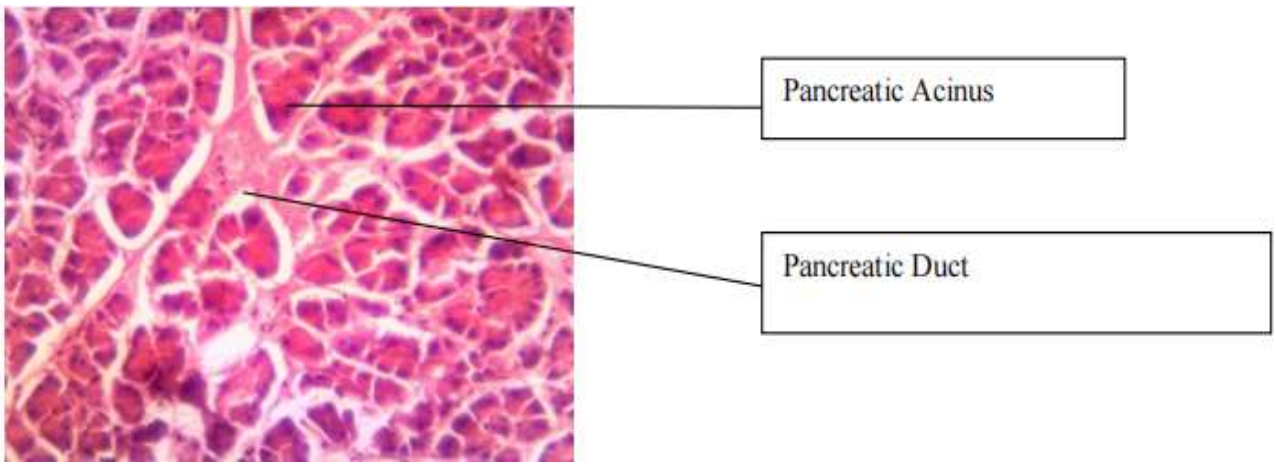
**GROUP 1**



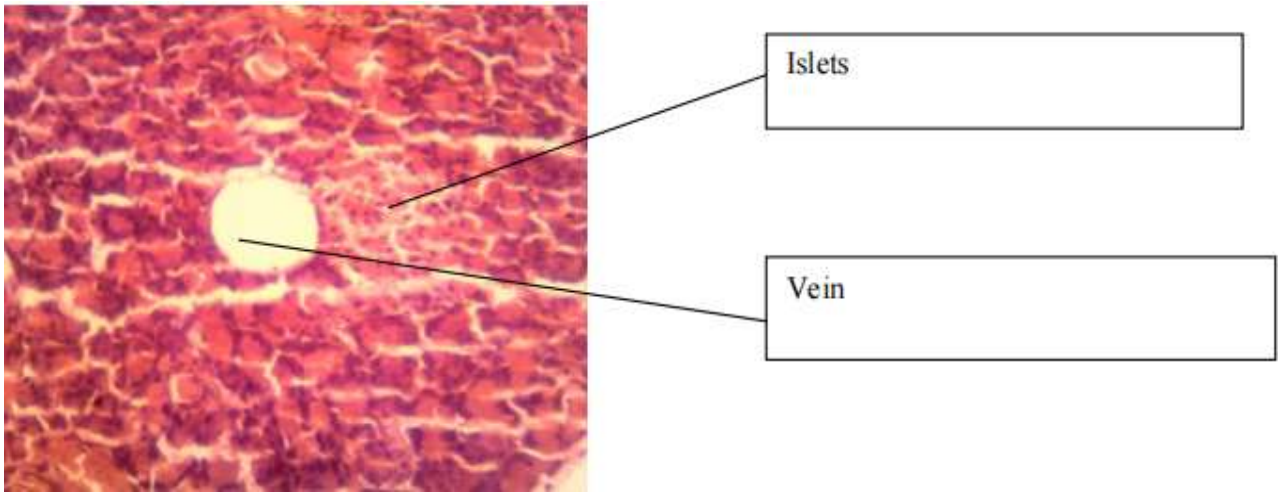

**GROUP 2**



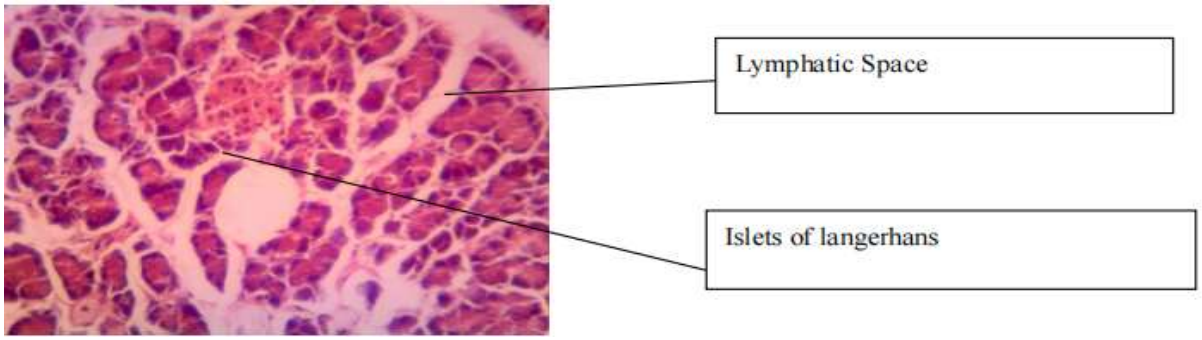

**GROUP 3**



**GROUP 4**




**GROUP 5**




The *in vivo* anti-diabetic study conducted on experimental rats demonstrated the effects of *Dianthus chinensis* extract on body weight, blood glucose levels, and biochemical parameters.The body weight of diabetic control rats (Group II) gradually decreased over the 14-day period,while the ratstreated with *Dianthus chinensis* (Group IV and V) showed a decrease in bodyweight, especially in the 400 mg/kg dose group, which exhibited a more significant reductioncompared to the 200 mg/kg dose group. In terms of blood glucose levels, rats in the diabeticcontrol group showed consistentlyhigh glucose levels, while those treated with *Dianthuschinensis* exhibited a reduction in blood glucose, particularly in the 400 mg/kg dose group,which had the most substantial decrease by day 14. The biochemical parameters revealed that thediabetic rats had elevated levels of total cholesterol (TC), triglycerides (TG), VLDL, LDL-C, andreduced HDL-C. Treatment with *Dianthus chinensis* resulted in a moderate improvement inthese parameters, with the 400 mg/kg dose showing favourable changes in lipid profilescompared to the diabetic control group. These findings suggest that *Dianthus chinensis* maypossess anti-diabetic properties, particularly at the 400 mg/kg dose, by improving body weight,blood glucose regulation, and lipid metabolism.

### 3.10 Statistical Analysis

Data were first examined for normality using the Kolmogorov–Smirnov test. Continuous variables with normal distributions were summarized as means ± standard deviations, while non-normal variables were described using medians and interquartile ranges. Categorical variables were presented as frequencies and percentages. For comparisons among treatment groups, independent two-sample *t*-tests were performed for pairwise comparisons. To control for inflated Type I error due to multiple testing, a Bonferroni correction was applied—specifically, each pairwise comparison's significance threshold was set to α/m, where m equals the number of comparisons (e.g., α=0.05/number of tests). Following this adjustment, the differences observed between treatment groups were highly significant, with Bonferroni-adjusted $P < 0.001$, indicating a very low probability that these findings were due to chance, while maintaining rigorous control over Type I error. This conservative approach ensures the validity of the statistical inference, despite its reduced power when many comparisons are made. Statistical tests were two-tailed, and analyses were conducted using [software name, version, provider, location].

## 4. DISCUSSION

The study of *Dianthus chinensis* extracts reveals significant pharmacological and phytochemical potential, particularly in the context of diabetes management. The percentage yield data shows that methanol was a more efficient solvent (2.85%) compared to petroleum ether (0.40%),indicating higher extraction efficiency and suggesting that methanol can extract a broader range of bioactive compounds. Preliminary phytochemical screening demonstrated that methanolic extracts possessed a wide array of phytochemicals, including alkaloids, glycosides,carbohydrates, flavonoids, tannins, saponins, and steroids—compounds known for various therapeutic effects. In contrast, the petroleum ether extract was limited primarily to carbohydrates, proteins, and flavonoids.Quantitative analyses further confirmed the richness of methanolic extracts, with notable totalphenolic content (67.5 mg/g gallic acid equivalent) and flavonoid content (56.35 mg/g rutinequivalent), both of which are associated with strong antioxidant activity. The DPPH assaysupported this, showing moderate antioxidant potential for the methanolic extract ($IC_{50}$ = 51.32μg/ml), although it was less potent than standard ascorbic acid ($IC_{50}$ = 19.54 μg/ml).In vivo studies using streptozotocin-induced diabetic rats showed that the methanolic extract,particularly at a higher dose of 400 mg/kg, significantly helped in managing blood glucoselevels. This dose led to a marked reduction in glucose levels close to the standard drug,Glibenclamide, and showed better weight maintenance in diabetic rats, suggesting both antihyperglycaemicand possible anti-cachectic effects. Biochemical parameters further validatedthese findings: the 400 mg/kg dose of *D. chinensis* extract led to a noticeable reduction in totalcholesterol, triglycerides, LDL, and VLDL, while improving HDL levels compared to diabeticcontrols, showing strong hypolipidemic effects.Histopathological analysis supported the biochemical findings, with the 400 mg/kg treated groupshowing improved pancreatic architecture compared to the damaged islets seen in untreateddiabetic rats. Overall, the study provides compelling evidence that *Dianthus chinensis*methanolic extract

possesses potent antioxidant, anti-diabetic, and lipid-lowering properties,making it a promising candidate for further research and potential therapeutic development.

## 5. CONCLUSION

The findings of this study clearly indicate that the Stem extract of Dianthus chinensis possesses significant antioxidant and anti-diabetic properties. The extract is rich in bioactivephytochemicals,particularly phenolic compounds and flavonoids, which are known to exert powerful antioxidant effects. These constituents likely contribute to the extract's ability to scavenge free radicals, reduce oxidative stress, and improve metabolic functions in diabetic conditions. In the STZ-induced diabetic rat model, the administration of *Dianthus chinensis* extract not only reduced blood glucose levels but also improved various biochemical parameters, including lipid profiles and liver/kidney function markers. It also mitigated common diabetes-related complications such as weight loss and tissue damage, particularly in the pancreas. The extract's protective effect on pancreatic β-cells was evident from Histopathological observations, suggesting it may aid in preserving or restoring insulin secretion capacity. The study supports the traditional use of *Dianthus chinensis* in herbal medicine and offers scientific evidence for its potential as a natural anti-diabetic agent. It provides a foundation for the development of plant-based therapeutic alternatives for managing diabetes and its complications. However, while the in vivo results are promising, further studies including isolation of specific active compounds, mechanism of action studies, and clinical trials in humans are necessary to validate these findings and assess safety, efficacy, and dosage requirements for therapeutic applications.

### Author Contribution

H.S: Investigation, Data Curation, Writing Original Draft, Conceptualization; D.K.T: Reviewing, Supervision; R.M: Editing; S.K.J: Supporting, Reviewing.

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### Declaration of competing interest
None to declare.

### Ethical Approval
Yes, ethical approval.
Reg. No. **1824/PO/RcBi/S/15/CPCSEA**
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