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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND ANTI-DIABETIC ACTIVITY OF *DIANTHUS CHINENSIS* EXTRACT ON EXPERIMENTAL ANIMAL

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Keywords:

Dianthus chinensis, Phytochemical screening, Antioxidant activity, Anti-diabetic activity, Streptozotocin, Methanol extract, Flavonoids, Phenolic compounds, Lipid profile, Histopathology

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ABSTRACT: The study aimed to evaluate the phytochemical composition, antioxidant potential, and anti-diabetic activity of *Dianthus chinensis* flower extract. Petroleum ether and methanol were used for extraction, with the methanolic extract showing higher yield and richer phytochemical content. Quantitative analysis revealed notable levels of total phenolic content (78.9 mg/g gallic acid equivalent) and total flavonoid content (71.8 mg/g rutin equivalent), suggesting strong antioxidant capacity. This was supported by the DPPH free radical scavenging assay, where the methanol extract exhibited an IC_{50} value of 48.46 µg/mL, indicating potent antioxidant activity. The anti-diabetic effect was assessed in a streptozotocin-induced diabetic rat model. Oral administration of the methanolic extract at 400 mg/kg significantly reduced fasting blood glucose levels, improved body weight, and normalized lipid parameters compared to diabetic control rats. Histopathological findings showed restoration of pancreatic architecture, confirming its protective effect. In summary, *Dianthus chinensis* extract demonstrated notable phenolic and flavonoid content, strong antioxidant capacity, and significant glucose-lowering activity in diabetic rats. These results indicate its potential as a natural source of bioactive compounds for managing oxidative stress and diabetes mellitus.

INTRODUCTION: Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is one of the most prevalent non-communicable diseases worldwide and poses serious health challenges due to its association with long-term complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy [1]. According to the World Health Organization (WHO), the global prevalence of diabetes has been increasing steadily, emphasizing the urgent need for effective and affordable therapeutic interventions [2].

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Conventional antidiabetic drugs, including insulin and oral hypoglycemic agents such as sulfonylureas, biguanides, and thiazolidinediones, can effectively control blood glucose levels but are often associated with adverse effects such as hypoglycemia, gastrointestinal discomfort, and hepatic or renal toxicity [3, 4]. Moreover, their long-term use may lead to reduced efficacy and increased dependency, prompting the search for safer and more sustainable alternatives.

Medicinal plants have long been used in traditional medicine for managing diabetes and are rich sources of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, glycosides, and phenolics many of which exhibit antioxidant and hypoglycemic activities [5, 6]. Since oxidative stress plays a key role in the pathogenesis of diabetes and its complications, investigating the antioxidant capacity and phytochemical composition of medicinal plants offers valuable insight into their potential mechanisms of action [7]. Despite extensive research on various antidiabetic plants, limited scientific evidence exists regarding the pharmacological potential of *Dianthus chinensis*, a species traditionally used for its medicinal properties. Therefore, the present study was undertaken to evaluate the phytochemical composition, antioxidant potential, and antidiabetic activity of *Dianthus chinensis* extract in streptozotocin-induced diabetic rats. The study aims to establish a correlation between its phytochemical profile and therapeutic efficacy in modulating glucose levels, lipid metabolism, and oxidative stress markers, thereby validating its ethnopharmacological relevance and exploring its potential as a natural antidiabetic agent [8, 10].

MATERIAL AND MTHODS:

Chemicals: All chemicals and reagents used were of analytical grade. Ninety-five percent alcohol, chloroform, and concentrated hydrochloric acid were obtained from Clorofilt Ind. Ammonia, glacial acetic acid, sodium hydroxide, and nitroprusside were purchased from Merck, while concentrated sulfuric acid was procured from Fizmerck. Petroleum ether was supplied by Research Lab, and additional alcohol by Molychem.

Selection of Plant Material: A total of 335 g of *Dianthus chinensis* plant material was collected, washed thoroughly to remove debris, and shade-dried at room temperature for three days. It was further oven-dried at 45°C to eliminate residual moisture and stored in sterile, airtight containers in a cool, dry place. The plant material (flower) of *Dianthus chinensis* (Family: Caryophyllaceae) was authenticated by Dr. Jagrati Tripathi, Department of Botany, Government College Khimlasa, Sagar (M.P.), and a voucher specimen was deposited for future reference (Authentication No. AC/090/25, dated 05.04.2025).

Extraction by Soxhlation Process: In this study, a continuous hot percolation method utilizing a Soxhlet apparatus was used to extract bioactive compounds from finely powdered *Dianthus chinensis*, following standard extraction protocols. Initially, petroleum ether, a non-polar solvent, was employed at approximately 60°C to def at the plant material. The extraction process continued until the siphon tube became clear, signifying the complete removal of non-polar constituents. The defatted residue was subsequently air-dried and re-extracted with methanol under the same Soxhlet conditions to isolate polar phytochemicals. The Soxhlet extraction system provides multiple advantages, including continuous solvent recycling that enhances extraction efficiency, reduces solvent usage, and enables uninterrupted operation without manual handling. Moreover, it allows high-temperature reflux, improving solubility and mass transfer of compounds while removing the need for post-extraction filtration. After extraction, the solvents were evaporated under reduced pressure using a rotary evaporator at 40°C. The concentrated extracts were then weighed, and the percentage yield was determined using the specified formula.

$$\%\text{Yield} = \text{Weight of extract} / \text{Weight of plant material used} \times 100$$

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 [11].

Objective of Phytochemical Screening: The primary goal of this screening was to identify the bioactive constituents present in *Dianthus chinensis* extracts obtained through successive Soxhlet extraction using petroleum ether (non-polar) and methanol (polar) solvents. These phytochemicals are crucial due to their established roles in various therapeutic activities such as antimicrobial, anti-inflammatory, and antioxidant effects.

Qualitative Analysis: Dried extracts from both solvents were subjected to standard qualitative phytochemical tests to detect major classes of primary and secondary metabolites. The tests followed well-established protocols involving specific reagents that indicate the presence of phytochemicals through distinct color changes or precipitate formation [12].

Quantitative Estimation of Phytochemical Constituents: To complement the preliminary screening, quantitative analysis was performed to measure the concentration of key bioactive compounds present in the methanolic and petroleum ether extracts of *Dianthus chinensis*. This step is essential for correlating phytochemical content with potential pharmacological activities.

Target Phytochemicals:

Determination of Total Phenolic Content (TPC): The Folin–Ciocalteu assay is based on the reaction between the reagent and phenolic hydroxyl groups, forming a blue-colored complex whose intensity correlates with the phenolic concentration. The total phenolic content of *Dianthus chinensis* extracts was determined using the Folin–Ciocalteu colorimetric method due to its sensitivity and reliability. In this procedure, 0.2 ml of plant extract was mixed with 2.5 ml of Folin–Ciocalteu reagent, followed by 2 ml of 7.5% sodium carbonate after 5 minutes. The mixture was diluted to 7 ml with distilled water and incubated for 2 hours at room temperature. Absorbance was then measured at 760 nm using a UV–Visible spectrophotometer, and phenolic content was quantified using a gallic acid standard curve (20–100 µg/ml), expressed as mg gallic acid equivalents per gram of dry extract (mg GAE/g extract) [13].

Determination of Total Flavonoid Content (TFC): The aluminium chloride colorimetric assay is based on the formation of a stable flavonoid–aluminium complex that exhibits maximum absorbance at 510 nm, with the color intensity directly proportional to the flavonoid concentration. The total flavonoid content of *Dianthus chinensis* extracts was determined using this method due to its specificity and simplicity. For the assay, 0.5 ml of plant extract was mixed with 2 ml of distilled water, followed by the addition of 0.15 ml of 5% sodium nitrite, left for 6 minutes, then 0.15 ml of 10% aluminium chloride, and again incubated for 6 minutes. Finally, 2 ml of 4% sodium hydroxide was added, and the mixture was thoroughly shaken. The absorbance of the pink-colored complex was measured at 510 nm using a UV–Visible spectrophotometer. Quantification was done using a rutin standard curve (20–100 µg/ml), and results were expressed as mg rutin equivalents per gram of dry extract (mg RE/g extract) [14].

Antioxidant Assay (DPPH Radical Scavenging Activity): The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a common method used to assess the free radical scavenging activity of plant extracts. DPPH•, a stable violet-colored radical with maximum absorbance at 517 nm, is reduced to yellow diphenyl picrylhydrazine upon reaction with antioxidants that donate hydrogen or electrons, resulting in a measurable decrease in absorbance. In this study, a 0.004% DPPH methanolic solution (5 ml) was mixed with 50 µl of *Sonchus oleraceus* extract at concentrations ranging from 20–100 µg/ml and incubated in the dark for 30 minutes at room temperature. Absorbance was then measured at 517 nm using a UV–Visible spectrophotometer, with a blank serving as the control. The percentage of DPPH radical scavenging activity was calculated using the absorbance values of the control (A_0) and test sample (A_1). This simple and rapid assay effectively indicates antioxidant strength, where higher inhibition percentages reflect stronger free radical scavenging and potential therapeutic effects [15].

$$\text{Inhibition (\%)} = (Ao - A1 / Ao) \times 100$$

Acute Oral Toxicity: The acute oral toxicity of the test extract was assessed using the OECD 423 Acute Toxic Class Method, a stepwise approach designed to estimate toxicity with minimal animal use. In this method, groups of three animals per sex receive a single oral dose of 5, 50, 300, or 2000 mg/kg and are observed for 14 days for signs of toxicity, including behavioral changes, weight loss, and mortality.

Dose selection depends on observed outcomes absence of deaths leads to a higher dose, one death repeats the same dose, and two or more deaths indicate toxicity, prompting dose reduction or discontinuation. This procedure enables estimation of the LD_{50} range and classification of toxicity while adhering to ethical standards, providing a reliable safety profile for further pharmacological and toxicological studies [16].

Anti-Diabetic Evaluation:

Experimental Animals and Ethical Approval: Healthy adult Wistar rats of either sex (250 ± 70 g) were used. The study was conducted in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC approval no.: PBRI/IAEC/09-05-25/024). Animals were maintained under controlled conditions (22 ± 2°C, 55 ± 10% relative humidity, 12 h light/dark cycle) with free access to food and water.

Animal used:

Weight*:* 250±70 gm

Strain*:* Wistar rat

Sex*:* Either sex

Streptozotocin Induced Diabetes: Streptozotocin (STZ) was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. For diabetes induction, STZ was freshly dissolved in 0.1 M citrate buffer (pH 4.5) and administered orally at a dose of 50 mg/kg body weight. The solution was used within 15 minutes to preserve its stability and injected using a 1 mL tuberculin syringe with a 24-gauge needle. Each animal received a 0.4 mL injection. Control animals were given only the citrate buffer (0.4 mL) under the same conditions. On the third day after administration, fasting blood glucose levels were measured to confirm diabetes induction. Animals with glucose levels above 250 mg/dL were classified as diabetic and selected for further pharmacological testing [18].

Experimental Setup for the Study: Diabetic rats were randomly divided into five groups (n = 6 per group) and treated orally once daily for 14 days as shown in **Table 1** follows:

TABLE 1: EXPERIMENTAL DESIGN

Group	Treatment	Dose	Duration
I	Normal Control	Distilled water (1 ml/kg)	14 days
II	Diabetic Control	STZ (50 mg/kg)	—
III	Standard	Glibenclamide (10 mg/kg/day, p.o.)	14 days
IV	Test I	*Dianthus chinensis* extract (250 mg/kg/day, p.o.)	14 days
V	Test II	*Dianthus chinensis* extract (500 mg/kg/day, p.o.)	14 days

Evaluation Parameters of Antidiabetic Activity:

Collection and Monitoring of Blood Glucose Levels: Blood glucose was monitored by collecting samples from the tail vein of rats that had been fasted overnight (12 hours). The first reading was taken three days after streptozotocin administration (considered as day 0) to verify the induction of diabetes.

Further glucose measurements were taken on days 1, 3, 5, 7, and 14 throughout the treatment period. For each measurement, a small cut was made at the tip of the tail, and the initial drop of blood was discarded to avoid contamination.

The next drop was placed on a glucose test strip and read using an Accu-Chek glucometer (Roche Diagnostics, Germany). Following each sampling, the site was cleaned with ethanol to reduce the risk of infection and ensure hygienic handling.

Evaluation of Lipid Profile Parameters: To evaluate the effects of treatment on lipid metabolism, blood samples were collected from overnight-fasted rats on days 0 and 14 *via* the retro-orbital plexus under light diethyl ether anesthesia. After clotting and centrifugation at 2500 rpm for 10 minutes at 25°C, serum was separated and analyzed for total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C).

Very low-density lipoprotein cholesterol (VLDL-C) was estimated as:

$$\text{VLDL-C} = \text{TG} / 5$$

Low-density lipoprotein cholesterol (LDL-C) was calculated as

$$\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{VLDL-C})$$

These parameters were used to assess diabetes-induced lipid alterations and the lipid-lowering potential of *Dianthus chinensis* extract [18].

RESULTS:

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 2: PERCENTAGE YIELD OF CRUDE EXTRACTS OF *DIANTHUS CHINENSIS* EXTRACT

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
Dianthus chinensis	Pet ether	335	1.10	0.32%
	Methanol	280.89	4.60	1.63%

TABLE 3: PHYTOCHEMICAL TESTING OF EXTRACT PETROLEUM ETHER

Experiment	Presence or absence of phytochemical test
	Petroleum ether extract
Alkaloids	
Dragendroff's test	Positive
Mayer's reagent test	Positive
Wagner's reagent test	Positive
Hager's reagent test	Positive
Glycoside	
Borntrager test	Negative
Legal's test	Negative
Killer-Killiani test	Negative
Carbohydrates	
Molish's test	Negative
Fehling's test	Negative
Benedict's test	Negative
Barfoed's test	Negative
Proteins and Amino Acids	
Biuret test	Negative
Ninhydrin test	Negative
Flavonoids	
Alkaline reagent test	Present (+ ve)
Lead Acetate test	Present (+ ve)
Tannin and Phenolic Compounds	
Ferric Chloride test	Present (+ ve)
Saponin	
Foam test	Negative
Test for Triterpenoids and Steroids	
Salkowski's test	Negative
Libbermann-Burchard's test	Negative

TABLE 4: PHYTOCHEMICAL TESTING EXTRACT OF METHANOL

Experiment	Presence or absence of phytochemical test
	Methanol extract
Alkaloids	
Dragendroff's test	Negative
Mayer's reagent test	Negative
Wagner's reagent test	Negative
Hager's reagent test	Negative
Glycoside	
Borntrager test	Positive
Legal's test	Positive
Killer-Killiani test	Positive
Carbohydrates	
Molish's test	Positive
Fehling's test	Positive
Benedict's test	Positive
Barfoed's test	Positive
Proteins and Amino Acids	
Biuret test	Negative
Ninhydrin test	Negative
Flavonoids	
Alkaline reagent test	Positive
Lead Acetate test	Positive
Tannin and Phenolic Compounds	
Ferric Chloride test	Positive
Saponin	
Foam test	Positive
Test for Triterpenoids and Steroids	

Salkowski's test	Positive
Libbermann-Burchard's test	Positive

Quantitative Analysis: The crude extract of the plant was found to contain appreciable amounts of key secondary metabolites, particularly flavonoids and phenolic compounds.

To quantify these bioactive constituents, specific spectrophotometric methods were utilized.

The analyses focused on determining the total phenolic content (TPC) and total flavonoid content (TFC), which are essential indicators of the extract's potential antioxidant and therapeutic efficacy.

Total Phenolic Content (TPC) Estimation:

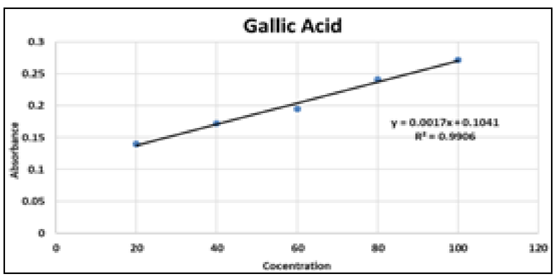


FIG. 1: REPRESENT STANDARD CURVE OF GALLIC ACID

Total Phenolic Content in Extract:

TABLE 5: TOTAL PHENOLIC CONTENT

Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.142	
0.189	78.9 mg/gm
0.225	

TABLE 6: TOTAL PHENOLIC CONTENT OF EXTRACT *DIANTHUS CHINENSIS*

Extracts	Total Phenolic content (mg/gm equivalent of Gallic acid)
Methanol	78.9

Total Flavonoids Content (TFC) Estimation:

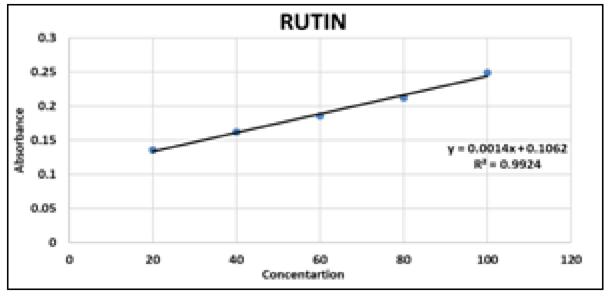


FIG. 2: REPRESENT STANDARD CURVE OF RUTIN

Total Flavonoid Content in Extract:

TABLE 7: TOTAL FLAVONOID CONTENT

Absorbance	TFC in mg/gm equivalent of Rutin
0.135	
0.190	71.8 mg/gm
0.210	

Anti-Oxidant Activity:

DPPH 2, 2- diphenyl-1-picryl hydrazyl Assay:

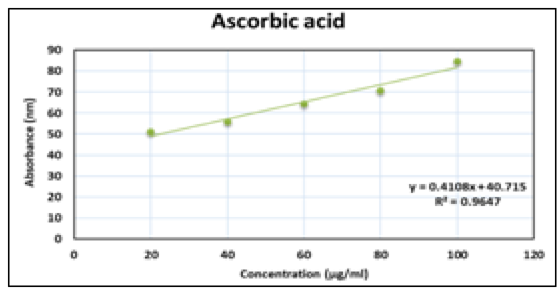


FIG. 3: DPPH RADICAL SCAVENGING ACTIVITY STD. ASCORBIC ACID

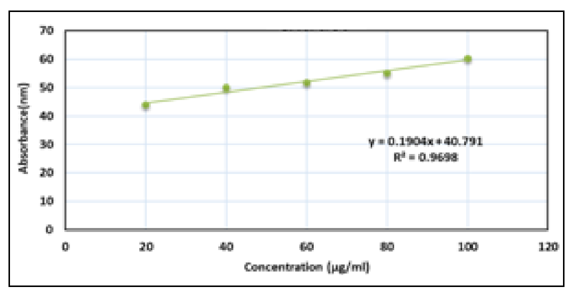


FIG. 4: REPRESENTS THE PERCENTAGE INHIBITION *VS* CONCENTRATION OF EXTRACT

In vivo Acute Oral Toxicity (OECD 423):

TABLE 8: GENERAL APPEARANCE AND BEHAVIORAL OBSERVATIONS OF ACUTE ORAL TOXICITY STUDY FOR CONTROL AND TREATED GROUPS

Observations	Control	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
Food intake	Normal	Normal	Normal	Normal	Normal
Body weight	Normal	Normal	No change	No change	No change
Temperature	Normal	Normal	Normal	Normal	Normal
Changes in skin and fur	Nill	Nill	Nill	Nill	Nill
Urination	Normal	No effect	No effect	No effect	No effect
Diarrhoea	Absent	Absent	Absent	Absent	Absent
Death	Alive	Alive	Alive	Alive	Alive

Streptozotocin Induced Diabetes Model:

FIG. 5: ORAL DOSING OF STREPTOZOTOCIN INDUCED DIABETES MODEL IN RATS

In-vivo Anti -diabetic Study:

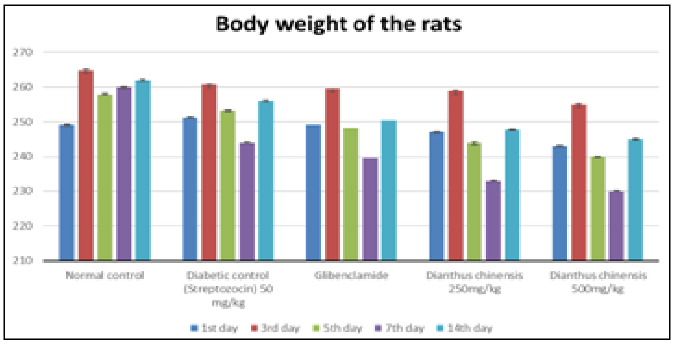


FIG. 6: GRAPHICAL REPRESENTATION OF EFFECT OF _DIANTHUS CHINENSIS_ EXTRACT ON BODY WEIGHT OF THE RATS

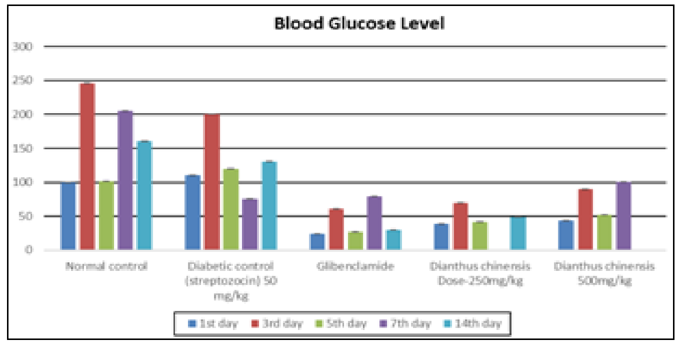


FIG. 7: GRAPHICAL REPRESENTATION OF EFFECT OF _DIANTHUS CHINENSIS_ EXTRACT ON BLOOD GLUCOSE LEVEL OF THE RATS

Biochemical Parameters:

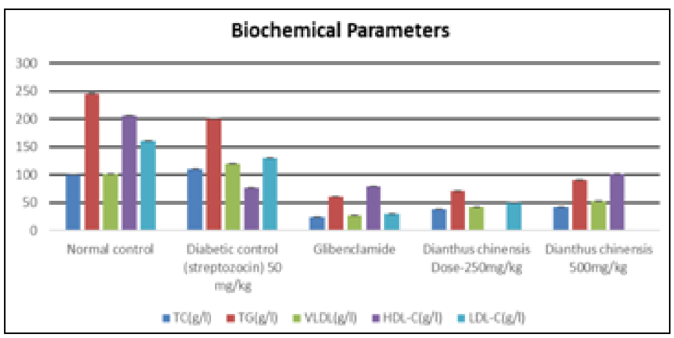
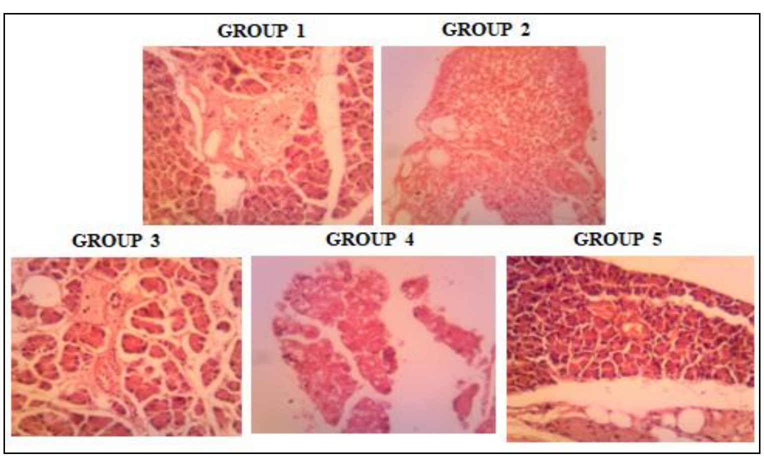


FIG. 8: GRAPHICAL REPRESENTATION OF EFFECT OF _DIANTHUS CHINENSIS_ EXTRACT ON BIOCHEMICAL PARAMETERS OF THE RATS

Histopathological Examination:



The *in-vivo* anti-diabetic study on experimental rats evaluated the impact of *Dianthus chinensis* flower extract on body weight, blood glucose levels, and key biochemical markers. Over the 14-day period, diabetic control rats (Group II) experienced a steady decline in body weight. In contrast, rats treated with *Dianthus chinensis* (Groups IV and V) also showed weight reduction, with the 500 mg/kg dose group exhibiting a more pronounced decrease than the 250 mg/kg group. Regarding blood glucose, untreated diabetic rats maintained elevated glucose levels throughout the study, whereas those administered *Dianthus chinensis* extract showed a notable decline, particularly in the 500 mg/kg group, which achieved the most significant reduction by day 14. Biochemical analysis indicated that diabetic rats had increased levels of total cholesterol (TC), triglycerides (TG), very-low-density lipoprotein (VLDL), and low-density lipoprotein cholesterol (LDL-C), along with decreased high-density lipoprotein cholesterol (HDL-C). Treatment with *Dianthus chinensis* led to moderate improvements in these lipid parameters, with the higher dose producing more favourable results. These outcomes highlight the potential of *Dianthus chinensis* seed extract, especially at 500 mg/kg, to exert anti-diabetic effects by improving blood glucose control, lipid metabolism, and mitigating weight loss associated with diabetes.

CONCLUSION: The methanolic extract of *Dianthus chinensis* shows strong potential as a natural therapeutic option for managing diabetes mellitus. It significantly lowers blood glucose levels in streptozotocin-induced diabetic rats, improves lipid profiles, and preserves pancreatic tissue integrity. These effects are linked to its rich phytochemical composition, particularly flavonoids and phenolic compounds, which contribute to its pronounced antioxidant activity. The extract remains safe up to 2000 mg/kg, indicating a wide margin of safety for experimental use. Its combined antioxidant and antihyperglycemic effects suggest that *Dianthus chinensis* may help reduce oxidative stress while improving metabolic balance, offering an integrated approach to diabetes management. Given its promising pharmacological profile, the extract represents a valuable lead for developing plant-based therapies for diabetes and its complications. However, further studies on chronic toxicity, mechanisms of action, pharmacokinetics, and clinical validation are essential to support its potential therapeutic application.

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CONFLICTS OF INTEREST: Nil

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