Project Report

Effect of DB-1 on streptozotocin- Nicotinamide induced diabetes mellitus in rats

Sponsor

Herbal Luxe Pvt. Ltd.

Performing Laboratory

Wellia Labs Pvt. Ltd
Office No. 3, First floor, Mauli krupa,
Opposite hotel Atulya, Nanded Phata,
Pune-411041

&

Department of Pharmacology,
Bharati Vidyapeeth (Deemed to be University)
Poona College of Pharmacy,
Erandwane, Pune 411038
Maharashtra

Study Director

Dr. Prajakta Vaishampayan

Report submission date: 02/01/2025

Sample code: WLS130_DB-1

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Report on

Non-Clinical Pharmacology

Performed by	: Wellia labs Pvt. Ltd & Poona college of Pharmacy
Reviewed By	: Mr. Shriram Kulkarni
Project Initiation date	: 11/07/2024
Project End date	: 22/10/2024
Report date	: 02/01/2025
Customer code	: WLPCA039
Test facility	: Department of Pharmacology, Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, Pune
Contact	**:** +91 8788262637/ 9370984374
Email id	: info@wellialabs.com
Animal ethics commitee protocol number and approval date	**:** PCP/IAEC/2024/2-8

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Signature page

Report No. : WLRP_24_130

Conducted by

Wellia labs Pvt. Ltd.,
Office No. 3, First floor, Mauli krupa,
Opposite hotel Atulya, Nanded Phata,
Pune-411041, Maharashtra, India

The following report is true and accurate with the results obtained. We hereby confirm the authenticity of the same.

Designation	Name	Signature	Date
Project Analyst	Ms. Apeksha Gangurde		
Project Director	Dr. Prajakta Vaishampayan		
Quality personnel	Mr. Shriram Kulkarni		

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Contents

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Statement of Compliance

Study Code: WELLIA 1-2024-2025_WLS130

Test Substance: Wellia 1_DB-1

We hereby attest to the authenticity of the study and guarantee that the data is correct and accurate to the best of our knowledge and that the study was performed by the procedure described in the Standard Operating Procedures of Department of Pharmacology, Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy, Pune.

The study complies with the protocol mutually agreed and under the regulations of the Committee for Control and Supervision of Experiments on Animals (CCSEA Registration No. 1703/PO/Re/S/13/ CCSEA)

Study director	Signature	Date
Dr. Prajakta Vaishampayan		

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Ethical committee permission and confidentiality

We hereby confirm that research protocol was approved by Institutional Animal Ethics Committee (IAEC), Protocol number PCP/IAEC/2024/2-8 (Copy of approval Certificate is enclosed).

All the animals were euthanized after the completion of the study. The study was conducted using recommended/approved anesthetics and under the supervision of a veterinarian. The report is confidential in nature and access is restricted to authorized people only.

Study director	Signature	Date
Dr. Prajakta Vaishampayan		

Certificate on completion of project

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Project Title	Effect of DB 1 on Streptozotocin-Nicotinamide induced diabetes mellitus in rats.
Sponsored by	Herbal Luxe Pvt. Ltd
Research conducted at	Department of Pharmacology, Bharati Vidyapeeth (Deemed to be University) Poona College of Pharmacy, Pune.
Project compiled by	Wellia Labs Pvt. Ltd.

We hereby confirm that research project is successfully completed and report has been submitted to Herbal luxe Pvt. Ltd.

Study director	Signature	Date
Dr. Prajakta Vaishampayan		

List of abbreviations

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Term	Full Form
CCSEA	Committee for Control and Supervision of Experiments on Animals
IAEC	Institutional Animal Ethics Committee
Mg	Milligram
Kg	Kilogram
dL	Deciliter
i.p.	Intra peritoneal route
STZ	Streptozotocin
NIC	Nicotinamide
VLDL	Very Low density lipoprotein
LDL	Low density lipoprotein
HDL	High density lipoprotein
AST/SGOT	Aspartate transaminase
ALT/SGPT	Alanine transaminase
ALP	Alkaline Phosphatase
BUN	Blood Urea Nitrogen

List of materials

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Name of the chemical/material	Make
Streptozotocin	Sigma
Nicotinamide	Sigma
Metformin Hydrochloride 500 mg	Cipla

1. Aim: The aim of the present study is to evaluate DB-1 in Streptozotocin -Nicotinamide induced diabetes mellitus in rats.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

2. Objective:

I. To induce diabetes mellitus by intraperitoneal administration of Streptozotocin-Nicotinamide.

II. To determine the effect of treatment of DB-1 on blood glucose, lipid profile, hepatic profile, kidney profile, blood profile, tissues and bone strength.

III. To compare the effect of DB-1 with standard metformin.

3. Materials and Methods:

3.1. Materials:

Streptozotocin was procured from Sigma Aldrich, nicotinamide was procured. Metformin was procured from marketed formulation.

The study protocol (Protocol approval number: PCP/IAEC/2024/2-8 was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, which is registered with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), under registration number 1703/PO/Re/S/13/CCSEA, dated 03/01/22. Male Wistar rats, weighing between 150-180 g, were sourced from the Global Bio Research Ltd., Pune. The animals were housed under standard environmental conditions, with free access to a standard pellet diet and water. All procedures followed the guidelines set forth by CCSEA for animal care and experimentation.

3.2. Methods:

The animals were divided into 5 groups of 10 animals each as follows:

Group I – Vehicle Control

Group II – Diabetic Control (streptozotocin 65 mg/kg ip. (STZ)+ nicotinamide 110 mg/kg (NIC)) induced treated with water orally once daily.

Group III – STZ-NIC Diabetic animals treated with standard (metformin 500 mg/kg, orally once daily)

Group IV – STZ-NIC Diabetic animals treated with DB 1 (998 mg/kg) orally once daily

Group V – Prefeed with STZ-NIC Diabetic animals treated with DB 1 (998 mg/kg) orally once daily

3.3. Induction of Experimental Diabetes Mellitus:

Streptozotocin was dissolved in a freshly prepared 0.1 M citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline. The overnight fasted (16 h fasting with free access

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

to water) animals were induced with diabetes by intraperitoneal injection of 65 mg/kg of Streptozotocin (STZ), 15 min after the intraperitoneal injection of 110 mg/kg of nicotinamide. The vehicle control group (Group I) was injected with buffer alone. After 72 h, blood was withdrawn by retroorbital puncture under anesthesia and the blood glucose level was estimated to confirm the induction of diabetes mellitus. After 1 week of induction, blood glucose level was estimated again and a fasting blood glucose level more than 200 mg/dL was considered as diabetic.

The animals were given with the respective treatment for 2 weeks. The blood glucose and body weight were determined weekly. At the end of two weeks of treatment, the lipid profile and hepatic profile were assessed. Histopathological examinations of the liver and kidneys were conducted on two animals from each group, which were sacrificed for this purpose. This marked the conclusion of Phase 1.

Phase 2 began with six animals from each group continuing the treatment for an additional two months. At the end of the 10th week of treatment, the animals were placed in metabolic cages for urine collection to assess urine volume. Serum creatinine and blood urea nitrogen (BUN) levels were determined. The animals were then anesthetized for blood pressure measurement. Subsequently, the animals were sacrificed, and the femur bone was evaluated for bone strength. Histopathological examinations of the kidneys and blood vessels were also performed to assess structural changes.

4. Results:

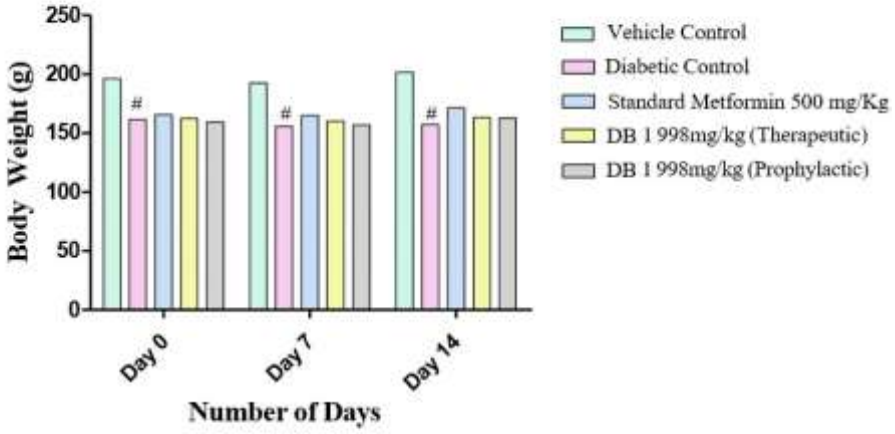
4.1. Phase 1:

4.1.1. Effect of DB 1 on body weight (g) in STZ-NIC induced diabetes in rats

On day 0, the body weight of all the groups of animals were between 150 to 200 g and there was no significant difference observed between the groups on day 0. After induction of diabetes in two weeks, the body weight of the diabetic control had significantly ($p<0.001$) decreased. Treatment with DB 1 shows no significant change in weight (Table 1: Figure 1) when compared to diabetic control on day 7 and 14 of treatment. The observed effect was as comparable to that of standard treated group. The decrease in body weight with diabetes mellitus has been attributed to the gluconeogenesis i.e.) catabolism of proteins and fats, which is associated with the muscle wasting in diabetic patients.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Figure 1: Effect of DB 1 on Body weight (g) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 10.
Two-way ANOVA followed by Bonferroni t-test.
#p<0.001 when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally.

Table 1: Effect of DB 1 on Body Weight and Fasting Blood Glucose in Streptozotocin-Nicotinamide induced diabetes mellitus in rats:

Parameter	Vehicle Control	Diabetic control	Standard Metformin 300 mg/Kg	DB 1 (998mg/kg) (Therapeutic)	DB 1 (998mg/kg) (Prophylactic)
Body Weight (g)					
DAY 0	196.3±5.08	161.5±7.54#	165.7±6.93	162.7±4.87	159.2±7.32
DAY 7	192.7±5.51	155.8±7.54#	165.2±6.46	160.3±4.94	157±7.76
DAY 14	201.8±5.12	157.3±6.96#	171.5±6.07	163.2±4.73	162.9±6.77
FBGS (mg/dL)					
DAY 1	132.4±6.44	364.9±10.74 #	332.9±20.70	336.5±21.65	319.5±10.44
DAY 7	114.9±2.98	367.3±10.11 #	328.8±17.40	326.9±21.34*	314.9±10.30

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

DAY 14	128.1±2.36	370.6±9.42#	297.2±12.07**	319.6±21.16*	308.4±10.11*

Values are expressed as mean ± SEM, n= 10.

Two-way ANOVA followed by Bonferroni t-test.

#p<0.001 when compared to Vehicle control;

*p<0.05, **p<0.01, when compared to Diabetic control

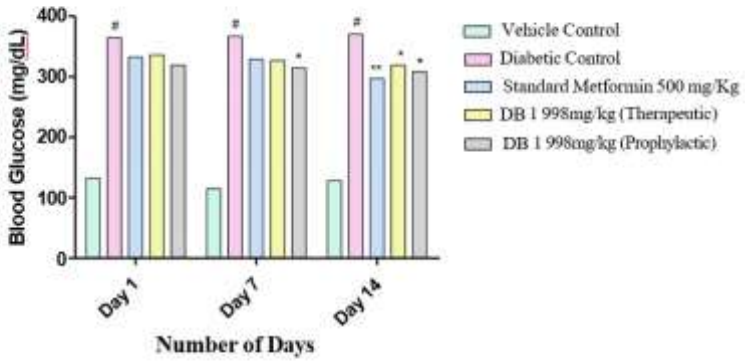
DB 1 and Metformin were administered orally.

FBS – Fasting Blood Glucose

4.1.2. Effect of DB 1 on blood glucose (mg/dL) in STZ-NIC induced diabetes in rats

Upon induction with diabetes mellitus on day 0, the blood glucose level of all the test groups of animals were between 200 and 400 mg/dl post 72 h of induction. There was no significant difference in glucose levels observed between the groups on this day. Treatment with wellie 1 significantly (p<0.05) decreased the blood glucose level from day 7 onwards, which was further decreased on day 14 when compared to diabetic control group of animals. The observed effect was as comparable to that of standard treated group which had significantly (p<0.01) decreased the blood glucose level by day 14 and on day 21 when compared to diabetic control (Table 1: Figure 2). This confirms the antihyperglycemic potential of wellie 1 which is comparable to that of the standard.

Figure 2: Effect of DB 1 on Blood Glucose (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



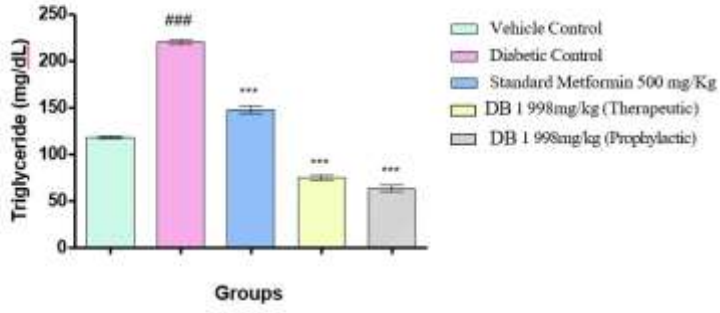
Values are expressed as mean ± SEM, n= 10.
Two-way ANOVA followed by Bonferroni t-test.
#p<0.001 when compared to Vehicle control;
*p<0.05, **p<0.01 when compared to Diabetic control
DB 1 and Metformin were administered orally.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

4.1.3. Effect of DB 1 on Lipid Profile (mg/dL) in STZ-NIC induced diabetes in rats *Serum triglycerides (mg/dL)*

The normal level of serum triglycerides is less than 150 mg/dL. There was an increase in the serum triglycerides level of the diabetic control group, which was found to be 220.50 mg/dL in 2 weeks of induction of diabetes in diabetic control. Treatment with DB 1 for 2 weeks significantly ($p<0.001$) decreased the serum triglycerides levels when compared to diabetic control group of animals. The observed effect was similar of standard treated group which had decreased the serum triglycerides levels when compared to diabetic control (Table 2: Figure 10).

Figure 10: Effect of DB 1 on Triglyceride (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 2.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

Table 2: Effect of DB 1 on Liver Profile and Lipid Profile in Streptozotocin-Nicotinamide induced diabetes mellitus in rats:

Parameter	Vehicle Control	Diabetic control	Standard Metformin 300 mg/Kg	DB 1 998 mg/Kg (Therapeutic)	DB 1 998 mg/Kg (Prophylactic)
AST (U/L)	5445±127.99	6237±78.84#	7616±17.68**	7440±85.56**	1817±4.24***
ALT (U/L)	3001±7.78	6614.5±61.16###	4201±57.98**	8224±214.25**	8524±104.30**
ALP (U/L)	13.5±0.35	23.5±0.35##	49.5±1.06***	20±0.71	41±0.71***
TC (mg/dL)	62±0.71	80±0.71###	73±0.71*	66.5±0.35***	78.5±0.35
TG (mg/dL)	118.5±1.06	220.5±1.77###	148±2.83***	75.5±1.77***	63.5±2.47***
HDL (mg/dL)	37.5±0.35	37±1.41	38.5±0.35	40.5±1.06	44±0.71

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

LDL (mg/dL)	2.5±0.07	33.75±1.03###	5.25±0.04***	12.6±0.14***	23.5±0.35**
VLDL (mg/dL)	23.85±0.32	44.9±0.21###	28.1±0.49***	15.75±0.11***	12±0.18***

Values are expressed as mean ± SEM, n= 2.

One way ANOVA followed by Bonferroni t-test.

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ when compared to Vehicle control;

*p<0.05, **p<0.01, ***p<0.001 when compared to Diabetic control

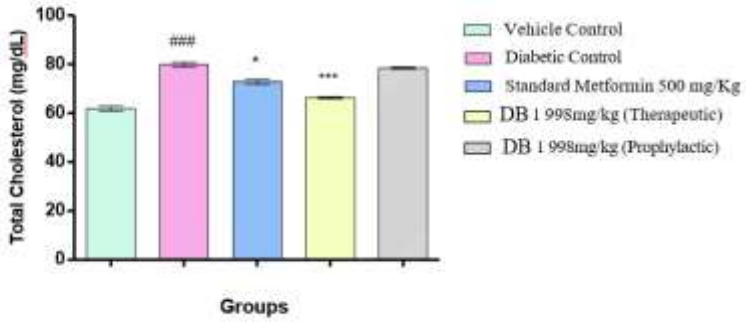
DB 1 and Metformin were administered orally.

AST- Aspartate Aminotransferase; ALT- Alanine Aminotransferase; ALP - Alkaline Phosphatase; TC – Total Cholesterol, LDL – Low density lipoprotein; TG – Triglycerides; HDL – High density lipoprotein; VLDL- Very low-density lipoprotein

Total cholesterol (mg/dL)

The normal level of total cholesterol is less than 10-54 mg/dL. There was an increase in the serum total cholesterol level of the diabetic control group, which was found to be 80.71 mg/dL in 2 weeks of induction of diabetes in diabetic control. Treatment with wellie 1 for 2 weeks significantly (p<0.001) decreased the serum total cholesterol levels when compared to diabetic control group of animals. The observed effect was better that of standard treated group which had significantly (p<0.05) decreased the serum total cholesterol levels when compared to diabetic control (Table 2: Figure 9).

Figure 9: Effect of DB 1 on Total Cholesterol (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



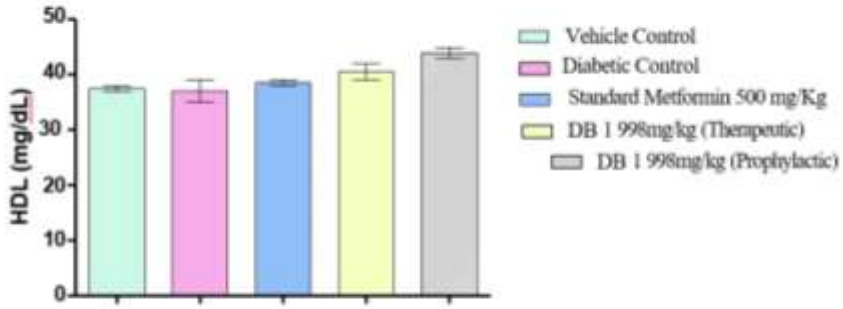
Values are expressed as mean ± SEM, n= 2.
One-way ANOVA followed by Bonferroni's t-test.
***p<0.001 when compared to Vehicle control;
*p<0.05, ***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

HDL cholesterol (mg/dL)

The normal level of serum HDL cholesterol is more than 40 mg/dL. There was a decrease in the serum HDL level of the diabetic control group, which was found to be 37 mg/dL in 2 weeks of induction of diabetes in diabetic control. Treatment with DB-1 or standard for 2 weeks did alter the HDL cholesterol levels when compared to diabetic control (Table 2: Figure 6).

Figure 6: Effect of DB 1 on HDL (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



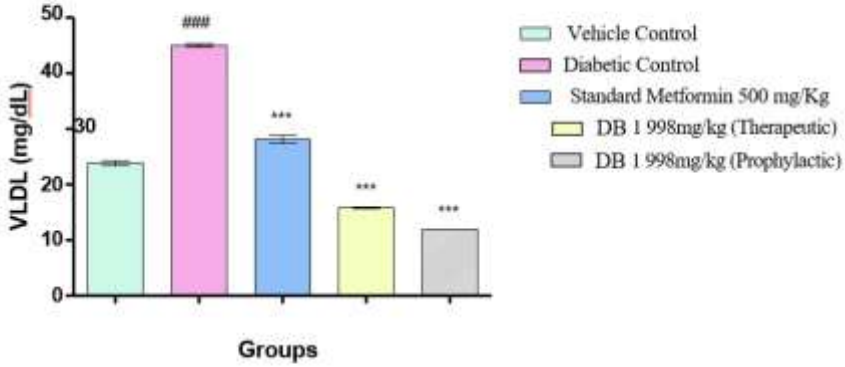
Values are expressed as mean ± SEM, n= 2.
One-way ANOVA followed by Bonferroni's t-test.
No significant change when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally

VLDL cholesterol (mg/dL)

The normal level of serum VLDL cholesterol is 15-30 mg/dL. There was significant change in the serum VLDL cholesterol level of the diabetic control group, which was found to be 44.9 mg/dL in 2 weeks of induction of diabetes in diabetic control. Treatment with DB 1 for 2 weeks significantly ($p<0.001$) decreased the serum VLDL cholesterol when compared to diabetic control group of animals. The observed effect was similar of standard treated group which had decreased the serum VLDL cholesterol levels when compared to diabetic control (Table 2: Figure 8).

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Figure 8: Effect of DB 1 on VLDL (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



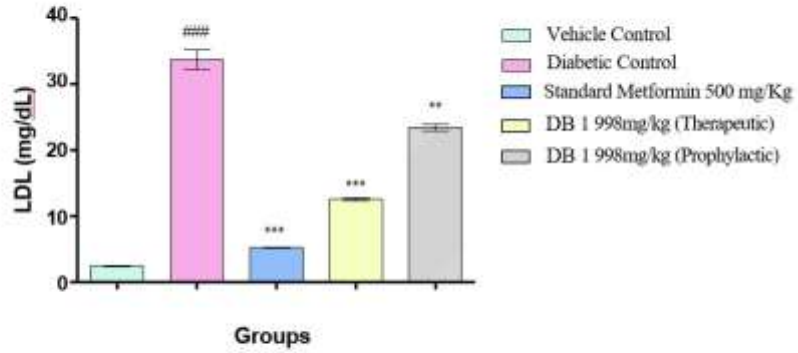
Values are expressed as mean ± SEM, n= 2.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
**p<0.01, ***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

LDL cholesterol (mg/dL)

The normal level of serum LDL cholesterol is 38-85 mg/dL. There was no significant change in the serum LDL cholesterol level of the diabetic control group as per the normal range but in comparison with vehicle control group significant change has been observed in 2 weeks of induction of diabetes in diabetic control. Treatment with DB 1 for 2 weeks significantly ($p<0.001$) decreased the serum LDL cholesterol when compared to diabetic control group of animals. The observed effect was similar of standard treated group when compared to diabetic control (Table 2: Figure 7). Though the LDL levels were decreased upon treatment with DB 1 and standard, the observed serum LDL level in diabetic animals were not elevated considering clinically normal LDL values in the diabetic control group of animals.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Figure 7: Effect of DB 1 on LDL (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 2.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
**p<0.01, ***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. A marked increase in triglyceride levels were observed in the diabetic control animals which confirms the failure to activate lipoprotein lipase in diabetics which lead to hypertriglyceridemia. The decrease in the lipid profile upon treatment with DB 1 confirms the antidiabetic potential of DB 1. The observed alterations in lipid profile of DB 1 treated groups is similar with the alterations observed in the standard treated group.

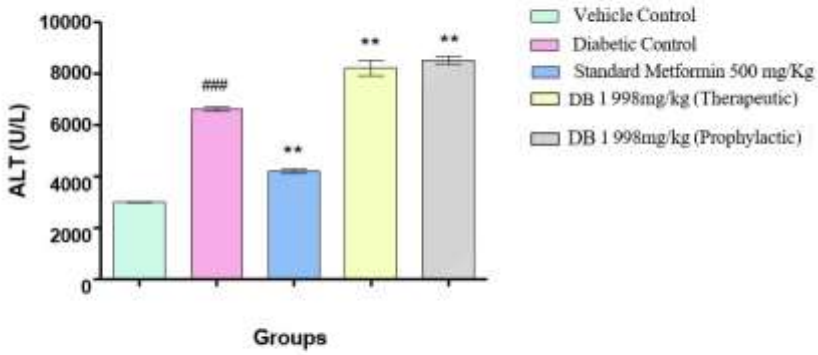
4.1.4. Effect of DB 1 on Hepatic Profile in STZ-NIC induced diabetes in rats

Alanine Transaminase ALT (U/L)

The normal level of serum ALT is 10-40 U/L. There was a tremendous increase in the ALT level of the diabetic control group, which was found to be 6614.5 U/L in 2 weeks of induction of diabetes in diabetic control. Treatment with DB 1 for 2 weeks significantly ($p<0.01$) decreased the serum ALT levels when compared to diabetic control group of animals. The observed effect was comparable to that of standard treated group which had significantly ($p<0.01$) decreased ALT levels when compared to diabetic control (Table 2: Figure 4). However, the observed decrease in both treatment groups did not reverse ALT to clinically normal levels.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Figure 4: Effect of DB 1 on ALT (U/L) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



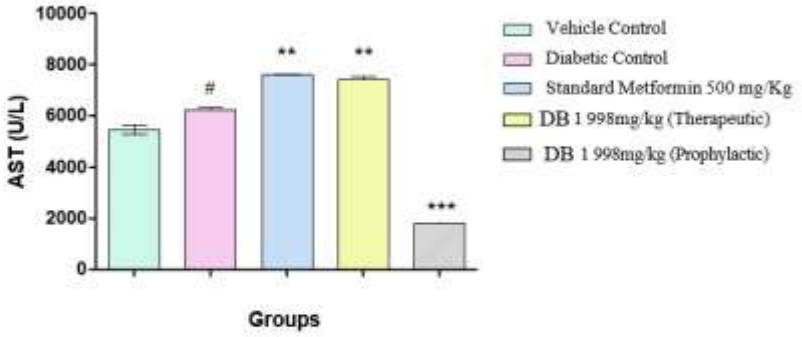
Values are expressed as mean ± SEM, n= 2.
One-way ANOVA followed by Bonferroni's t-test.
***p<0.001 when compared to Vehicle control;
**p<0.01 when compared to Diabetic control
DB 1 and Metformin were administered orally

Aspartate Transaminase AST (U/L)

The normal level of serum AST is 50-150 U/L. There was a drastic increase in the AST level of the diabetic control group, which was found to be 6237.5 U/L in 2 weeks of induction of diabetes in diabetic control. Treatment with DB 1 for 2 weeks significantly ($p<0.001$) decreased the AST levels when compared to diabetic control group of animals. The observed effect was superior to the standard treated group which had significantly ($p<0.01$) decreased the AST levels when compared to diabetic control (Table 2: Figure 3). However the observed decrease in both treatment groups did not reverse AST to clinically normal levels.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Figure 3: Effect of DB 1 on AST (U/L) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 2.
One way ANOVA followed by Bonferroni's t-test.
#p<0.05 when compared to Vehicle control;
**p<0.01, ***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally.

Alkaline Phosphatase ALP (U/L)

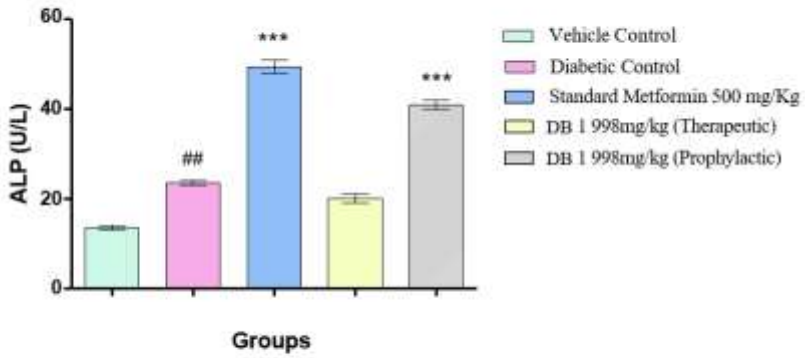
The normal level of serum ALP is 30-130 U/L. There was no significant change in the ALP level of the diabetic control group as per the normal range but in comparison with vehicle control group significant change has been observed in 2 weeks of induction of diabetes in diabetic control. Treatment with DB 1 for 2 weeks significantly ($p<0.001$) altered the ALP levels when compared to diabetic control group of animals. The observed effect was similar to the standard treated group which had significantly ($p<0.001$) altered the ALP levels when compared to diabetic control (Table 2: Figure 5). Furthermore, the observed values in both treatment groups did not reversed the ALP to clinically normal levels.

Aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are intracellular hepatic enzymes that leak into the circulation, serving as markers of hepatocyte injury. Elevations in ALT, AST, and alkaline phosphatase (ALP) are hypothesized to be predictors of diabetes. Furthermore, increased levels of these gluconeogenic enzymes, whose gene transcription is normally suppressed by insulin, may indicate impaired insulin signalling and elevated blood glucose levels rather than solely hepatocyte injury.

In diabetic rats, the elevated activities of ALT, AST, and ALP in the serum are likely due to the leakage of these enzymes from liver cytosol into the bloodstream, resulting from the hepatotoxic effects of streptozotocin (STZ). However, treatment with DB 1 for two weeks appeared to modulate the activity of these enzymes, suggesting a potentially protective effect. Further study is needed to comprehensively evaluate the impact of DB 1 and its mechanisms of action.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Figure 5: Effect of DB 1 on ALP (U/L) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 2.
One-way ANOVA followed by Bonferroni's t-test.
**p<0.01 when compared to Vehicle control;
***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

5. Conclusion:

1. DB 1 treatment did not significantly prevent weight loss in diabetic rats during the two-week study. The observed effects on body weight were comparable to those seen in the standard treatment group, indicating limited influence on diabetes-induced muscle wasting and gluconeogenesis.
2. DB 1 demonstrated significant ($p<0.05$) antihyperglycemic potential by reducing blood glucose levels from day 7 onwards, with further reductions observed on day 14. The effect was comparable to that of the standard treatment, confirming its potential as an effective agent in managing hyperglycaemia.
3. DB 1 significantly ($p<0.001$) improved the lipid profile by reducing serum triglycerides, total cholesterol, VLDL cholesterol, and LDL cholesterol levels. The effects were similar to or better than those of the standard treatment group, reinforcing the antidiabetic properties of DB 1. However, it did not alter HDL cholesterol levels within the two-week period.
4. Treatment with DB 1 significantly reduced elevated ALT and AST levels ($p<0.01$ and $p<0.001$, respectively), indicative of its potential to mitigate STZ-induced hepatocyte injury. However, the reductions did not fully restore enzyme levels to clinically normal ranges, suggesting partial protection against hepatic damage.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Phase 2 of the study was initiated, involving the continuation of treatment for an additional two months. This phase focused on further evaluating the long-term effects of DB 1 on biochemical parameters, physiological outcomes, and histopathological changes to provide a comprehensive understanding of its therapeutic potential.

Phase 2

From Day 21 onward, the body weight of animals in the vehicle control group remained stable, averaging 216 ± 10.12 g, while the diabetic control group exhibited a significant weight reduction, averaging 156.17 ± 3.33 g. This decrease in weight highlights the adverse metabolic effects of diabetes, such as gluconeogenesis and muscle wasting caused by the catabolism of proteins and fats.

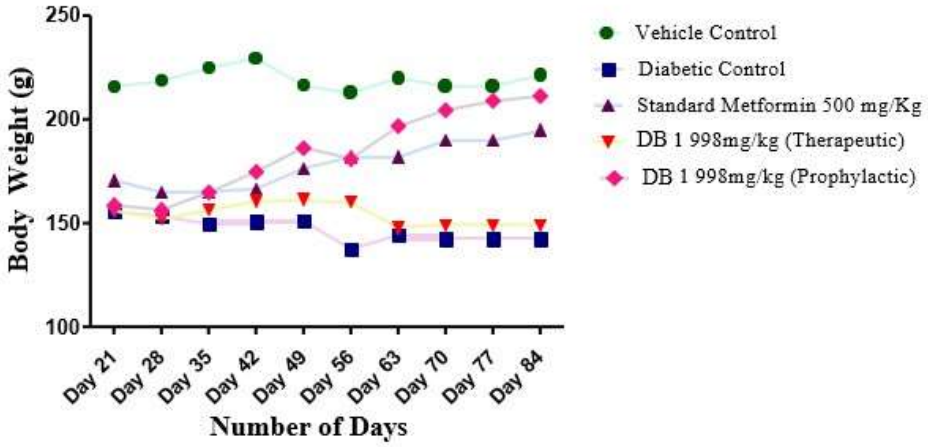
The standard-treated group (Metformin 500 mg/kg) showed a gradual improvement in body weight from Day 49, with significant gains observed from Day 56 onward ($p<0.05$). By Day 84, the body weight of this group had increased to 195 ± 14 g, demonstrating its efficacy in mitigating diabetes-induced weight loss.

The therapeutic group treated with DB 1 (998 mg/kg) showed slight improvements in body weight over the course of the study. However, these changes were not statistically significant when compared to the diabetic control group, suggesting limited therapeutic efficacy in preventing weight loss associated with diabetes. By Day 84, the body weight of this group was 149.25 ± 12.50 g, similar to the diabetic control group.

In contrast, the prophylactic group treated with DB 1 (998 mg/kg) exhibited a significant improvement in body weight from Day 56 onward ($p<0.01$, $p<0.001$). By Day 84, the body weight of this group reached 211.50 ± 23.41 g, which was comparable to and slightly superior to the standard-treated group. (Table 1: Figure 1).

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Figure 1: Effect of DB 1 on Body weight (g) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
Two-way ANOVA followed by Bonferroni t-test.
DB 1 and Metformin were administered orally.

The findings indicate that while DB 1 has limited therapeutic effects in improving body weight in diabetic animals, its prophylactic administration shows significant potential in preventing weight loss associated with diabetes mellitus

Effect of DB 1 on blood glucose (mg/dL) in STZ-NIC induced diabetes in rats

On Day 21, the blood glucose levels in the vehicle control group averaged 134.33 ± 3.26 mg/dL, while the diabetic control group exhibited a significant increase to 340.17 ± 11.56 mg/dL ($p<0.001$), confirming the hyperglycemic state induced by STZ-NIC. The standard-treated group (Metformin 500 mg/kg) showed a marked reduction in blood glucose levels, which decreased from 254.8 ± 9.92 mg/dL on Day 21 to 161.08 ± 8.80 mg/dL by Day 84 ($p<0.001$), demonstrating the effectiveness of Metformin in glycemic control.

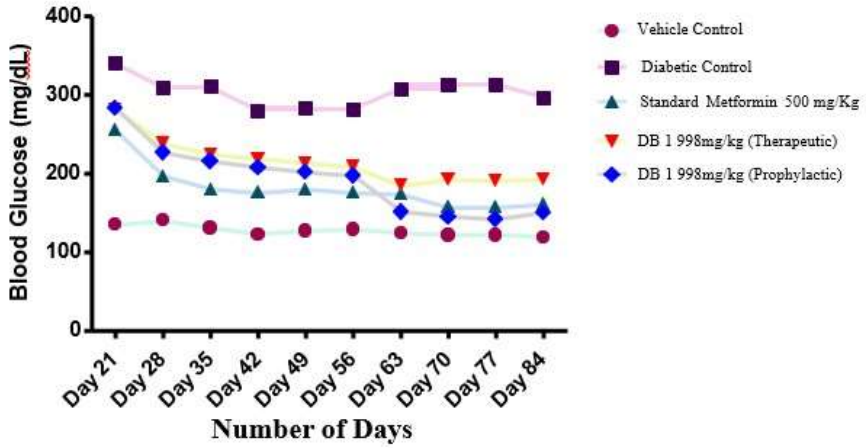
The therapeutic group treated with DB 1 (998 mg/kg) exhibited a gradual reduction in blood glucose levels over time. Starting at 282.17 ± 2.72 mg/dL on Day 21, levels reduced to 192.25 ± 9.66 mg/dL by Day 84 ($p<0.001$). While the improvement was significant compared to the diabetic control group, it was less pronounced than the reduction observed in the standard-treated group.

The prophylactic group treated with DB 1 (998 mg/kg) showed significant efficacy in preventing hyperglycemia. Blood glucose levels decreased consistently from 282.67 ± 5.98 mg/dL on Day 21 to 150.00 ± 18.44 mg/dL by Day 84 ($p<0.01$, $p<0.001$). By the end of the study, the prophylactic group

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

achieved blood glucose levels close to those of the vehicle control group, suggesting robust preventive potential against diabetes-induced hyperglycemia. (Table 1: Figure 2).

Figure 2: Effect of DB 1 on Blood glucose (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
Two-way ANOVA followed by Bonferroni t-test.
DB 1 and Metformin were administered orally.

Table 1

Parameter	Vehicle Control	Diabetic control	Standard Metformin 300 mg/Kg	Wellia 1 (998mg/kg) (Therapeutic)	Wellia 1 (998mg/kg) (Prophylactic)
Body Weight (gm)					
DAY 21	216± 10.12	156.17± 3.33#	170.67± 5.75	156.17± 6.93	158.50 ± 9.69
DAY 28	218.83± 10.30	153.67 ± 4.61#	164.83 ± 4.47	152.17± 7.12	156.33 ± 10.07
DAY 35	225± 10.68	150 ± 2.90#	165.17± 6.13	156.50± 7.67	164.83 ± 11.56
DAY 42	229.67± 11.43	150.83± 3.32#	166.50± 4.71	161.00± 8.15	174.80 ± 7.20
DAY 49	216.50±	151.20±	176.67±	161.50±	186.50 ±

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

	7.01	6.22#	6.18	5.89	10.61
DAY 56	213.17± 5.22	137.40± 11.94#	182± 9.83*	160.33± 9.02	181.25 ± 16.24
DAY 63	220.33 ± 4.69	144.40± 11.18#	182± 10.71*	148.00± 8.98	196.75± 17.31**
DAY 70	216.20 ± 5.33	142.60 ± 8.61#	190.25± 13.15*	149.25± 12.50	204.75 ± 18.91**
DAY 77	216.20 ± 5.33	142.60± 8.61#	190.25± 13.15*	149.25± 12.50	209.00 ± 20.82***
DAY 84	221.60 ± 6.30	142.60± 6.64#	195± 14*	149.25± 12.50	211.50± 23.41***
Blood Glucose (mg/dL)					
DAY 21	134.33± 3.26	340.17± 11.56#	254.8± 9.92***	282.17± 2.72**	282.67± 5.98**
DAY 28	140.33± 4.13	309.67 ± 6.04#	196.8± 17.07***	238.00 ± 12.60***	226.50± 13.69***
DAY 35	130.50 ± 7.16	310.50 ± 10.08#	180.2± 15.38***	224.00 ± 10.82***	215.50± 9.73***
DAY 42	122.17 ± 6.68	280.00± 12.45#	175.3± 14.34***	219.17± 10.45**	208.00± 11.82***
DAY 49	126.67 ± 10.95	282.20± 8.33#	179.8± 11.72***	213.33± 10.68***	201.50± 15.96***
DAY 56	128.50 ± 8.82	281.20 ± 7.76#	175.3± 10.70***	208.17± 10.77***	197.00± 13.57***
DAY 63	123.50 ± 6.67	308.00 ± 14.95#	173.4± 10.90***	184.60 ± 14.16***	151.00± 15.60***
DAY 70	121.00 ± 5.66	313.60 ± 12.30#	156.8± 9.06***	192.75± 11.46***	145.00± 15.38***
DAY 77	121.00 ± 5.66	313.60 ± 12.30#	159.5± 8.82***	191.00± 11.12***	141.50± 15.11***

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

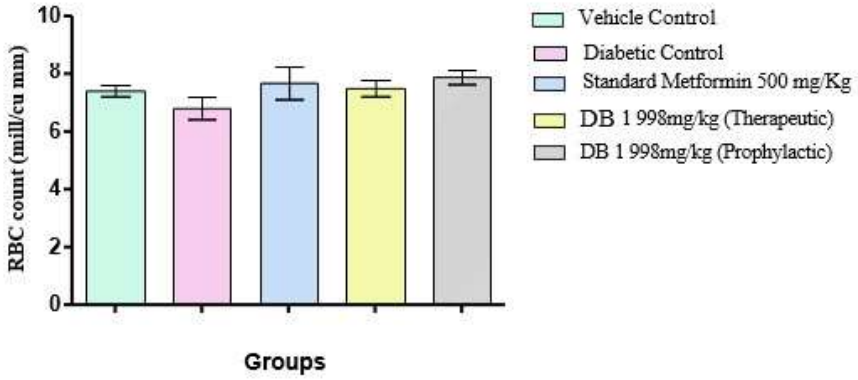
DAY 84	118.40 ± 3.87	296.20 ± 5.09#	161.08 ± 8.80***	192.25± 9.66***	150.00± 18.44***

Values are expressed as mean ± SEM, n= 6.
Two-way ANOVA followed by Bonferroni t-test.
#p<0.001 when compared to Vehicle control;
*p<0.05, **p<0.01, ***p<0.001 when compared to Diabetic control DB 1 and Metformin were administered orally.

Effect of DB 1 on complete blood count in Streptozotocin-Nicotinamide induced diabetes mellitus in rats:

Red Blood Cell (RBC) Count The RBC count in the vehicle control group was 7.43 ± 0.18 million/cu mm, which significantly decreased to 6.82 ± 0.34 million/cu mm in the diabetic control group. Standard treatment with Metformin (500 mg/kg) restored the RBC count to 7.72 ± 0.49 million/cu mm. The therapeutic group treated with DB 1 (998 mg/kg) showed an RBC count of 7.51 ± 0.25 million/cu mm, while the prophylactic group exhibited a slight improvement, achieving 7.92 ± 0.21 million/cu mm. (Table 2: Figure 3).

Figure 3: Effect of DB 1 on RBC (mill/cu mm) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

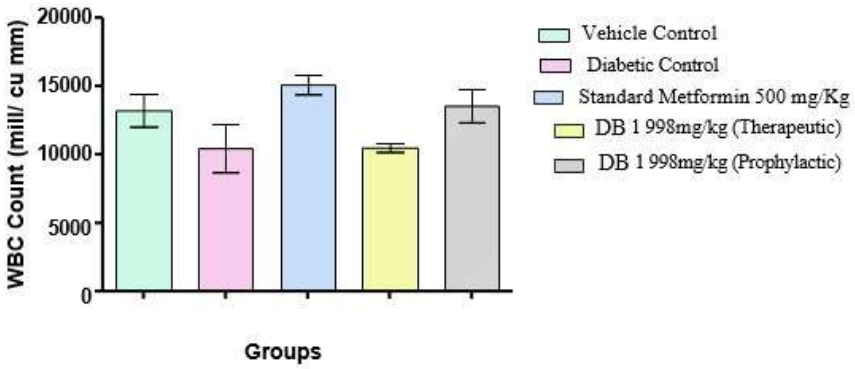


Values are expressed as mean ± SEM, n= 6.
One way ANOVA followed by Bonferroni's t-test.
No significant change when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

White Blood Cell (WBC) Count The diabetic control group showed a reduction in WBC count (10480 ± 1573.4/cu mm) compared to the vehicle control (13160 ± 1067.2/cu mm). The standard-treated group demonstrated a significant increase in WBC count (15035.25 ± 615.3/cu mm). Both the therapeutic (10477.5 ± 278.2/cu mm) and prophylactic (13575 ± 1047.8/cu mm) groups exhibited improved WBC counts, with the prophylactic group showing levels closer to the vehicle control. (Table 2: Figure 4).

Figure 4: Effect of DB 1 on WBC (mill/cu mm) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

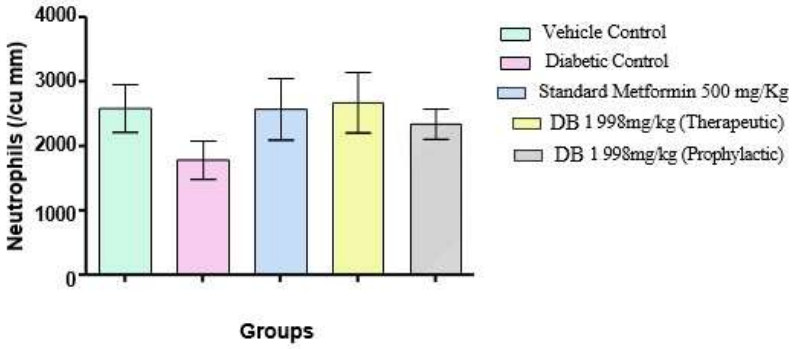


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
No significant change when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally

Neutrophil Count Neutrophil count decreased in the diabetic control group (1788.2 ± 265.49/cu mm) compared to the vehicle control (2581 ± 329.66/cu mm). Treatment with DB 1 (therapeutic: 2667.5 ± 404.38/cu mm; prophylactic: 2336 ± 202.41/cu mm) significantly improved neutrophil counts, with the therapeutic group achieving levels comparable to the standard-treated group (2570 ± 411.62/cu mm). (Table 2: Figure 5).

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

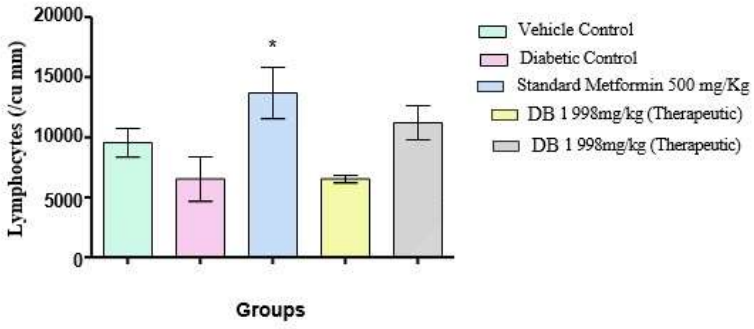
Figure 5: Effect of DB 1 on Neutrophils count (/cu mm) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
No significant change when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally

Lymphocyte Count A significant reduction in lymphocyte count was observed in the diabetic control group (6504 ± 1630.68/cu mm) compared to the vehicle control (9553.4 ± 1058.8/cu mm). The prophylactic group treated with DB 1 (11224 ± 1233.18/cu mm) demonstrated a marked improvement, surpassing both the standard-treated group (13684 ± 1837.85/cu mm) and the therapeutic group (6537.5 ± 263.69/cu mm). (Table 2: Figure 6).

Figure 6: Effect of DB 1 on Lymphocytes count (/cu mm) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

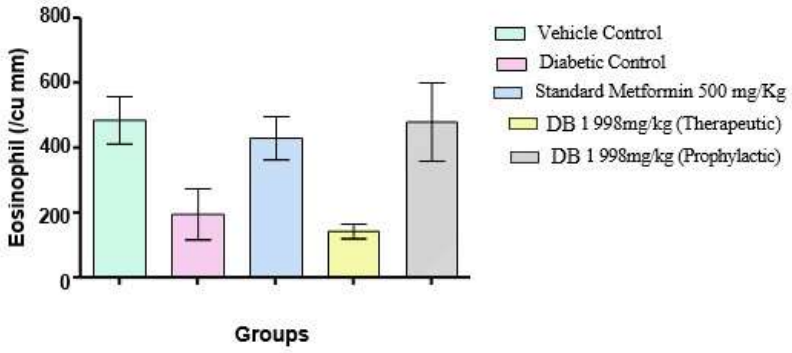


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
No significant change when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Eosinophil Count Eosinophil count was notably reduced in the diabetic control group (195.2 ± 69.73/cu mm) compared to the vehicle control (486.2 ± 65.35/cu mm). The prophylactic administration of DB 1 significantly improved eosinophil count (479.5 ± 104.32/cu mm), closely aligning with the vehicle control group, whereas the therapeutic group showed minimal improvement (145.25 ± 19.60/cu mm). (Table 2: Figure 7).

Figure 7: Effect of DB 1 on Eosinophil count (/cu mm) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

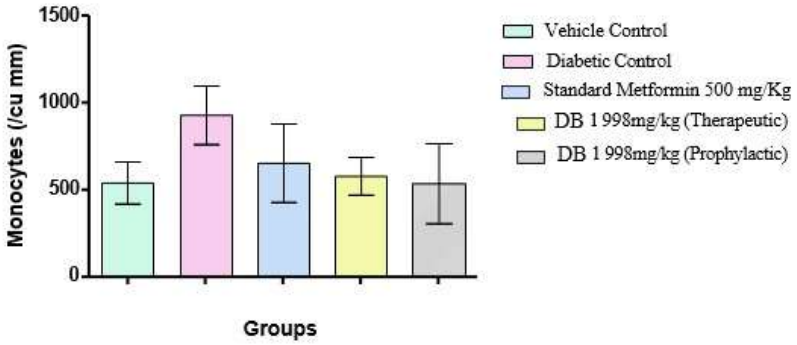


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
No significant change when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally

Monocyte Count The monocyte count increased significantly in the diabetic control group (932.6 ± 150.42/cu mm) compared to the vehicle control (539.4 ± 107.50/cu mm). Treatment with DB 1 reduced the monocyte count in both the therapeutic (582.25 ± 94.05/cu mm) and prophylactic (535.5 ± 198.40/cu mm) groups, approaching levels similar to the vehicle control group. (Table 2: Figure 8).

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

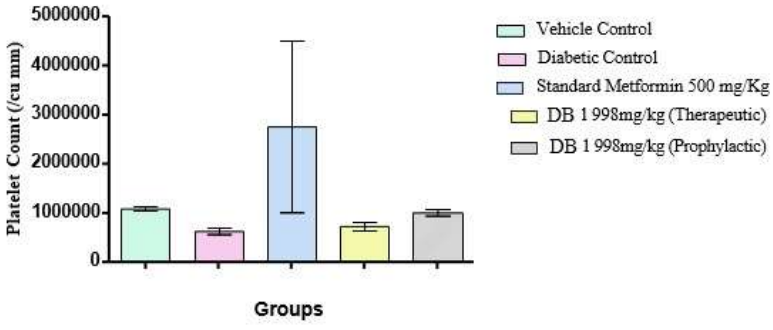
Figure 8: Effect of DB 1 on Monocyte count (/cu mm) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
No significant change when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally

Platelet Count The platelet count decreased substantially in the diabetic control group (623000 ± 59403.7/cu mm) compared to the vehicle control (1081820 ± 39583.25/cu mm). The prophylactic administration of DB 1 (1002750 ± 57779.08/cu mm) significantly restored platelet levels, outperforming the therapeutic group (721250 ± 72980.63/cu mm), though still below the vehicle control. (Table 2: Figure 9).

Figure 9: Effect of DB 1 on Platelet count (/cu mm) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

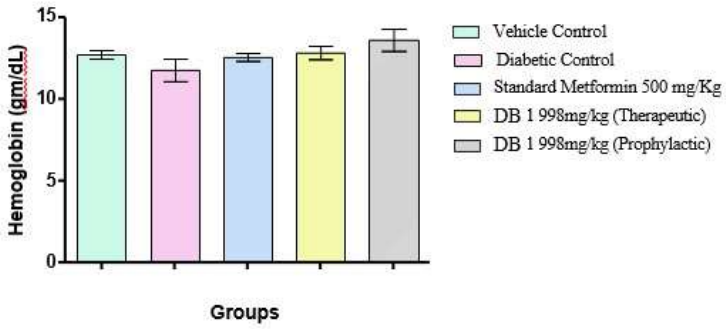


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
No significant change when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Hemoglobin Levels The diabetic control group showed a slight decrease in hemoglobin levels (11.8 ± 0.62 g/dL) compared to the vehicle control (12.72 ± 0.24 g/dL). The standard-treated group (12.58 ± 0.22 g/dL) and both therapeutic (12.83 ± 0.36 g/dL) and prophylactic (13.6 ± 0.59 g/dL) DB 1-treated groups demonstrated improvements, with the prophylactic group achieving the highest hemoglobin levels. (Table 2: Figure 10).

Figure 10: Effect of DB 1 on Hemoglobin (gm/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
No significant change when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally

Although hematological parameters showed improvement with treatment using DB 1 and the standard drug, the levels observed in diabetic animals remained below the clinically normal range for the diabetic control group.

Table 2

Parameter	Vehicle Control	Diabetic control	Standard Metformin 300 mg/Kg	Wellia 1 998 mg/Kg (Therapeutic)	Wellia 1 998 mg/Kg (Prophylactic)
RBC (mill/cu mm)	7.43± 0.18	6.82± 0.34	7.72± 0.49	7.51± 0.25	7.92± 0.21
WBC (mill/cu mm)	13160± 1067.2	10480± 1573.4	15035.25± 615.3	10477.5± 278.2	13575± 1047.8
Neutrophil count (/cu mm)	2581± 329.66	1788.2± 265.49	2570± 411.62	2667.5± 404.38	2336± 202.41
Lymphocyte count (/cu mm)	9553.4± 1058.80	6504± 1630.68	13684± 1837.85	6537.5± 263.69	11224± 1233.18

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Eosinophil count (/cu mm)	486.2± 65.35	195.2± 69.73	428.75± 57.45	145.25± 19.60	479.5± 104.32
Monocyte Count (/cu mm)	539.4± 107.50	932.6± 150.42	655.75± 194.67	582.25± 94.05	535.5± 198.40
Platelet Count (/cu mm)	1081820± 39583.25	623000± 59403.70	2757250± 1508141.96	721250± 72980.63	1002750± 57779.08
Hemoglobin (gm/dL)	12.72± 0.24	11.8± 0.62	12.58± 0.22	12.83± 0.36	13.6± 0.59

Values are expressed as mean ± SEM, n= 6.
One way ANOVA followed by Bonferroni t-test.
No significant change when compared to Vehicle control;
No significant change when compared to Diabetic control
DB 1 and Metformin were administered orally.

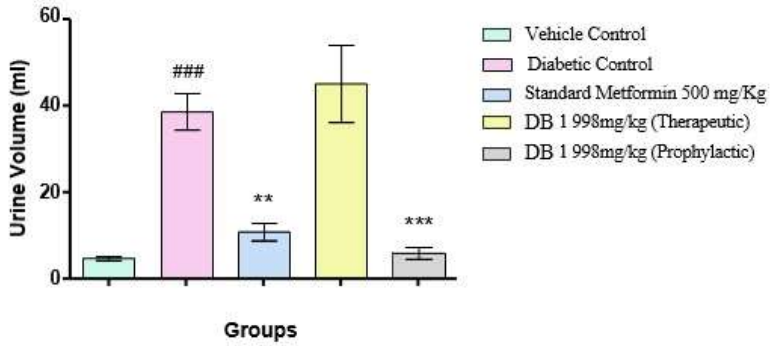
RBC- Red Blood Cell; WBC-White Blood Cell

Effect of DB 1 on Urine Volume, Bone strength and Blood Pressure in Streptozotocin-Nicotinamide induced diabetes mellitus in rat:

Urine Volume (ml): Urine volume significantly increased in the diabetic control group (38.6 ± 3.72) compared to the vehicle control (4.8 ± 0.44), indicating diabetic complications like polyuria. Treatment with DB 1 (therapeutic: 45.25 ± 7.71; prophylactic: 6 ± 1.17) significantly reduced urine volume in the prophylactic group ($p<0.001$), suggesting potential protective effects against diabetic nephropathy. (Table 3: Figure 11).

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

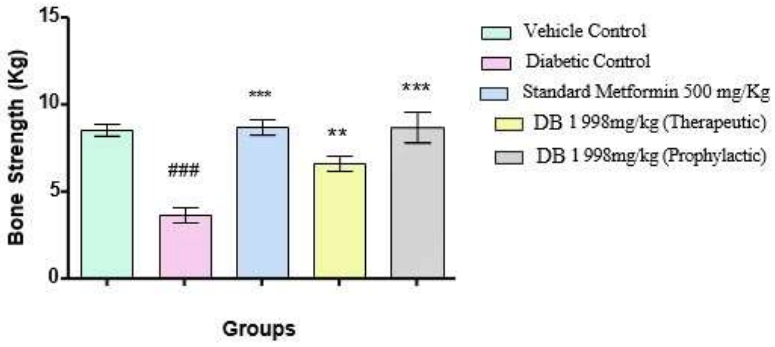
Figure 11: Effect of DB 1 on Urine Volume (ml) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
**p<0.01, ***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

Bone Strength (Kg): Bone strength significantly declined in diabetic controls (3.68 ± 0.39) compared to vehicle controls (8.56 ± 0.31). Prophylactic treatment with DB 1 (8.7 ± 0.76, p<0.001) restored bone strength to levels comparable to vehicle controls, highlighting its potential in mitigating diabetes-induced bone fragility. (Table 3: Figure 12).

Figure 12: Effect of DB 1 on Bone strength (Kg) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

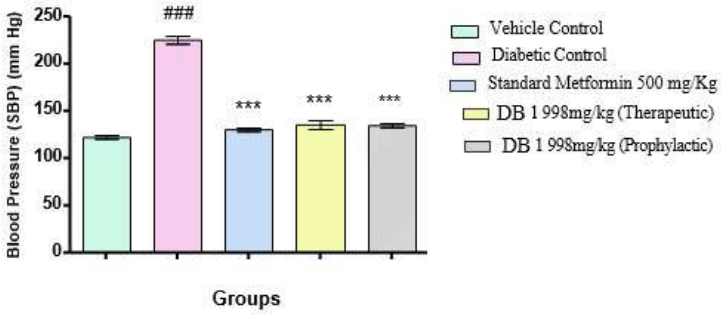


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
**p<0.01, ***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Systolic Blood Pressure (SBP) (mmHg): SBP was markedly elevated in diabetic controls (224.2 ± 3.76) compared to vehicle controls (122.2 ± 1.78). Both DB 1 treatment groups significantly reduced SBP (therapeutic: 135 ± 4.11, prophylactic: 135.25 ± 1.85, p<0.001), similar to Metformin's effect. (Table 3: Figure 13).

Figure 13: Effect of DB 1 on Systolic Blood Pressure (mm Hg) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

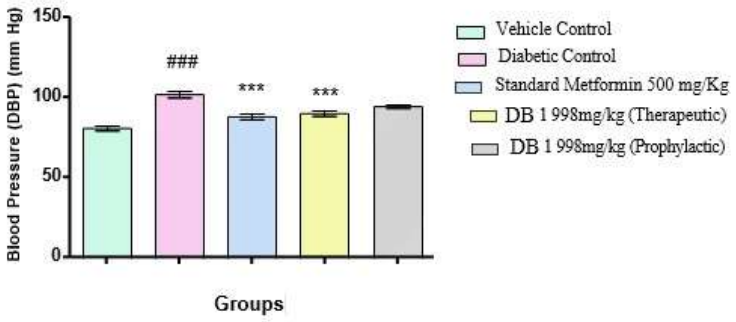


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

Diastolic Blood Pressure (DBP) (mmHg): DBP followed a similar pattern, rising in diabetic controls (102 ± 1.90) and being significantly reduced in DB 1-treated groups (therapeutic: 89.75 ± 1.47; prophylactic: 94.25 ± 0.89, p<0.001). This suggests DB 1 effectively mitigates diabetes-induced hypertension. (Table 3: Figure 14).

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Figure 14: Effect of DB 1 on Diastolic Blood Pressure (mm Hg) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

Table 3

Parameter	Vehicle Control	Diabetic control	Standard Metformin 300 mg/Kg	Wellia 1 998 mg/Kg (Therapeutic)	Wellia 1 998 mg/Kg (Prophylactic)
Urine Volume (ml)	4.8± 0.44	38.6± 3.72###	11± 1.77**	45.25± 7.71	6± 1.17***
Bone Strength (Kg)	8.56± 0.31	3.68± 0.39###	8.73± 0.38***	6.6± 0.37**	8.7± 0.76***
SBP (mm Hg)	122.2± 1.78	224.2± 3.76###	130.75± 1.60***	135± 4.11***	135.25± 1.85***
DBP (mm Hg)	81.2± 1.37	102± 1.90###	88.25± 1.56***	89.75± 1.47***	94.25± 0.89

Values are expressed as mean ± SEM, n= 6.
One way ANOVA followed by Bonferroni t-test.
###p<0.001 when compared to Vehicle control;
**p<0.01, ***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally.

SBP- Systolic Blood Pressure; DBP- Diastolic Blood Pressure

The above parameters demonstrate that DB 1 (998 mg/kg), particularly in its prophylactic administration, exerts significant protective effects against diabetes-induced physiological alterations in rats. It effectively reduces urine volume, indicating improved renal function, and

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

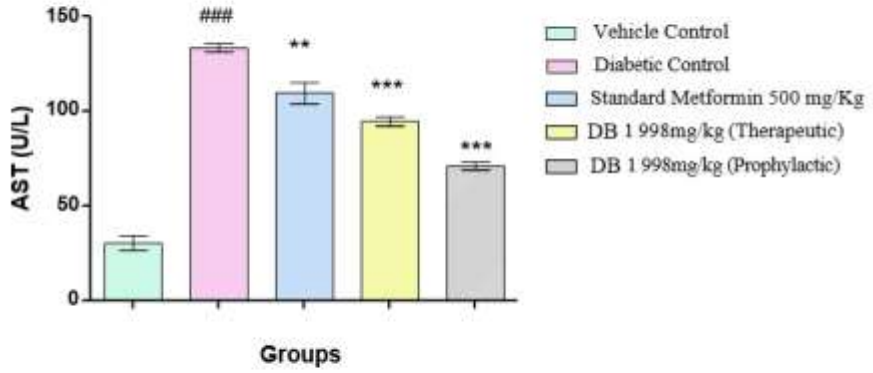
restores bone strength, showcasing its potential in mitigating diabetes-related bone fragility. Furthermore, the reduction in systolic and diastolic blood pressure reflects its role in managing diabetes-associated hypertension, contributing to cardiovascular health.

Effect of DB 1 on Liver, Kidney and Lipid profile Streptozotocin-Nicotinamide induced diabetes mellitus in rats:

Aspartate Transaminase (AST) (U/L):

AST levels were significantly elevated in the diabetic control group (133.6 ± 2.09) compared to the vehicle control (30.6 ± 3.55), indicating liver damage. DB 1 treatment significantly reduced AST levels (therapeutic: 94.5 ± 2.08, prophylactic: 71 ± 1.80, $p<0.001$), with prophylactic treatment showing a more pronounced effect. (Table 4: Figure 15).

Figure 15: Effect of DB 1 on AST (U/L) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



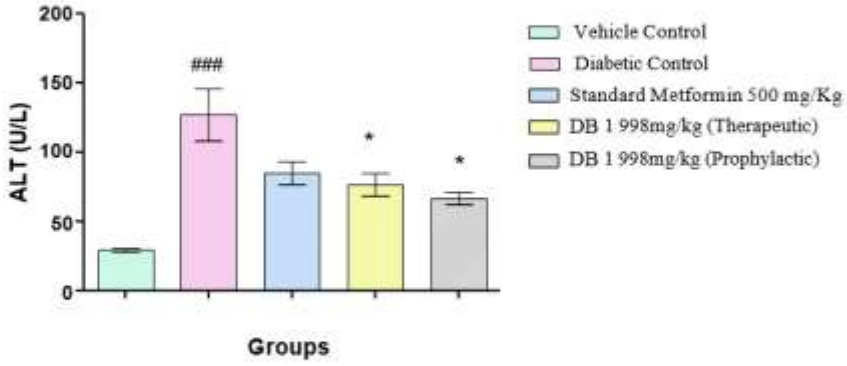
Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
**p<0.01, ***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

Alanine Transaminase (ALT) (U/L):

ALT levels followed a similar trend, rising significantly in the diabetic control group (127 ± 16.72) compared to the vehicle control (29 ± 1.06). DB 1 (therapeutic: 76.75 ± 7.09, prophylactic: 66.75 ± 3.91, $p<0.001$) reduced ALT levels, indicating its potential to protect liver function. (Table 4: Figure 16)

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Figure 16: Effect of DB 1 on ALT (U/L) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

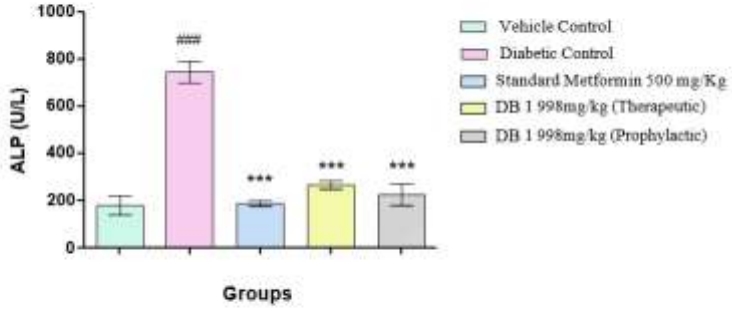


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
*p<0.05 when compared to Diabetic control
DB 1 and Metformin were administered orally

Alkaline Phosphatase (ALP) (U/L):

ALP levels were markedly elevated in diabetic rats (747.4 ± 41.68) compared to the vehicle control (180.86 ± 36.01). Both therapeutic (268.25 ± 15.64) and prophylactic (224.75 ± 39.68, p<0.001) DB 1 treatments significantly reduced ALP, indicating its ability to alleviate liver and biliary dysfunction. (Table 4: Figure 17)

Figure 17: Effect of DB 1 on ALP (U/L) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

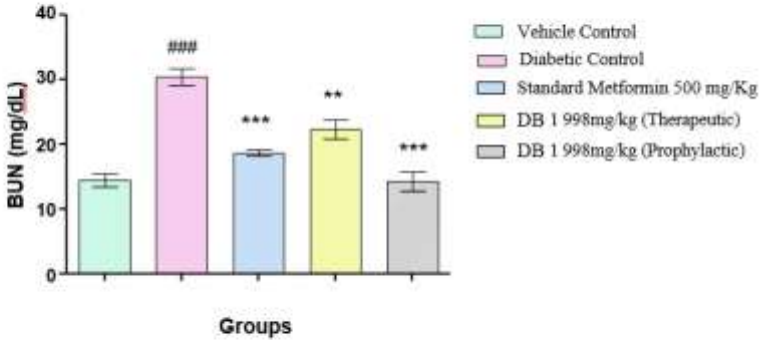


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Blood Urea Nitrogen (BUN) (mg/dL): BUN levels increased significantly in the diabetic control group (30.4 ± 1.15) compared to vehicle control (14.38 ± 0.89). While therapeutic DB 1 increased BUN (22.34 ± 1.25), prophylactic treatment significantly normalized levels (14.25 ± 1.29, $p<0.001$), indicating protective renal effects. (Table 4: Figure 18)

Figure 18: Effect of DB 1 on BUN (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

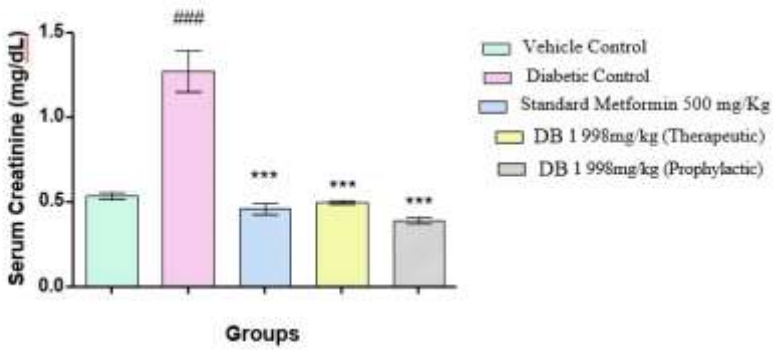


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
**p<0.01, ***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

Serum Creatinine (mg/dL): Serum creatinine levels were elevated in diabetic rats (1.27 ± 0.11) compared to vehicle controls (0.54 ± 0.02). Both DB 1 treatments significantly reduced creatinine (therapeutic: 0.50 ± 0.01, prophylactic: 0.39 ± 0.02, $p<0.001$), demonstrating improved kidney function. (Table 4: Figure 19)

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

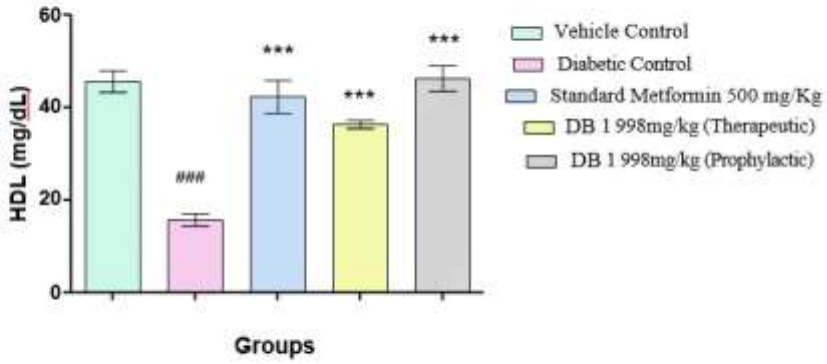
Figure 19: Effect of DB 1 on Serum Creatinine (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

High-Density Lipoprotein (HDL) (mg/dL): HDL levels significantly decreased in the diabetic control group (15.8 ± 1.18) compared to the vehicle control (45.6 ± 2.07). DB 1 treatment, especially prophylactic (46.25 ± 2.36, p<0.001), restored HDL levels, indicating its cardioprotective effects. (Table 4: Figure 20)

Figure 20: Effect of DB 1 on HDL (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

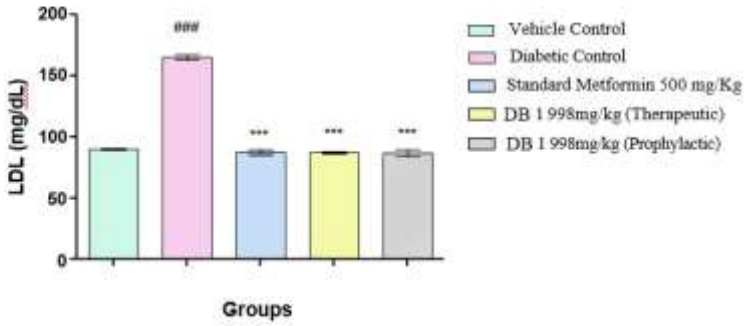


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Low-Density Lipoprotein (LDL) (mg/dL): LDL levels increased significantly in diabetic controls (165.2 ± 1.91) compared to vehicle controls (90.28 ± 0.54). Prophylactic DB 1 (86.9 ± 2.62, p<0.001) significantly reduced LDL, demonstrating its lipid-lowering potential. (Table 4: Figure 21)

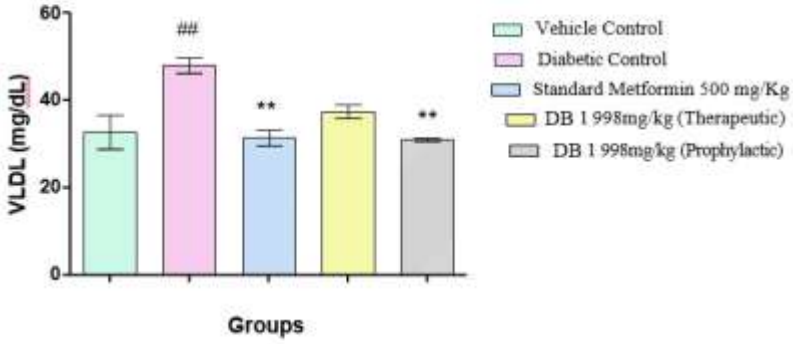
Figure 21: Effect of DB 1 on LDL (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally

Very Low-Density Lipoprotein (VLDL) (mg/dL): VLDL levels were elevated in diabetic rats (48.12 ± 1.62) but significantly reduced by DB 1 (therapeutic: 37.55 ± 1.37; prophylactic: 31.15 ± 0.34, p<0.01), indicating improved lipid metabolism. (Table 4: Figure 22)

Figure 22: Effect of DB 1 on VLDL (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats

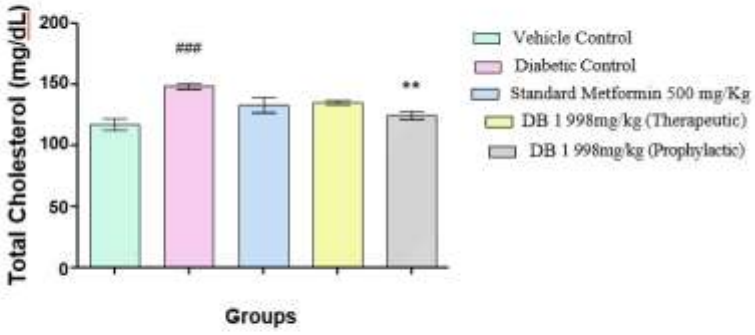


Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
##p<0.01 when compared to Vehicle control;
**p<0.01 when compared to Diabetic control
DB 1 and Metformin were administered orally

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Total Cholesterol (TC) (mg/dL): TC levels were significantly increased in diabetic controls (149 ± 2.15) compared to vehicle controls (117 ± 4.30). Prophylactic DB 1 (125 ± 3.02, p<0.01) effectively reduced TC levels, aligning them closer to normal. (Table 4: Figure 23)

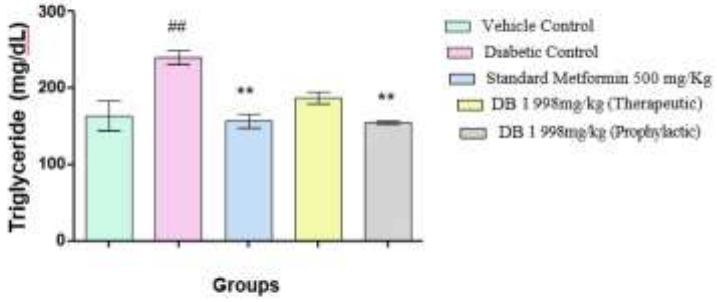
Figure 23: Effect of DB 1 on TC (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
###p<0.001 when compared to Vehicle control;
**p<0.01 when compared to Diabetic control
DB 1 and Metformin were administered orally

Triglycerides (TG) (mg/dL): TG levels were significantly higher in diabetic controls (240.6 ± 8.10) compared to vehicle controls (163.6 ± 17.51). DB 1 treatment (therapeutic: 187.75 ± 6.87; prophylactic: 155.75 ± 1.71, p<0.01) significantly reduced TG levels, with prophylactic treatment showing superior effects. (Table 4: Figure 24)

Figure 24: Effect of DB 1 on TG (mg/dL) in Streptozotocin-Nicotinamide induced diabetes mellitus in rats



Values are expressed as mean ± SEM, n= 6.
One-way ANOVA followed by Bonferroni's t-test.
##p<0.01 when compared to Vehicle control;
**p<0.01 when compared to Diabetic control
DB 1 and Metformin were administered orally

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Table 4

Parameter	Vehicle Control	Diabetic control	Standard Metformin 300 mg/Kg	Wellia 1 998 mg/Kg (Therapeutic)	Wellia 1 998 mg/Kg (Prophylactic)
AST (U/L)	30.6± 3.55	133.6± 2.09###	109.75± 4.81**	94.5± 2.08***	71± 1.80***
ALT (U/L)	29± 1.06	127± 16.72###	84.75± 7.22	76.75± 7.09*	66.75± 3.91*
ALP (U/L)	180.8± 36.01	747.4± 41.68###	188± 9.85***	268.25± 15.64***	224.75± 39.68***
BUN (mg/dL)	14.38± 0.89	30.4± 1.15###	18.65± 0.44***	22.34± 1.25**	14.25± 1.29***
Serum Creatinine (mg/dL)	0.54± 0.02	1.27± 0.11###	0.47± 0.03***	0.50± 0.01***	0.39± 0.02***
HDL (mg/dL)	45.6± 2.07	15.8± 1.18###	42.25± 3.07***	36.5± 0.75***	46.25± 2.36***
LDL (mg/dL)	90.28± 0.54	165.2± 1.91###	87.73± 1.98***	87.5± 0.74***	86.9± 2.62***
VLDL (mg/dL)	32.72± 3.50	48.12± 1.62##	31.5± 1.57**	37.55± 1.37	31.15± 0.34**
TC (mg/dL)	117.4± 4.30	149± 2.15###	133.25± 5.49	135.5± 1.48	125± 3.02**
TG (mg/dL)	163.6± 17.51	240.6± 8.10##	157.5± 7.83**	187.75± 6.87	155.75± 1.71**

Values are expressed as mean ± SEM, n= 6.
One way ANOVA followed by Bonferroni t-test.
##p<0.01, ###p<0.001 when compared to Vehicle control;
**p<0.01, ***p<0.001 when compared to Diabetic control
DB 1 and Metformin were administered orally.

AST- Aspartate Aminotransferase; ALT- Alanine Aminotransferase; ALP - Alkaline Phosphatase; BUN- Blood Urea Nitrogen; TC – Total Cholesterol, LDL – Low density lipoprotein; TG – Triglycerides; HDL – High density lipoprotein; VLDL- Very low-density lipoprotein

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

The parameter demonstrates that DB 1 (998 mg/kg), particularly in its prophylactic form, exerts significant protective effects against diabetes-induced biochemical alterations in rats. It effectively reduced elevated levels of AST, ALT, ALP, BUN, serum creatinine, LDL, VLDL, TC, and TG, indicating improved liver, renal, and lipid metabolism functions. Additionally, the restoration of HDL levels further highlights its cardioprotective potential. These findings underscore the therapeutic and prophylactic potential of DB 1 in managing diabetes and preventing its complications.

Conclusion:

1. Body Weight: DB 1 demonstrated limited therapeutic efficacy in improving body weight in diabetic rats, with the prophylactic treatment showing significant improvement, particularly from Day 56 onwards. Prophylactic DB 1 administration effectively prevented diabetes-induced weight loss, suggesting its potential as a preventive therapeutic.

2. Blood Glucose Levels: Prophylactic administration of DB 1 showed a significant reduction in blood glucose levels, reaching values close to those of the vehicle control group by Day 84. While therapeutic DB 1 showed improvement, it was less pronounced than Metformin, indicating better potential in preventing hyperglycemia rather than reversing it.

3. Hematological Parameters: DB 1 administration improved various blood parameters, including RBC, WBC, neutrophils, and lymphocytes. The prophylactic treatment was particularly effective in restoring normal levels, showcasing its potential to improve immune and hematological health in diabetic conditions.

4. Urine Volume and Renal Function: The prophylactic group treated with DB 1 significantly reduced urine volume, indicating protection against diabetic nephropathy. This suggests potential therapeutic value for renal health.

5. Bone Strength: Prophylactic treatment with DB 1 effectively restored bone strength to near-normal levels, highlighting its potential to mitigate diabetes-induced bone fragility.

6. Blood Pressure: DB 1 treatment, particularly prophylactic, significantly reduced both systolic and diastolic blood pressure, similar to the effects observed with Metformin, suggesting its potential in managing diabetes-associated hypertension.

7. Liver and Kidney Function: DB 1, especially in prophylactic administration, effectively reduced liver enzymes (AST, ALT, ALP) and improved renal function markers (BUN, serum creatinine), indicating protective effects on liver and kidney health in diabetic conditions.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

8. Lipid Profile: Prophylactic administration of DB 1 significantly improved lipid metabolism by reducing total cholesterol, triglycerides, LDL, and VLDL levels, while increasing HDL levels, thereby demonstrating its potential in managing diabetic dyslipidemia and providing cardioprotective benefits.

8788262637 info@wellialabs.com www.wellialabs.com
Registered office - 304, Amruta apartment, Ashok path, law college road, Erandwane, Pune - 411004

Histopathology Results:

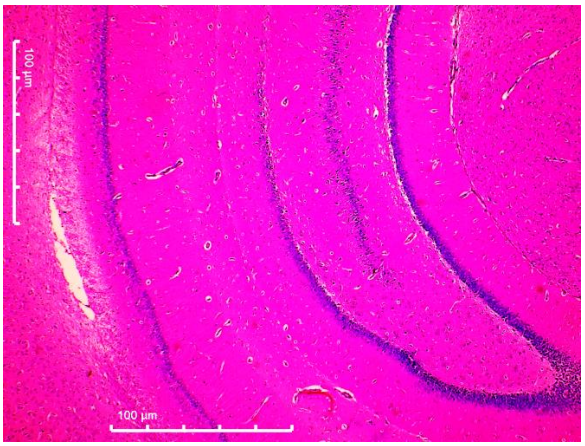
Procedure: The animal tissue sample was isolated and put in a 10% neutral buffer formalin solution. Specimens were cut into sections of 3-5-μm thickness and embedded in paraffin. Serial sections (3 μm) were cut using a microtome (Leica Biosystems, Germany) and stained with hematoxylin-eosin (H&E) stain. The prepared slides were examined under a microscope (Motic Microscopes, Tri-County Pkwy, Schertz, TX 78154, United States). The severity of the observed lesions was recorded as NAD = No Abnormality Detected, (+) Changes observed

Tissue processing protocol

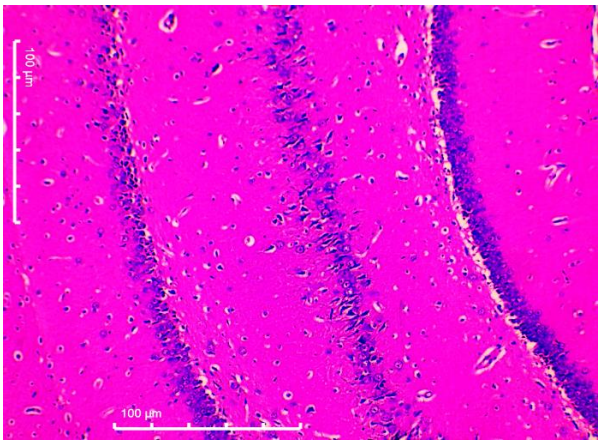
Deparaffinization and rehydration	Xylene I	5 min
	Xylene II	5 min
	90% Alcohol	5 min
	70% Alcohol	5 min
	Water Wash	10 min
Nuclear Staining	Harr's hematoxylin	8 min
	Water wash	2 min
Differentiation	Differentiation in 1% Acid	1 dip
	Alcohol	
	Water wash	10 min
Bluing	1% Lithium carbonate	1min
	Water wash	10 min
Cytoplasmic Staining	1% Eosin	1min
	90% Alcohol	30 sec
	70% Alcohol	30 sec
	Xylene I	5 min
	Xylene II	5 min
	Approximate time required	70-75 min

Brain:

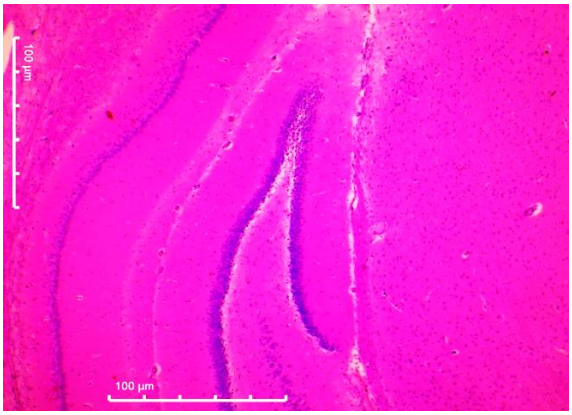
VC- Brain (4X)



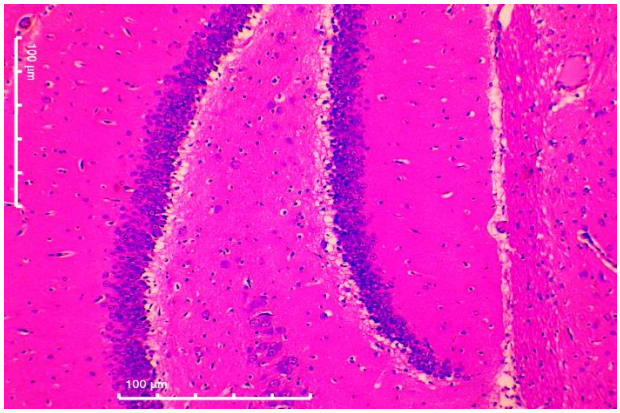
VC-Brain (10X)



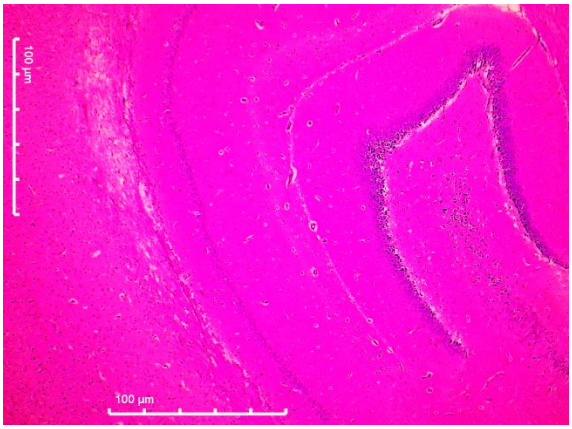
DC- Brain (4X)



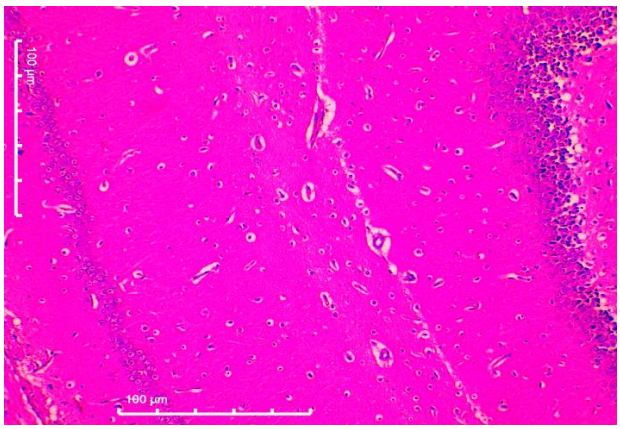
DC-Brain (10X)



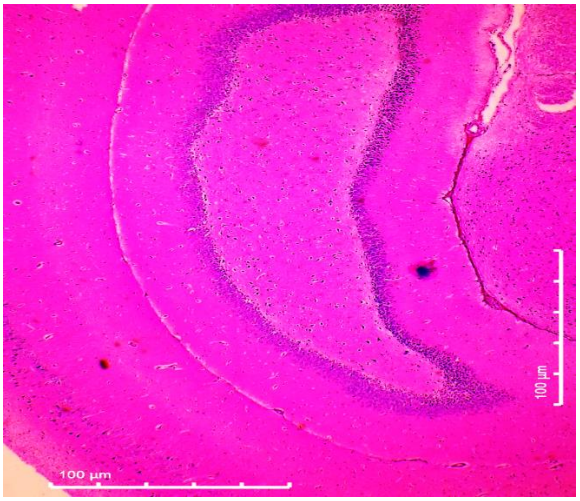
STD- Brain (4X)



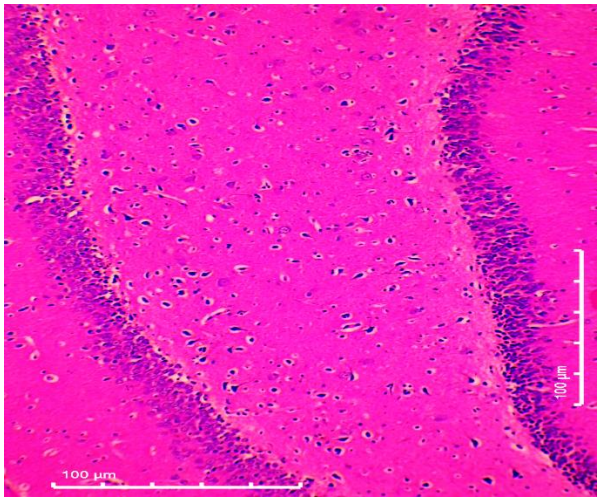
STD-Brain (10X)



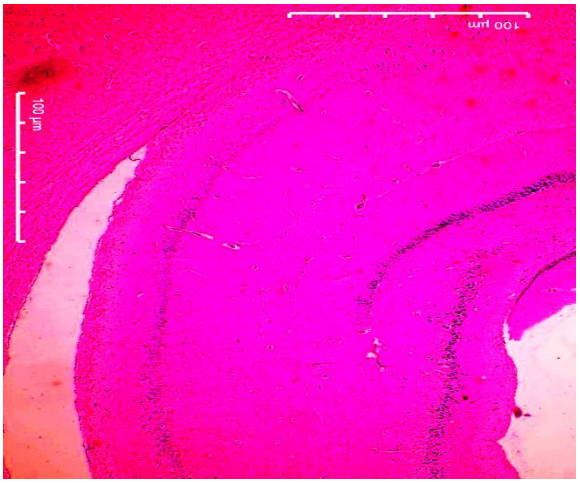
T1- Brain (4X)



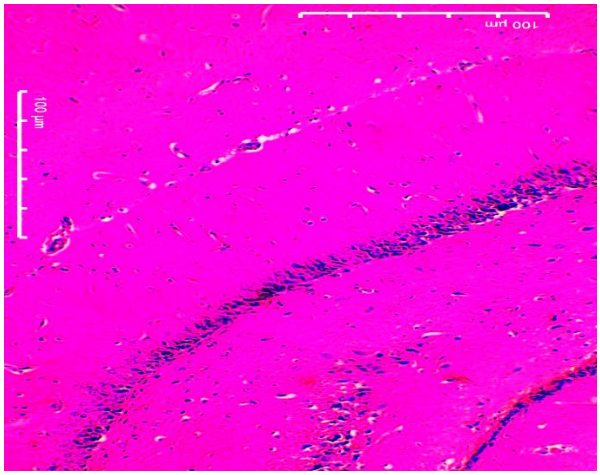
T1-Brain (10X)



T2- Brain (4X)



T2-Brain (10X)



Observations:

Sr. No	Group and Animal Code No.	Histopathological Observations – Brain			Overall Pathological grade / lesion score
		Vascular changes- Congestion/ Hemorrhages in brain tissue	Neuronal swelling and degenerative changes in neurons, dark neurons etc	Inflammatory changes with MNC infiltration in brain tissue	
1	VC	NAD	NAD	NAD	**NAD**
2	DC	Focal (+)	NAD	NAD	**NAD**
3	STD	NAD	NAD	NAD	**NAD**
4	T1	NAD	NAD	NAD	**NAD**
5	T2	NAD	NAD	NAD	**NAD**

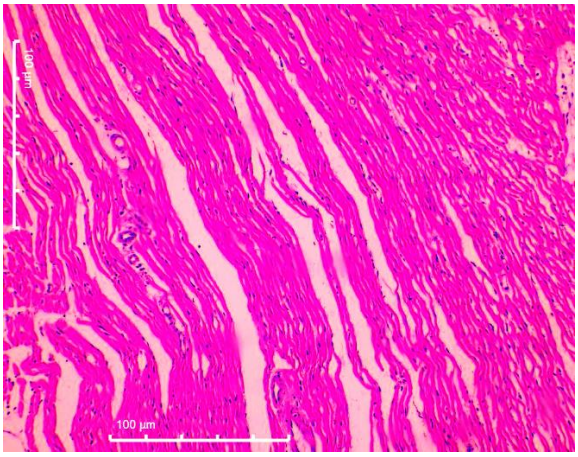
Note : NAD =No Abnormality Detected, (+) Changes observed

VC (Vehicle Control), STD (Standard Drug), T1 (Test Group 1), and T2 (Test Group 2) groups showed no abnormalities detected (NAD) in terms of vascular changes, neuronal swelling, degenerative changes, or inflammatory changes. The DC (Diabetic Control) group exhibited focal vascular changes, specifically congestion or hemorrhages, but no other abnormalities were detected.

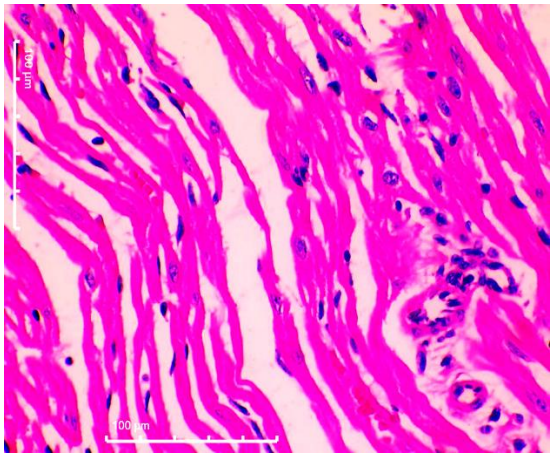
These findings suggest that the anti-diabetic treatments administered to the STD, T1, and T2 groups did not cause any adverse histopathological changes in the brain tissue of the rats. In contrast, the diabetic condition in the DC group led to some vascular changes, highlighting the potential protective effects of the treatments in preventing such changes.

Heart:

VC- Heart (10X)

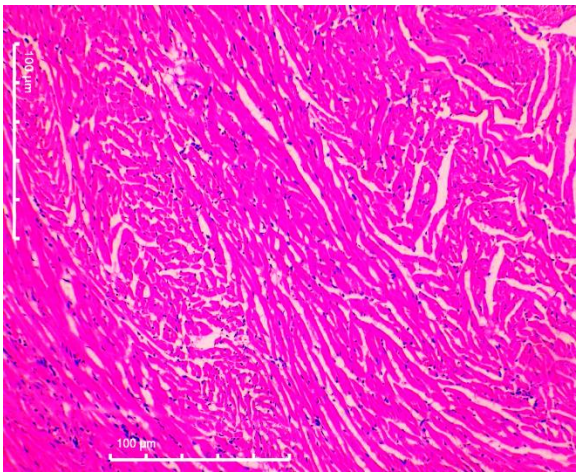


VC-Heart (40X)

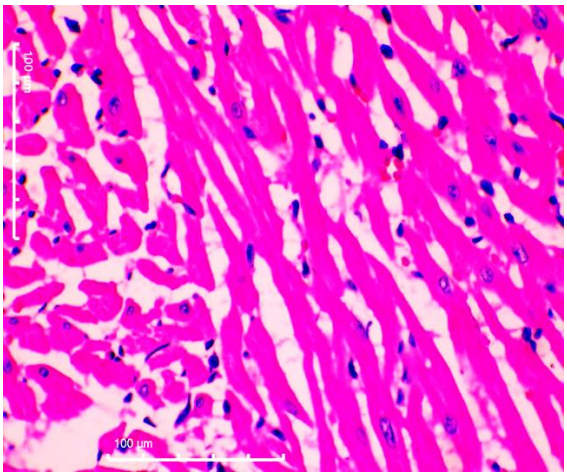


DC- Heart (10X)

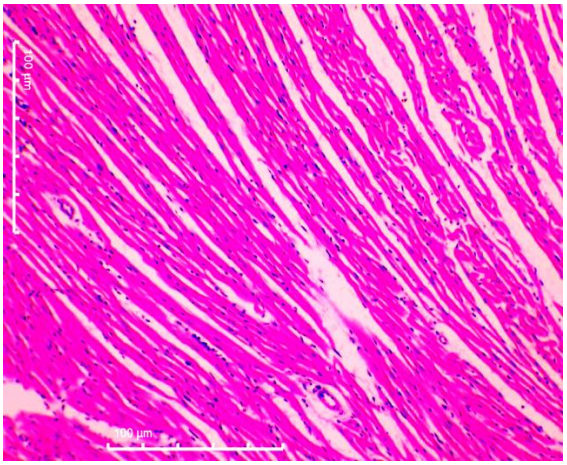
DC-Heart (40X)



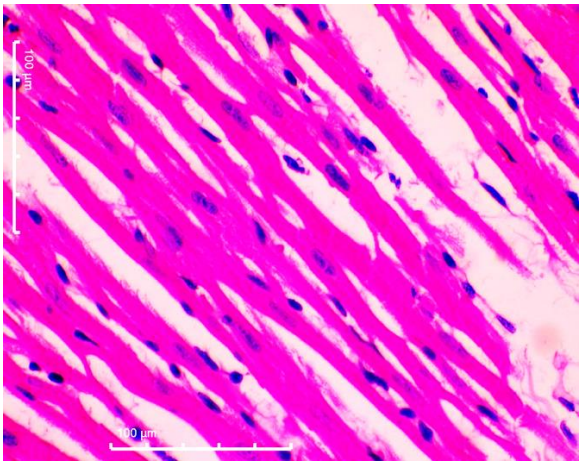
STD- Heart (10X)



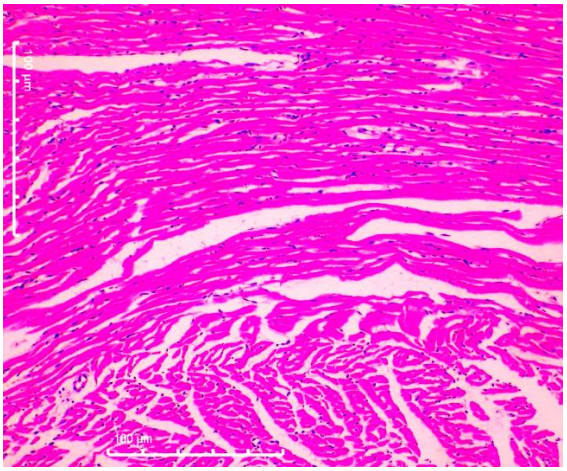
STD-Heart (40X)



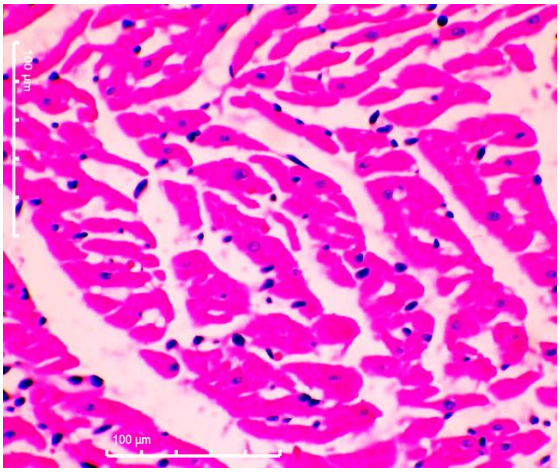
T1- Heart (10X)



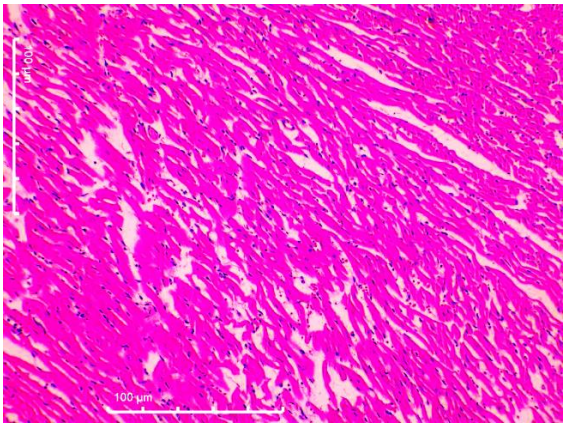
T1-Heart (40X)

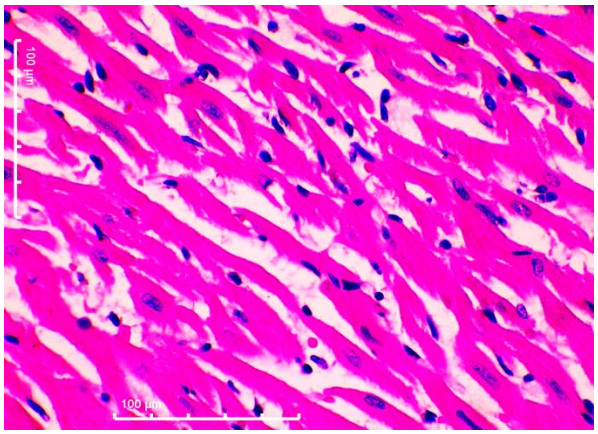


T2- Heart (10X)



T2-Heart (40X)





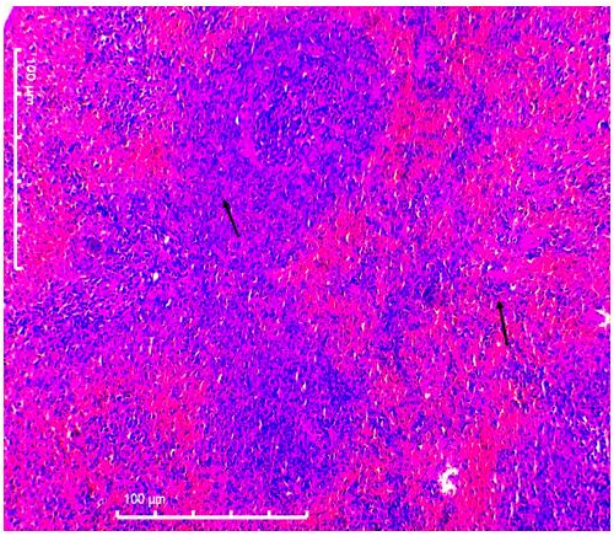
Sr. No	Group and Animal Code No.	Histopathological Observations – Heart				Overall Pathological grade / lesion score
		Vascular changes- Congestion / Hemorrhages in Cardiac tissue	Cardiomyopathy with Derangement and Dilation of Cardiac muscle fibers	Degenerative and necrotic changes with loss of muscle striations	Inflammatory cellular infiltration in cardiac tissue	
1	VC	NAD	NAD	NAD	NAD	**NAD**
2	DC	Focal (+)	Focal (+)	Focal (+)	NAD	**(+1)**
3	STD	NAD	NAD	Focal (+)	NAD	**NAD**
4	T1	NAD	Focal (+)	NAD	NAD	**NAD**
5	T2	NAD	NAD	NAD	Focal (+)	**NAD**
Note : NAD =No Abnormality Detected, (+) Changes observed Grades: 1- Mild, 2- Moderate, 3- Severe						

VC (Vehicle Control), STD (Standard Drug), T1 (Test Group 1), and T2 (Test Group 2) groups showed no abnormalities detected (NAD) in terms of vascular changes, cardiomyopathy, degenerative and necrotic changes, or inflammatory cellular infiltration. The DC (Diabetic Control) group exhibited focal vascular changes, cardiomyopathy, and degenerative changes, but no inflammatory cellular infiltration was detected.

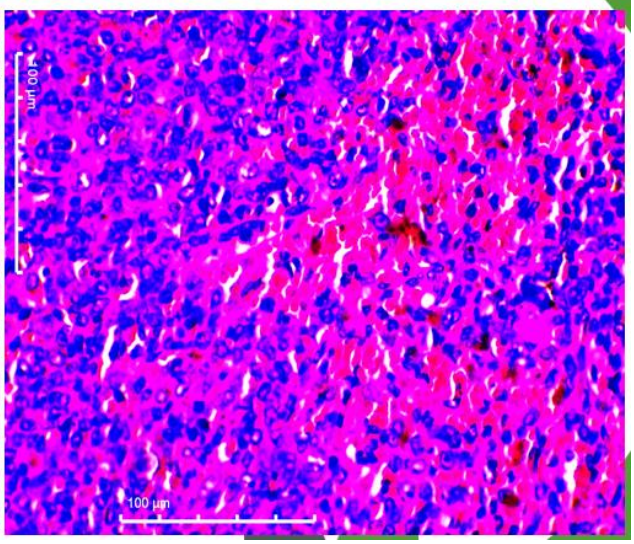
These findings suggest that the anti-diabetic treatments administered to the STD, T1, and T2 groups did not cause any adverse histopathological changes in the heart tissue of the rats. In contrast, the diabetic condition in the DC group led to some pathological changes, highlighting the potential protective effects of the treatments in preventing such changes.

Spleen:

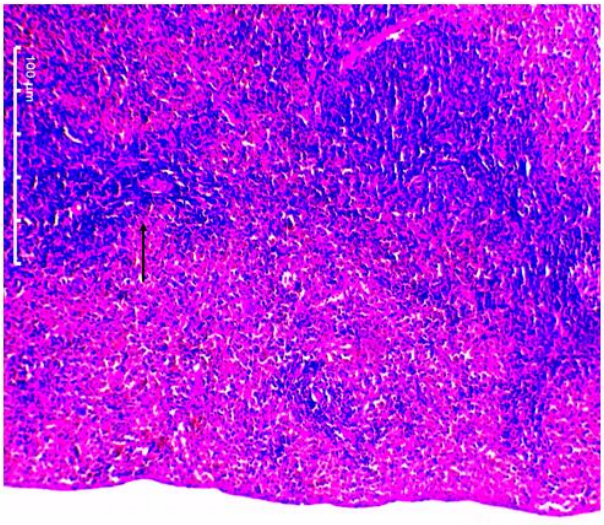
VC- Spleen (10X)



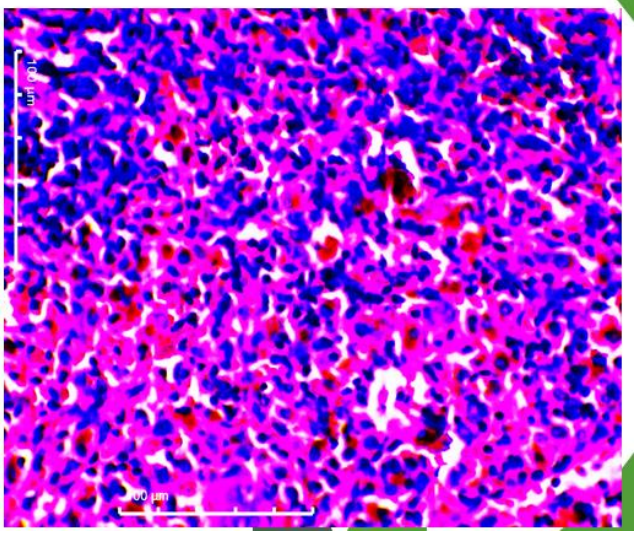
VC-Spleen (40X)



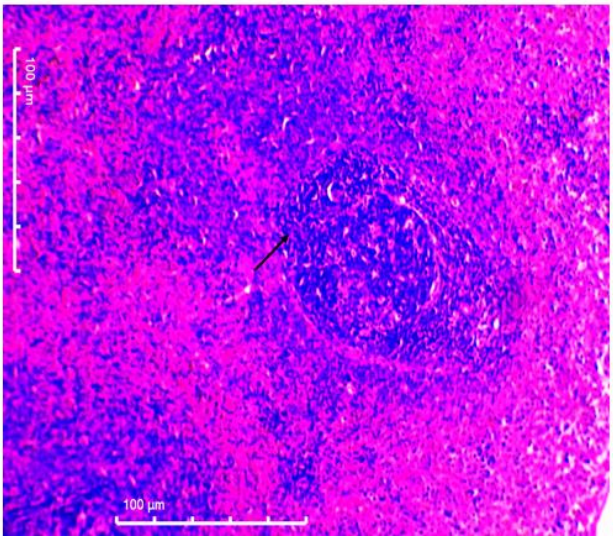
DC- Spleen (10X)



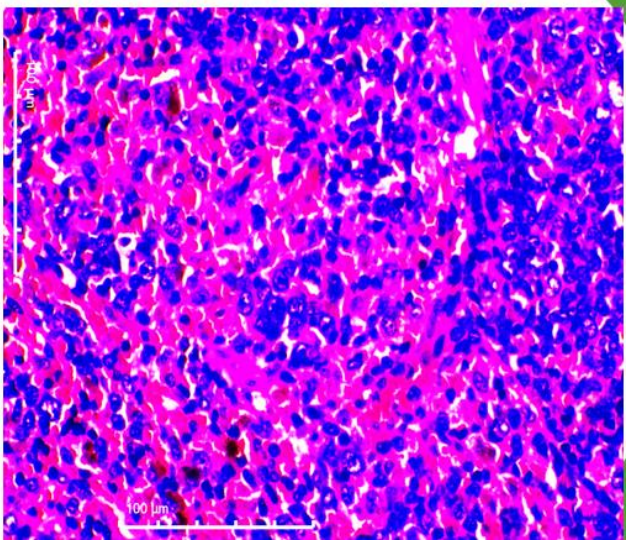
DC-Spleen (40X)



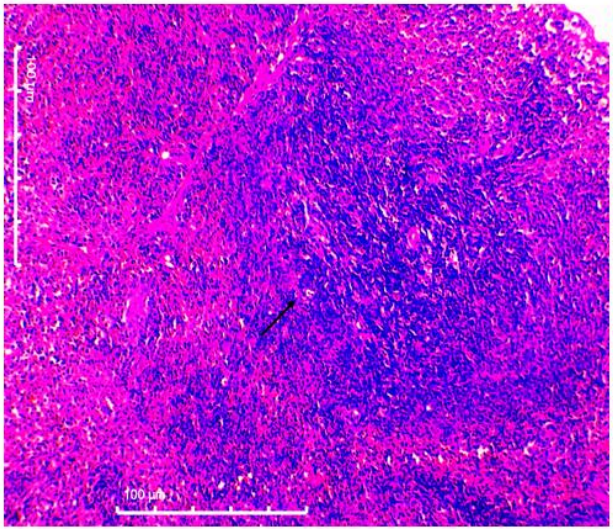
T1- Spleen (10X)



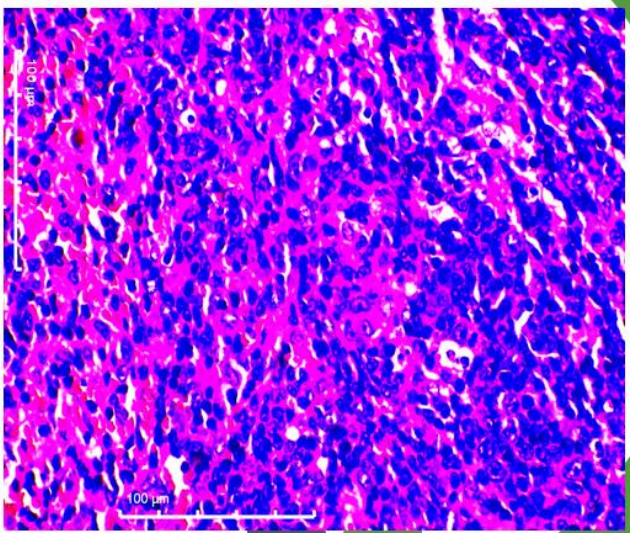
T1-Spleen (40X)



T2- Spleen (10X)



T2-Spleen (40X)



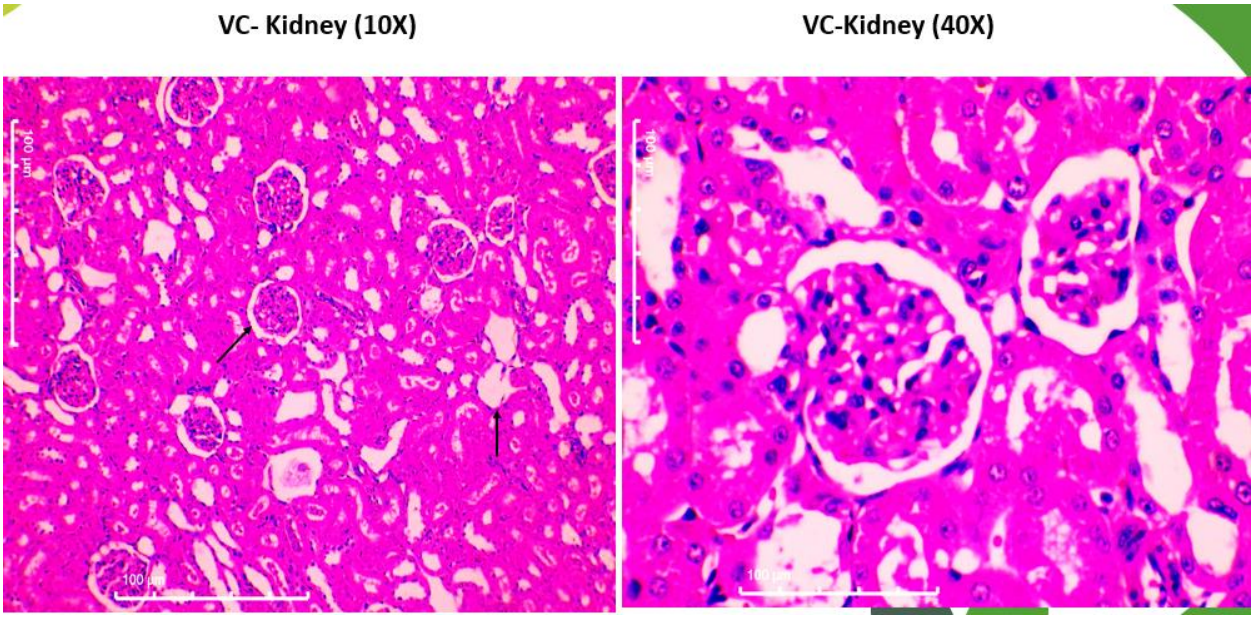
Sr. No.	Group and Animal Code No.	Histopathological Observations – Spleen			Overall Pathological grade / lesion score
		Congestion / Hemorrhages in Splenic tissue	Atrophic / hyperplastic changes of WHITE pulp/ Cellular changes with depletion of lymphocytes	Cellular changes in RED pulp with hemolysis, hemosiderosis etc.	
1	VC	NAD	NAD	NAD	NAD
2	DC	NAD	Focal (+)	Focal (+)	(+1)
3	STD	NAD	NAD	NAD	NAD

4	T1	Focal (+)	NAD	NAD	NAD
5	T2	NAD	NAD	NAD	NAD
Note : NAD =No Abnormality Detected, (+) Changes observed Grades: 1- Mild, 2- Moderate, 3- Severe					

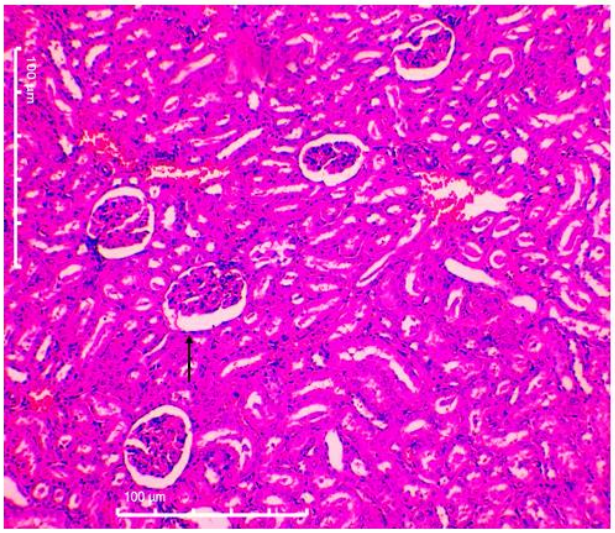
VC (Vehicle Control) and T1 (Test Group 1) groups showed focal vascular changes, specifically congestion or hemorrhages, but no other abnormalities were detected. The DC (Diabetic Control) group exhibited focal atrophic or hyperplastic changes in the white pulp and cellular changes in the red pulp, including hemolysis and hemosiderosis. STD (Standard Drug) and T2 (Test Group 2) groups showed no abnormalities detected (NAD) in terms of vascular changes, atrophic or hyperplastic changes, or cellular changes.

These findings suggest that the anti-diabetic treatments administered to the STD and T2 groups did not cause any adverse histopathological changes in the spleen tissue of the rats. In contrast, the diabetic condition in the DC group led to some pathological changes, highlighting the potential protective effects of the treatments in preventing such changes.

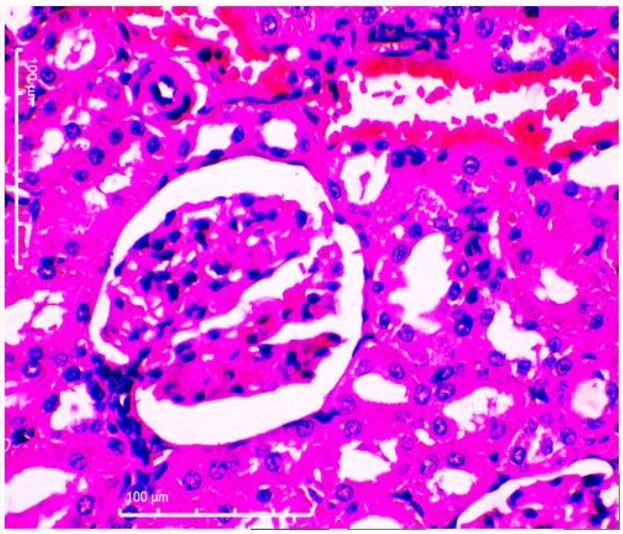
Kidney:



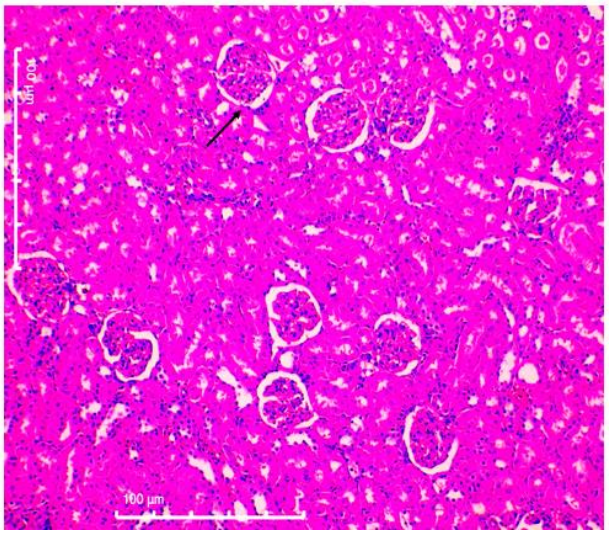
DC- Kidney (10X)



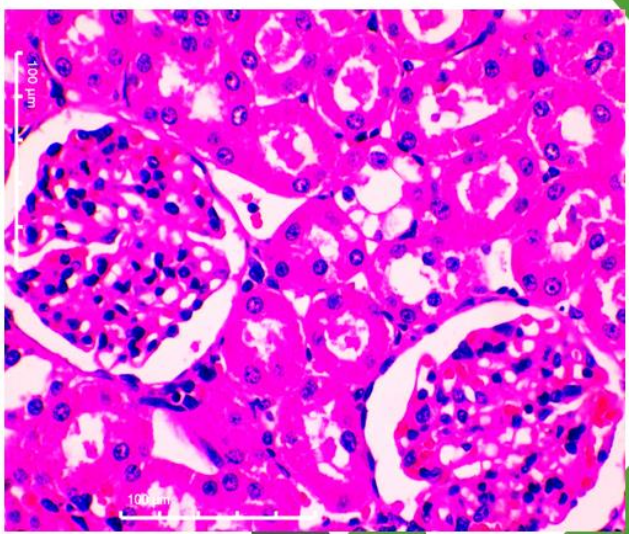
DC-Kidney (40X)



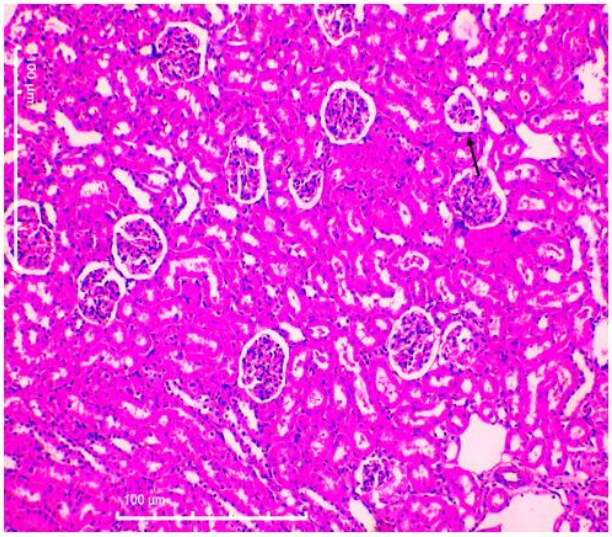
STD- Kidney (10X)



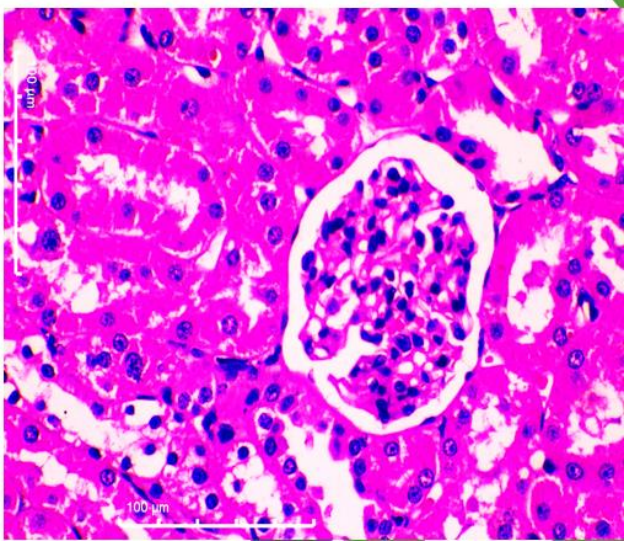
STD-Kidney (40X)



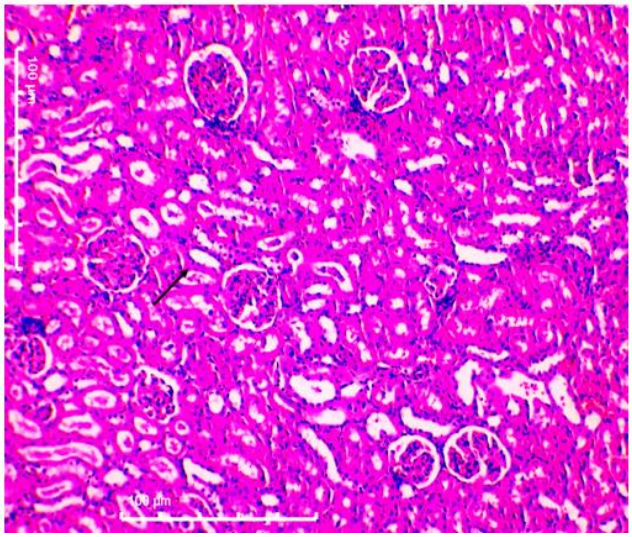
T1- Kidney (10X)



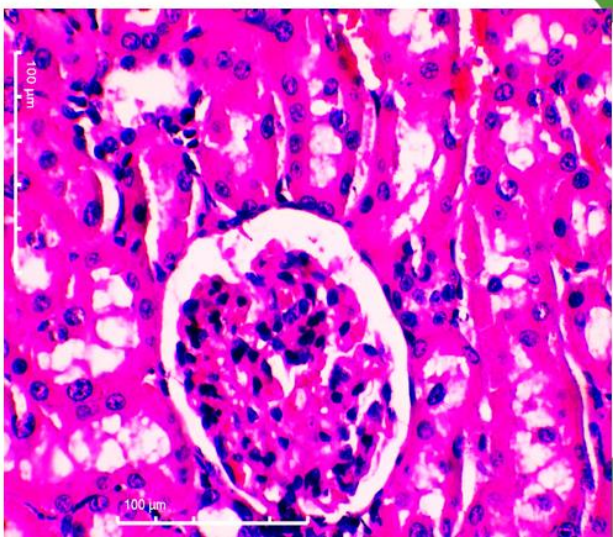
T1-Kidney (40X)



T2- Kidney (10X)



T2-Kidney (40X)



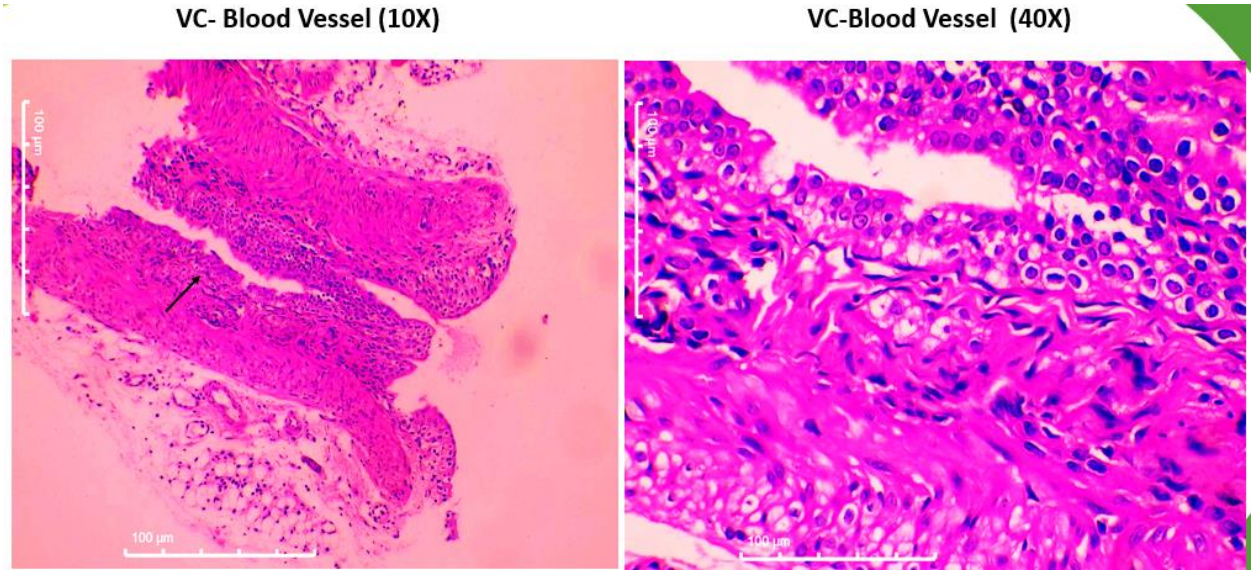
Sr. No.	Group and Animal Code No.	Histopathological Observations – Kidney			Overall Pathological grade/lesion score
		Vascular changes- Congestion / Hemorrhages in Renal tissue	Cellular changes / degenerative and necrotic changes of Renal Tubules and Glomerular changes	Inflammatory changes in Renal tissue	
1	VC	NAD	NAD	NAD	NAD
2	DC	NAD	Focal (+ 2)	Focal (+2)	Moderate (+2)

3	STD	NAD	NAD	NAD	NAD
4	T1	NAD	Focal (+ 1)	NAD	Mild (+1)
5	T2	Focal (+ 1)	NAD	NAD	Mild (+1)
Note : NAD =No Abnormality Detected, (+) Changes observed Grades: 1- Mild, 2- Moderate, 3- Severe					

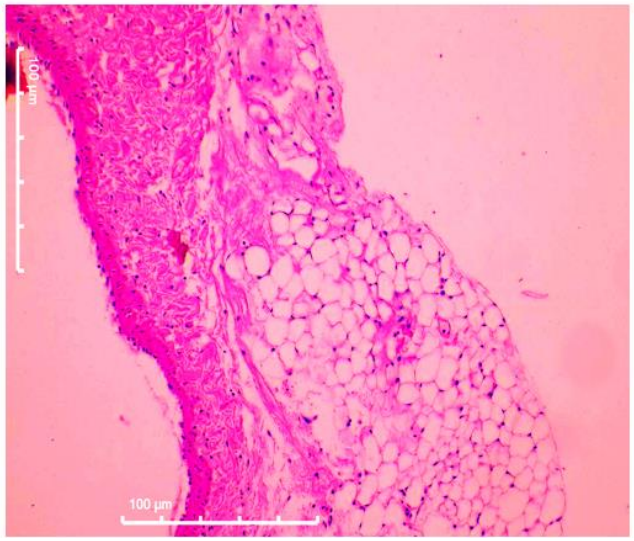
VC (Vehicle Control) and STD (Standard Drug) groups showed no abnormalities detected (NAD) in terms of vascular changes, cellular changes, or inflammatory changes. The DC (Diabetic Control) group exhibited focal degenerative and necrotic changes in renal tubules and glomerular changes, along with moderate inflammatory changes. T1 (Test Group 1) showed focal cellular changes and mild inflammatory changes, while T2 (Test Group 2) exhibited focal vascular changes and mild inflammatory changes.

These findings suggest that the anti-diabetic treatments administered to the STD, T1, and T2 groups did not cause significant adverse histopathological changes in the kidney tissue of the rats. In contrast, the diabetic condition in the DC group led to notable pathological changes, highlighting the potential protective effects of the treatments in preventing such changes.

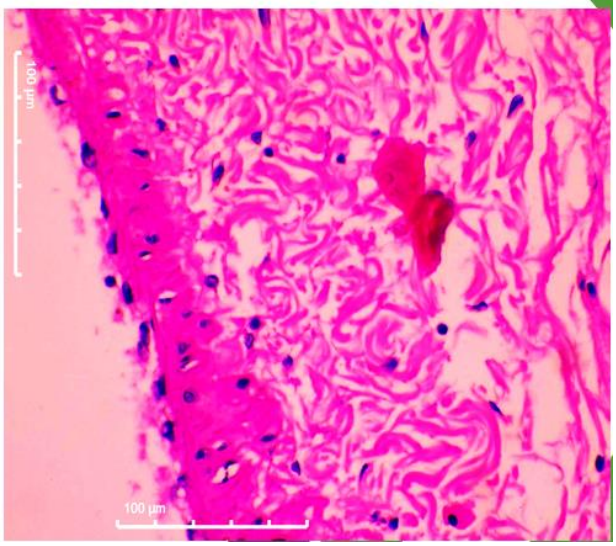
Blood Vessel:



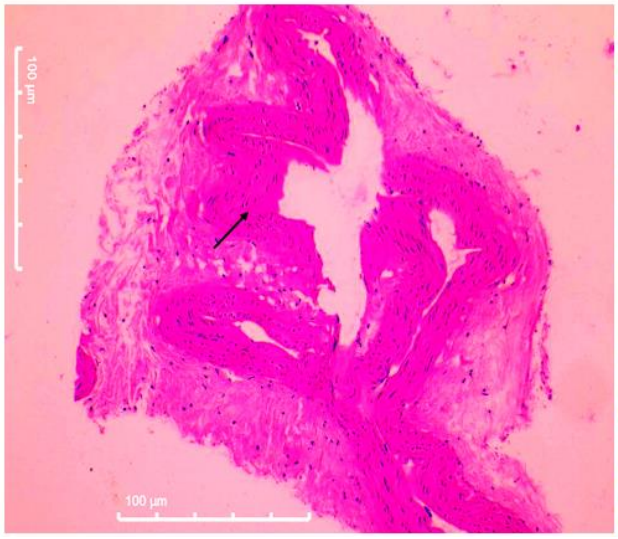
DC- Blood Vessel (10X)



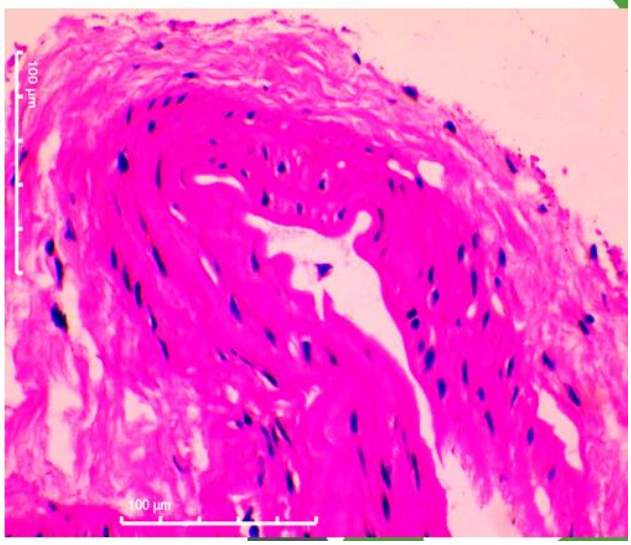
DC-Blood Vessel (40X)



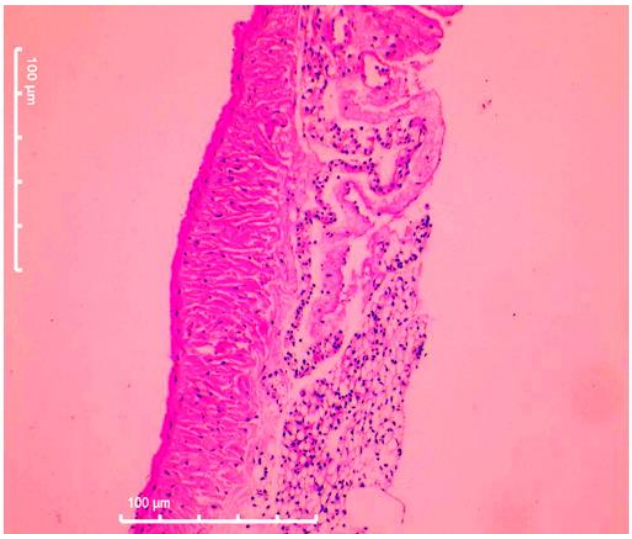
STD- Blood Vessel (10X)



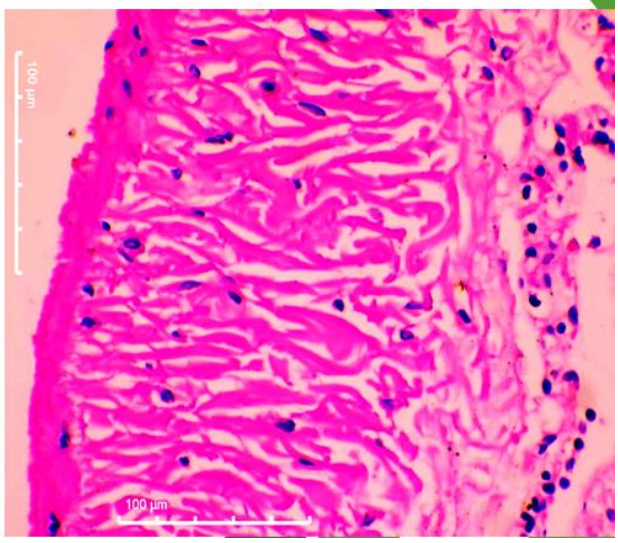
STD-Blood Vessel (40X)



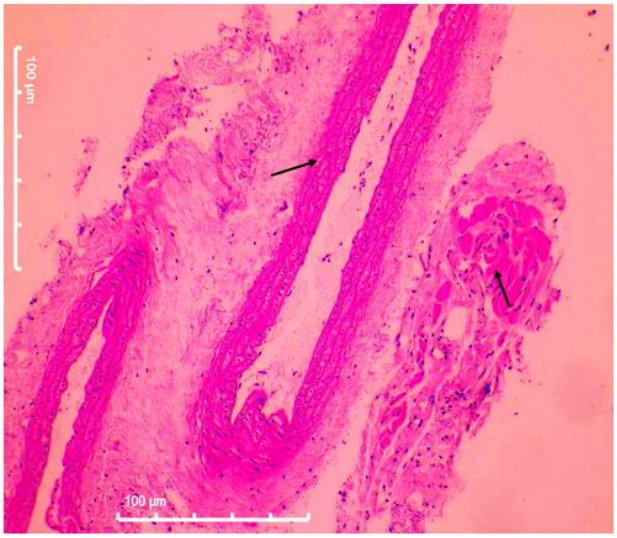
T1- Blood Vessel (10X)



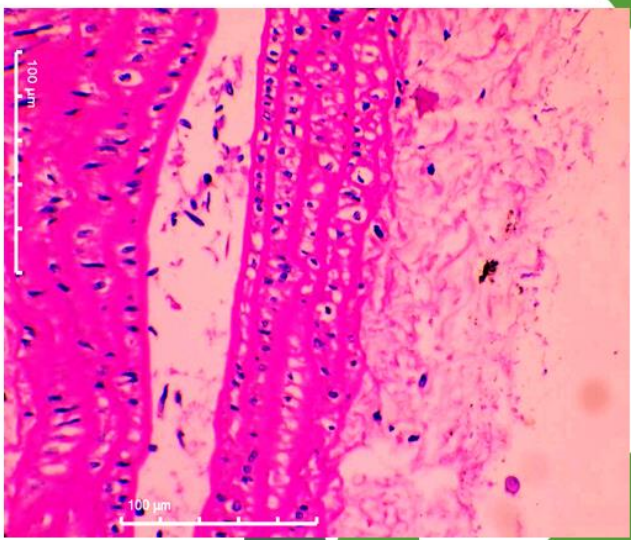
T1-Blood Vessel (40X)



T2- Blood Vessel (10X)



T2-Blood Vessel (40X)



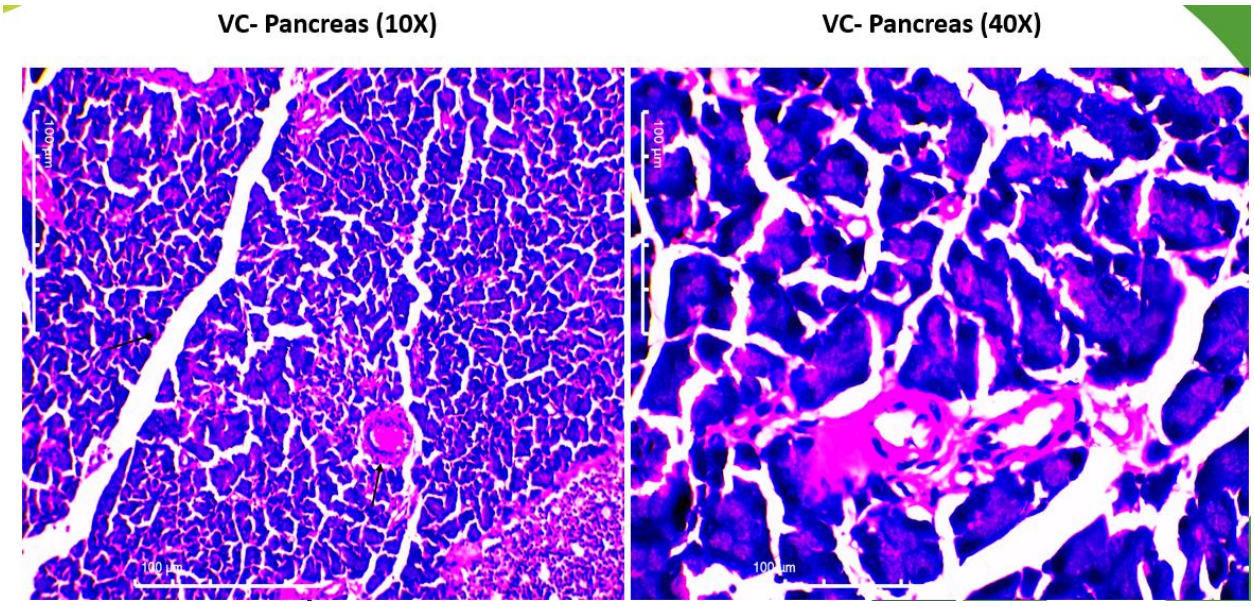
Sr. No.	Group and Animal Code No.	Histopathological Observations – Blood Vessel			Overall Pathological grade / lesion score
		Vascular changes- Congestion / Hemorrhages in vessel tissue	Cellular changes / degenerative and necrotic changes of Blood Vessel	Inflammatory changes in vessel tissue	
1	VC	NAD	NAD	NAD	**NAD**
2	DC	Focal (+2)	Focal (+2)	Focal (+1)	**Moderate (+2)**
3	STD	NAD	NAD	NAD	**NAD**

4	T1	NAD	NAD	NAD	**NAD**
5	T2	Focal (+ 1)	NAD	NAD	**Mild (+1)**
Note : NAD =No Abnormality Detected, (+) Changes observed Grades: 1- Mild, 2- Moderate, 3- Severe					

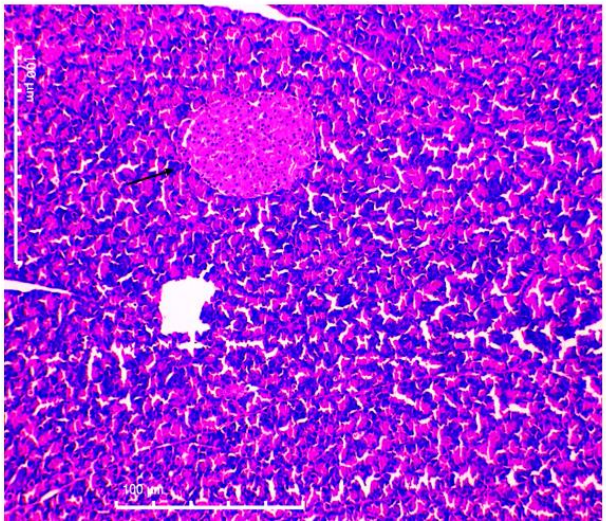
VC (Vehicle Control) and STD (Standard Drug) groups showed no abnormalities detected (NAD) in terms of vascular changes, cellular changes, or inflammatory changes. The DC (Diabetic Control) group exhibited focal vascular changes, including congestion or hemorrhages, and focal degenerative and necrotic changes, along with moderate inflammatory changes. T2 (Test Group 2) showed focal vascular changes and mild inflammatory changes, while T1 (Test Group 1) exhibited no abnormalities detected (NAD).

These findings suggest that the anti-diabetic treatments administered to the STD, T1, and T2 groups did not cause significant adverse histopathological changes in the blood vessel tissue of the rats. In contrast, the diabetic condition in the DC group led to notable pathological changes, highlighting the potential protective effects of the treatments in preventing such changes.

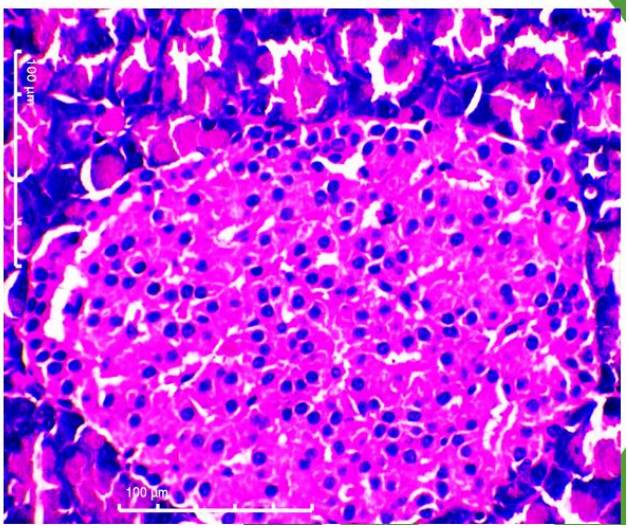
Pancreas:



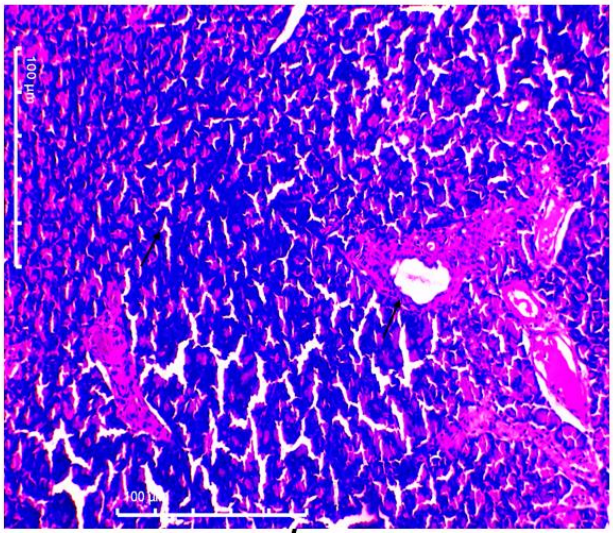
DC- Pancreas (10X)



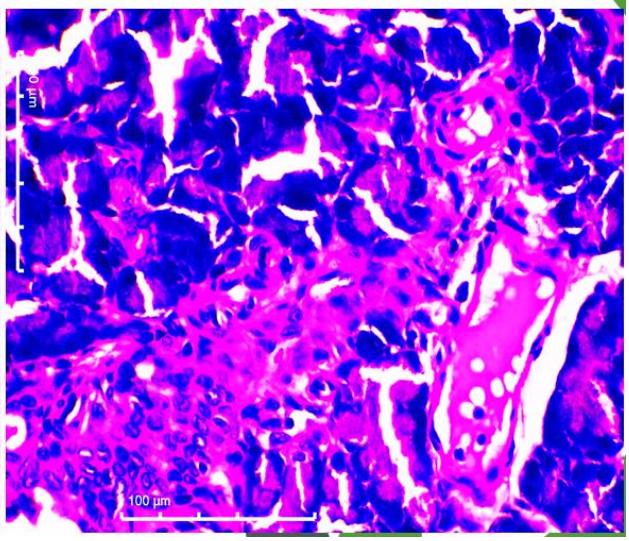
DC- Pancreas (40X)



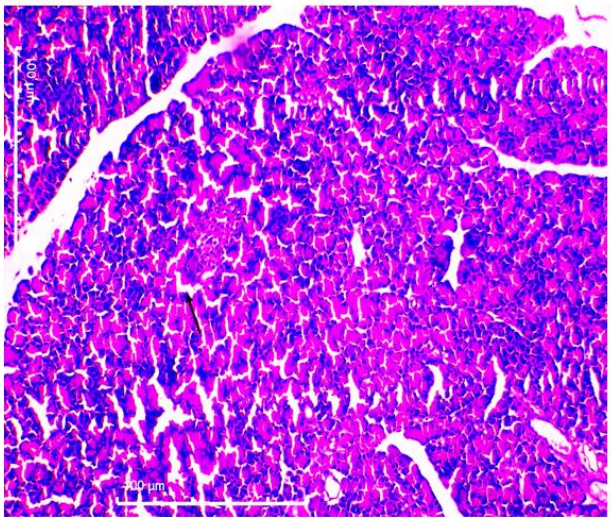
STD- Pancreas (10X)



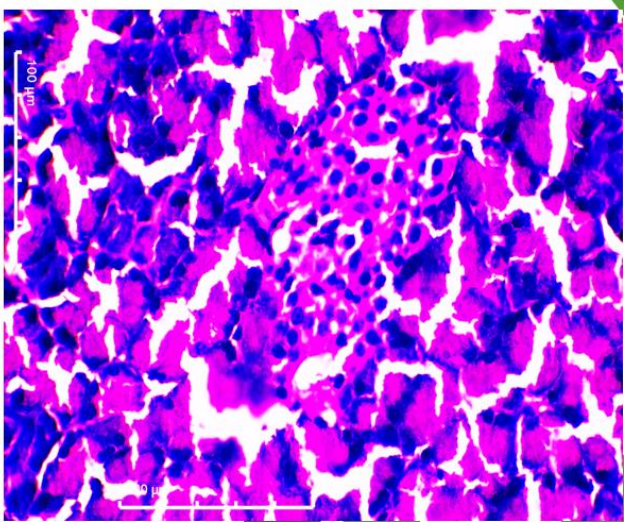
STD- Pancreas (40X)



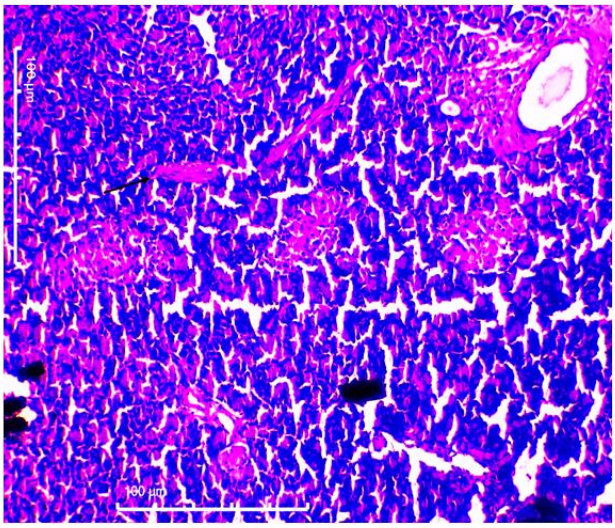
T1- Pancreas (10X)



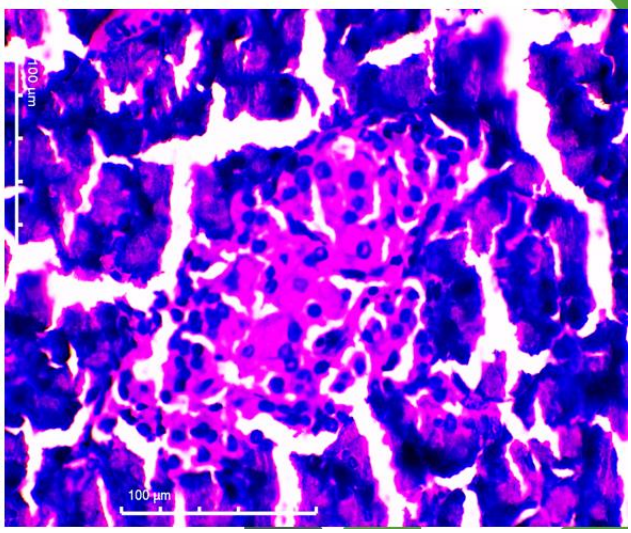
T1- Pancreas (40X)



T2- Pancreas (10X)



T2- Pancreas (40X)



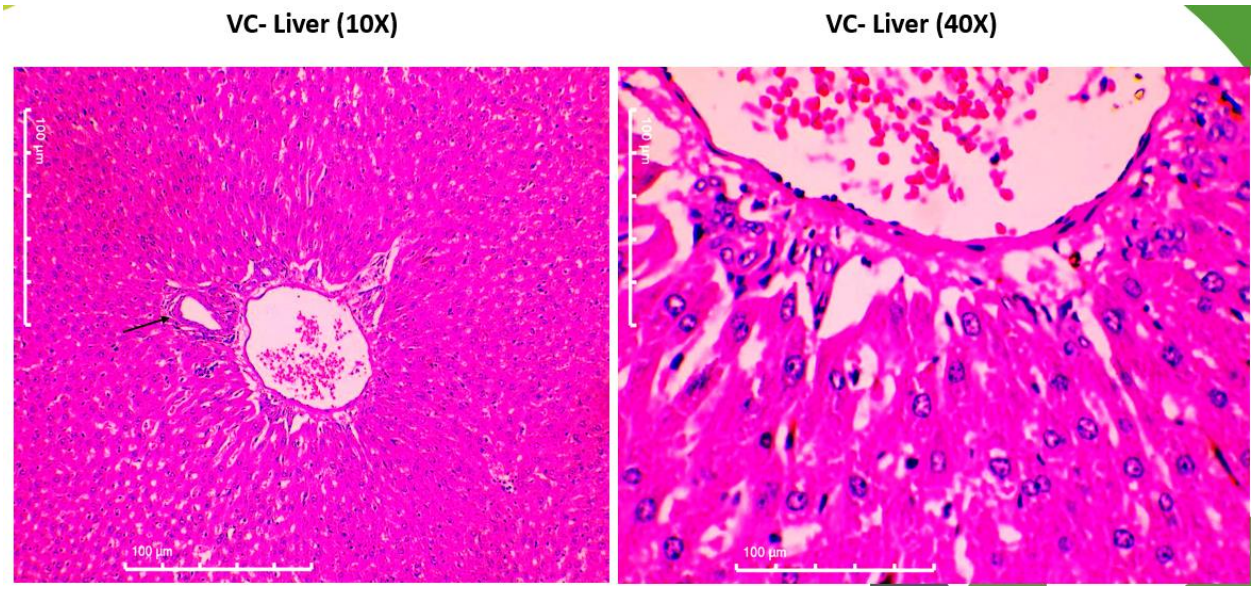
Sr. No.	Group and Animal Code No.	Histopathological Observations – Pancreas			Overall Pathological grade / lesion score
		Intact islets of the pancreas embedded in acinar cells and shrinkage of β-cells of islets of Langerhans	Reduction in the size of the islets of Langerhans and lymphocytic infiltration	Inflammatory changes and swelling in β-cells of islets of Langerhans	
1	VC	NAD	NAD	NAD	**NAD**
2	DC	Focal (+2)	Focal (+2)	Focal (+ 1)	**Moderate (+2)**
3	STD	NAD	NAD	NAD	**NAD**

4	T1	NAD	NAD	NAD	**NAD**
5	T2	NAD	Focal (+ 1)	NAD	**Mild (+1)**
Note : NAD =No Abnormality Detected, (+) Changes observed Grades: 1- Mild, 2- Moderate, 3- Severe					

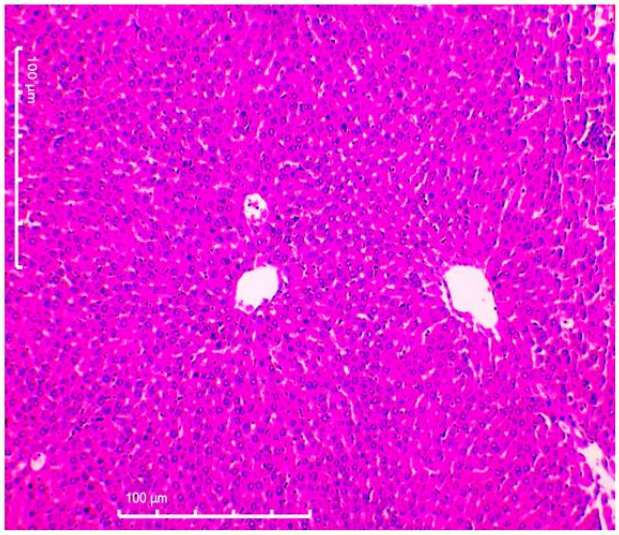
VC (Vehicle Control) and STD (Standard Drug) groups showed no abnormalities detected (NAD) in terms of intact islets, shrinkage of β-cells, reduction in the size of the islets, lymphocytic infiltration, or inflammatory changes. The DC (Diabetic Control) group exhibited focal shrinkage of β-cells, reduction in the size of the islets, lymphocytic infiltration, and moderate inflammatory changes. T2 (Test Group 2) showed focal reduction in the size of the islets and mild inflammatory changes, while T1 (Test Group 1) exhibited no abnormalities detected (NAD).

These findings suggest that the anti-diabetic treatments administered to the STD, T1, and T2 groups did not cause significant adverse histopathological changes in the pancreas tissue of the rats. In contrast, the diabetic condition in the DC group led to notable pathological changes, highlighting the potential protective effects of the treatments in preventing such changes.

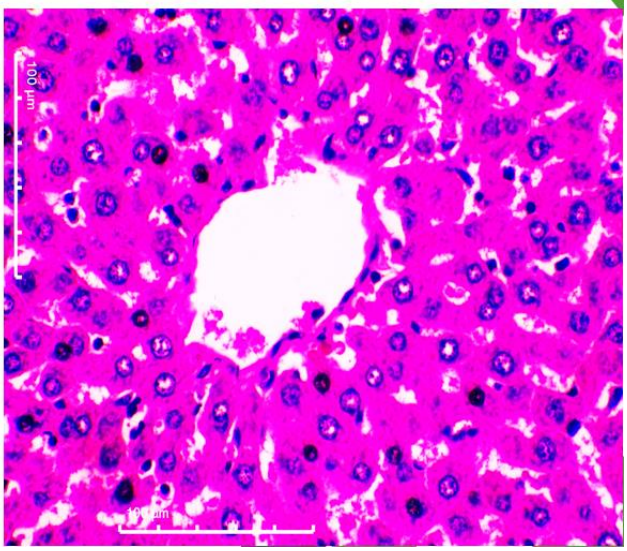
Liver:



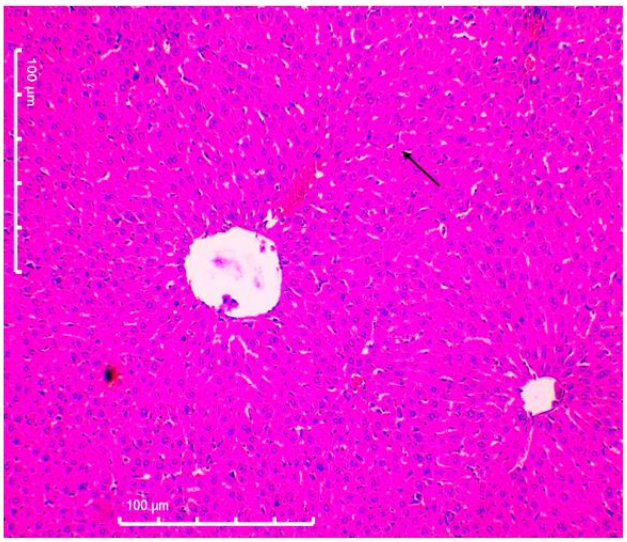
DC- Liver (10X)



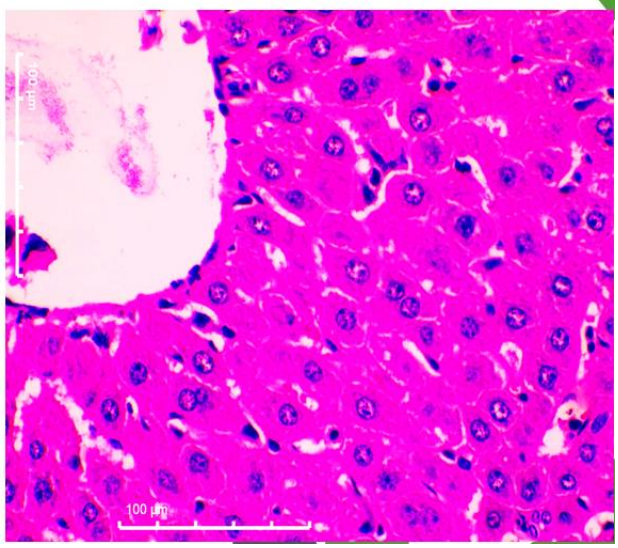
DC- Liver (40X)



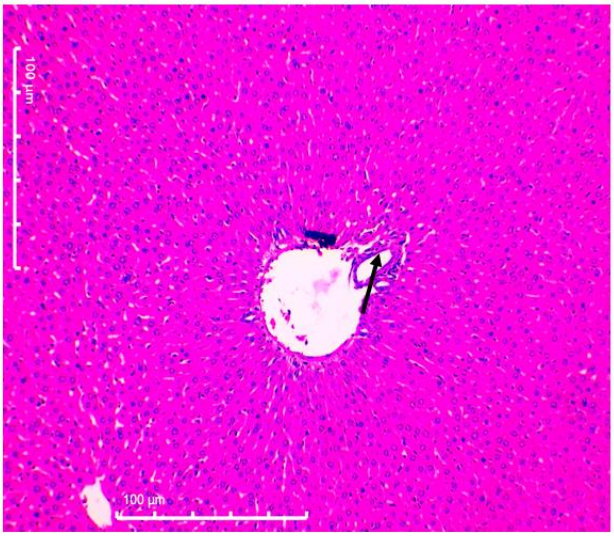
STD- Liver (10X)



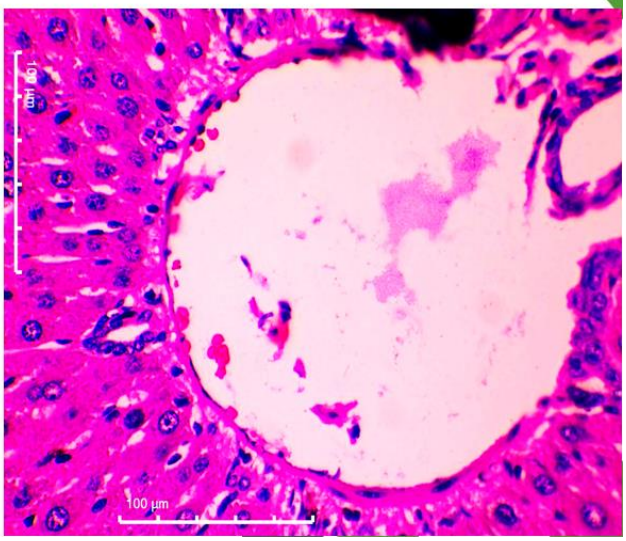
STD- Liver (40X)



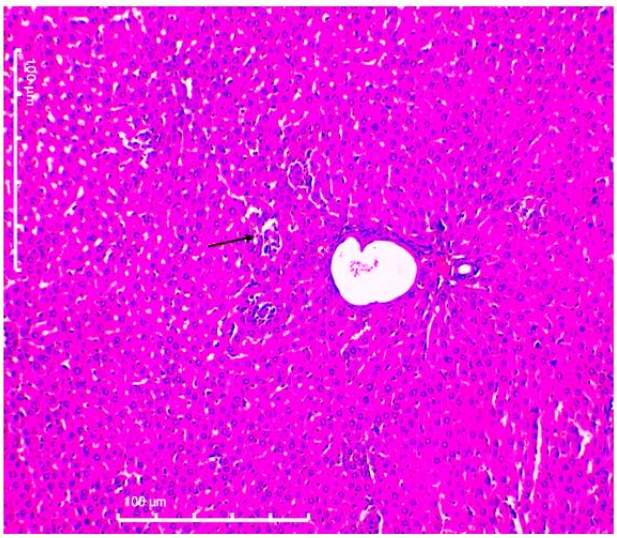
T1- Liver (10X)



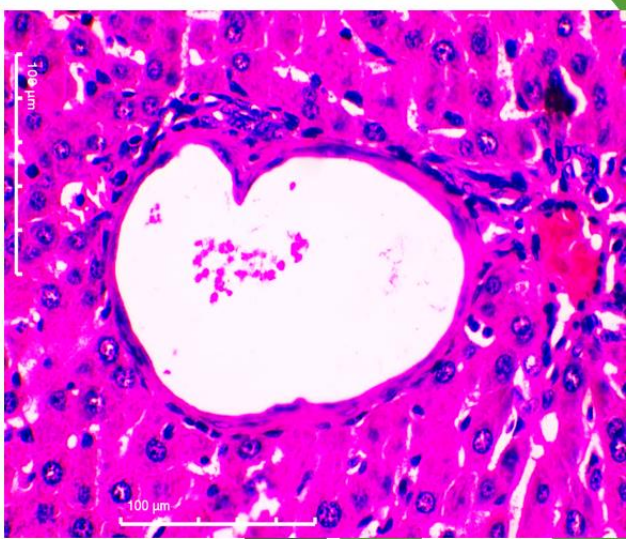
T1- Liver (40X)



T2- Liver (10X)



T2- Liver (40X)



Sr. No.	Group and Animal Code No.	Histopathological Observations – Liver			Overall Pathological grade / lesion score
		Vascular changes- Congestion / Hemorrhages in hepatic parenchyma	Cellular changes / degenerative and necrotic changes of hepatocytes	Inflammatory changes in hepatic tissue	
1	VC	NAD	NAD	NAD	NAD
2	DC	NAD	NAD	NAD	**NAD**
3	STD	NAD	NAD	NAD	**NAD**

4	T1	NAD	NAD	NAD	**NAD**
5	T2	Focal (+ 1)	NAD	NAD	**Mild (+1)**
Note : NAD =No Abnormality Detected, (+) Changes observed Grades: 1- Mild, 2- Moderate, 3- Severe					

VC (Vehicle Control) and T2 (Test Group 2) groups showed focal vascular changes, specifically congestion or hemorrhages, and mild inflammatory changes, but no other abnormalities were detected. The DC (Diabetic Control), STD (Standard Drug), and T1 (Test Group 1) groups showed no abnormalities detected (NAD) in terms of vascular changes, cellular changes, or inflammatory changes.

These findings suggest that the anti-diabetic treatments administered to the STD, T1, and T2 groups did not cause significant adverse histopathological changes in the liver tissue of the rats. In contrast, the diabetic condition in the DC group did not lead to any notable pathological changes, highlighting the potential protective effects of the treatments in preventing such changes.

Annexure:
Institutional Animal Ethics Committee Approval

CERTIFICATE

This is to certify that the project proposal no PCP/IAEC/2024/2- 8 entitled "Effect of Wellia-1 on Streptozotocin-Nicotinamide-induced diabetes mellitus in Wistar rats" submitted by Dr. Arulmozhi S. has been approved/recommended by the IAEC of Bharati Vidyapeeth (Deemed to be University), Poona College of Pharmacy in its meeting held on 30/04/24 (date) and 80 Wistar rats have been sanctioned under this proposal for a duration of next 1 year.

Authorized by	Name	Signature	Date
Chairman:	Dr A.P. Pawar	.....	30/4/24
Member Secretary:	Dr Urmila M. Aswar	.....	30/04/24
Main Nominee of CCSEA:	Dr Vijay Jagdale	.....	30/4/24

INSTITUTIONAL ANIMAL ETHICS COMMITTEE
Poona College of Pharmacy (BVDU)