IJBPAS, July, 2024, 13(7): 3585-3598

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFECT OF DAPTOMYCIN ON EXPERIMENTALLY INDUCED DIABETIC FOOT ULCER IN ANIMAL MODEL

PATIL PA[1] AND KAKADIYA JK[*2]

1: M. Pharm. Scholar, Department of Pharmacology, Parul Institute of Pharmacy and Research, Parul University, Vadodara, Gujarat, India

2: Associate Professor, Department of Pharmacology. Parul Institute of Pharmacy and Research, Parul University, Vadodara, Gujarat, India

***Corresponding Author: Mr. Prashant Patil: E Mail: pp9978485@gmail.com**

Received 15th March 2023; Revised 8th July 2023; Accepted 29th Oct. 2023; Available online 1st July 2024

https://doi.org/10.31032/IJBPAS/2024/13.7.8207

ABSTRACT

Daptomycin used as a treatment of Skin and soft tissue infections used in Diabetic foot ulcer.

Aim of the study: Daptomycin was investigated for its wound healing effects in a diabetic foot ulcer rat model.

Material and methods: To induce diabetic foot ulcers in Wistar rats, streptozotocin and Nicotinamide were utilized. Pentobarbitone sodium was used to anesthetize animals on day 7. On the dorsal surface of the foot, a rectangle was drawn, and the whole thickness of the skin (typical area 2–5 mm) was then excised. The ulcer area was calculated using ImageJ, a java-based programme.

Result: The additional change in wound area from day 1 to day 7 for the daptomycin treatment groups in comparison to the disease control group was calculated using ulcer area measurement information. In a study on microbial colonization, the daptomycin-treated group had very few colonies grow.

Conclusion: Following a seven-day course of daptomycin treatment, it is clear that the drug inhibits the development of significant consequences of diabetic foot ulcers. The study offers a rationale for daptomycin treatment in diabetic foot ulcers based on scientific evidence.

Keywords: Diabetic foot Ulcer, Ulcer area measurement, Wound healing

1. INTRODUCTION:

Diabetes is a chronic health condition that results in high levels of glucose in the blood.

This happens because the glucose cannot be processed by the cells due to either insufficient insulin production by the pancreas or the cells' inability to use the insulin effectively. This condition also causes disturbances in the metabolism of fats and proteins **[1-3]**.

Among the complications associated with diabetes, one of the most common is diabetic foot ulcers (DFU). This condition can be a source of discomfort due to its long-lasting nature, unattractive appearance, and negative psychological impact associated with having a persistent wound. As the condition worsens, it can also lead to difficulties with mobility, compounding the problem. Furthermore, amputation is a major potential consequence of DFU, resulting in permanent disability and the inability to carry out daily tasks. With the continued spread of diabetes, it is likely that DFU and similar complications will become more prevalent unless effective strategies are implemented at all levels. Therefore, it is crucial to educate and manage diabetes patients with a particular focus on foot care to prevent or detect DFU early on **[4]**.

Several studies have shown that DFU can result in various types of pathogenic infections, and S. aureus has been identified as the most frequently occurring pathogenic species in DFU. In a study involving 342 patients with diabetic foot infections, it was found that S. aureus accounted for 20.2% of isolates and was the most prevalent Gram-positive bacterium **[5]**. The development and spread of antibiotic resistance is making it more challenging to treat severe infections caused by Gram-positive bacteria. Antibiotics like penicillinase-resistant penicillin's, Vancomycin and teicoplanin are becoming less effective as a growing number of bacteria causing infections in hospitals are becoming resistant to these drugs **[6]**. The current antimicrobial drugs used to treat Gram-positive bacteria that have become resistant to other antibiotics have certain limitations from both a clinical and microbiological perspective. Linezolid is one such drug that is well-tolerated but can suppress bone marrow activity, leading to a risk of blood disorders. Therefore, treatment with linezolid requires close monitoring **[7]**.

The aim of this study is to evaluate the effect of daptomycin in a diabetic-induced foot ulcer animal model and also investigate its effect compared to linezolid.

2. MATERIAL AND METHODS:

Chemicals: Chemicals were procured from Sigma Aldrich, Mumbai.

Drugs: Daptomycin, Linezolid and Metformin are purchased from Vishal Chemist Tower, Vadodara.

Experimental Design:

ANIMALS:

The research conducted in this study involved using female Albino Wistar rats that were healthy and weighed between 170-220g. The Institutional Animal Ethics Committee (IAEC) of the Pharmacology department at Parul Institute of Pharmacy and Research approved all of the studies and procedures carried out in this study. Additionally, the Committee for the Control and Supervision of Experiments on Animals (CCSEA) granted permission for the research to be conducted.

Protocol No. PIPR 984/2022/02/14

Permitted Animals: 46

Animals were procured from **Jai Research Foundation, Vapi.**

HOUSING:

Prior to the start of the study, Albino Wistar rats were given seven days to adjust to their environment and were provided with standard pelleted rat food and water. The rats were housed in groups of three per cage in a controlled animal house environment where the temperature was maintained at 22±3ºC and humidity ranged from 30% to 70%. The rats were exposed to a 12-hour light and 12-hour dark cycle. Additionally, the rats were given access to R.O. drinking water through polypropylene water bottles with an SS spout, and were allowed to consume water as much as they desired (ad libitum).

ANIMAL GROUPINGS:

Animals were divided into 5 groups to check effect of Daptomycin on diabetic foot ulcer.

Table 2.1: Grouping of Animals

Sr. No.	Group	Drug	Dose	No. of Animals
1	Sham Control	Saline	0.5ml/Animal by Oral Route of Administration	6
2	Disease Control	Saline	0.5ml/Animal by Oral Route of Administration	10
3	Diabetic Control	Metformin	70mg/kg by Oral Route of Administration	10
4	Standard Drug	Linezolid + Metformin	50mg/kg By SC Route	10
5	Test Drug	Daptomycin + Metformin	12mg/kg By SC Route	10

INDUCTION:

Induction is done by two steps:

Step 1 Induction of Diabetes Mellitus

Streptozotocin and Nicotinamide could be used for the induction of diabetes mellitus in Albino Wistar Rats by an Intraperitoneal Route.

Step 2 Induction of wound Ulcer

Pentobarbitone Sodium was injected intraperitoneally to anaesthetize the rats. On the dorsal surface of the foot, a rectangle was marked, and after that, a complete thickness of

skin was removed.

Procedure:

1. Young healthy male Wistar rats weighing 170-220 g were used.
2. A single intraperitoneal dose of 60 mg/kg of STZ was used to establish type 2 diabetes mellitus in an animal model on day 1, followed by an injection of 120 mg/kg of NC 15 minutes later.
3. To induce ulcers, only rats with severe diabetes on day 7 (fasting blood glucose >250 mg/dl) will be used.
4. On the same day (Day 7), 70 mg/kg of pentobarbitone sodium was injected intraperitoneally to anaesthetize the rats.
5. A rectangle was drawn on the dorsal surface, and a full thickness (typical area 25 mm) layer of skin was then removed off.

Sham Control (Normal rats with no induction of diabetes only have ulcers induced.): Received vehicle for 7 days (Day 8 to Day 14)

Disease Control (Diabetic rats with ulcer induction): Received Vehicle for 7 days (Day 8 to 14)

Diabetic Control (Diabetic rats with ulcer induction): Received Metformin oral for 7 days (Day 8 to 14)

Test Control (Diabetic rats with ulcer induction): Received Daptomycin SC and Metformin oral for 7 days (Day 8 to 14)

Standard Control (Diabetic rats with ulcer induction): Received Linezolid SC and Metformin oral for 7 days (Day 8 to 14)

ESTIMATION PARAMETERS:

1. Ulcer Area Measurement:

The wound closure rate was assessed by tracing the wound on days 1 and day 7.

The steps were followed as given below for measurement of wound area:

1) The wound Images were clicked on day 1 and day 7.
2) The images were analysing using ImageJ (Java-based image processing program).

2. Microbial Colonization:

The procedure for microbial colonization can be complex and may involve multiple steps, depending on the specific sample and research.

The first step was to collect a sample from the site of interest. This involved swabbing the area.

The sample was then processed to isolate the microorganisms present. This involved culturing the sample on agar media, which could help to grow and identify the specific types of microbes present.

Once the microbes had been grown, the next step was to quantify the population. This involved counting the number of colonies present on a plate.

3. Cell Counting:

Cell counting is a common technique used in many biological and medical research applications to determine the number of cells in a sample. One of the most common methods of cell counting is the manual or automated cell count method. Here are the general steps involved in the manual cell count method.

STEP 1: Procedure for making slides from an agar plate colony:

1. Prepared a clean glass microscope slide and labeled it with the animal group name.
2. Used a sterile inoculating loop to gently scrape a small amount of the bacterial colony from the agar plate and transferred it onto the center of the microscope slide.
3. Moistened the colony with a drop of sterile water.
4. Heat-fixed the sample by passing the slide through the flame of a Bunsen burner or other heat source several times until the sample was completely dried.
5. Stained the sample with a suitable stain, such as crystal violet or Gram stain.
6. Washed the slide with distilled water to remove excess stain, and then blotted the slide dry with a clean paper towel or laboratory tissue.
7. Applied a drop of immersion oil to the stained area of the slide, and placed a cover slip over the sample.

STEP 2: Counting of Cell:

1. The sample under a microscope, using appropriate magnification and illumination
2. Positioned the sample in the field of view of the camera and adjusted the focus to ensure a clear image.
3. Then, the picture was analyzed under ImageJ (ImageJ is image processing tool for accurate counting of sample data).

4. Blood Glucose Level:

On Day 0, the animal model of type 2 diabetes mellitus was induced by a single intraperitoneal injection of 60 mg/kg of STZ, and afterwards, 120 mg/kg of NC was injected after 15 minutes.

On Day 3, blood samples were collected from the tail vein of the rat, and glucose levels were determined to confirm the development of diabetes. Only the animals that showed hyperglycemia (blood glucose level >250 mg/dl) were used in the experiment. The diabetic rats were randomly divided into four groups, each consisting of six rats. Blood glucose levels were estimated using the Dr. Morepen BG-03 Gluco One Glucometer.

On Days 7 and 14, blood samples were collected from the tail vein of the rat, and glucose levels were determined.

5. Histopathological Evaluation:

Skin was obtained by sacrificing animals. The

skin was cleaned with pH 7 buffer solutions before being buffered in 10% formalin solution for 24 hours. The skin was delivered to Vadodara Clinical Laboratories (VCL), Vadodara, after 24 hours for histological analysis.

3. RESULT:

1. Ulcer Area Measurement:

Ulcer Area Measurement on Day 1 and Day 7 **(Figure 3.1)**.

2. Microbial Colonization:

The procedure for microbial colonization can be complex and may involve multiple steps, depending on the specific sample and research **(Figure 3.3)**.

3. Cell Counting:

Cell counting is a common technique used in many biological and medical research applications to determine the number of cells in a sample. One of the most common methods of cell counting is the manual or automated cell count method. Here we used automated method of cell count by ImageJ tool **(Figure 3.4)**.

Day 1	Groups of Animal	Day 7
	**SHAM CONTROL**	
	**DISEASE CONTROL**	

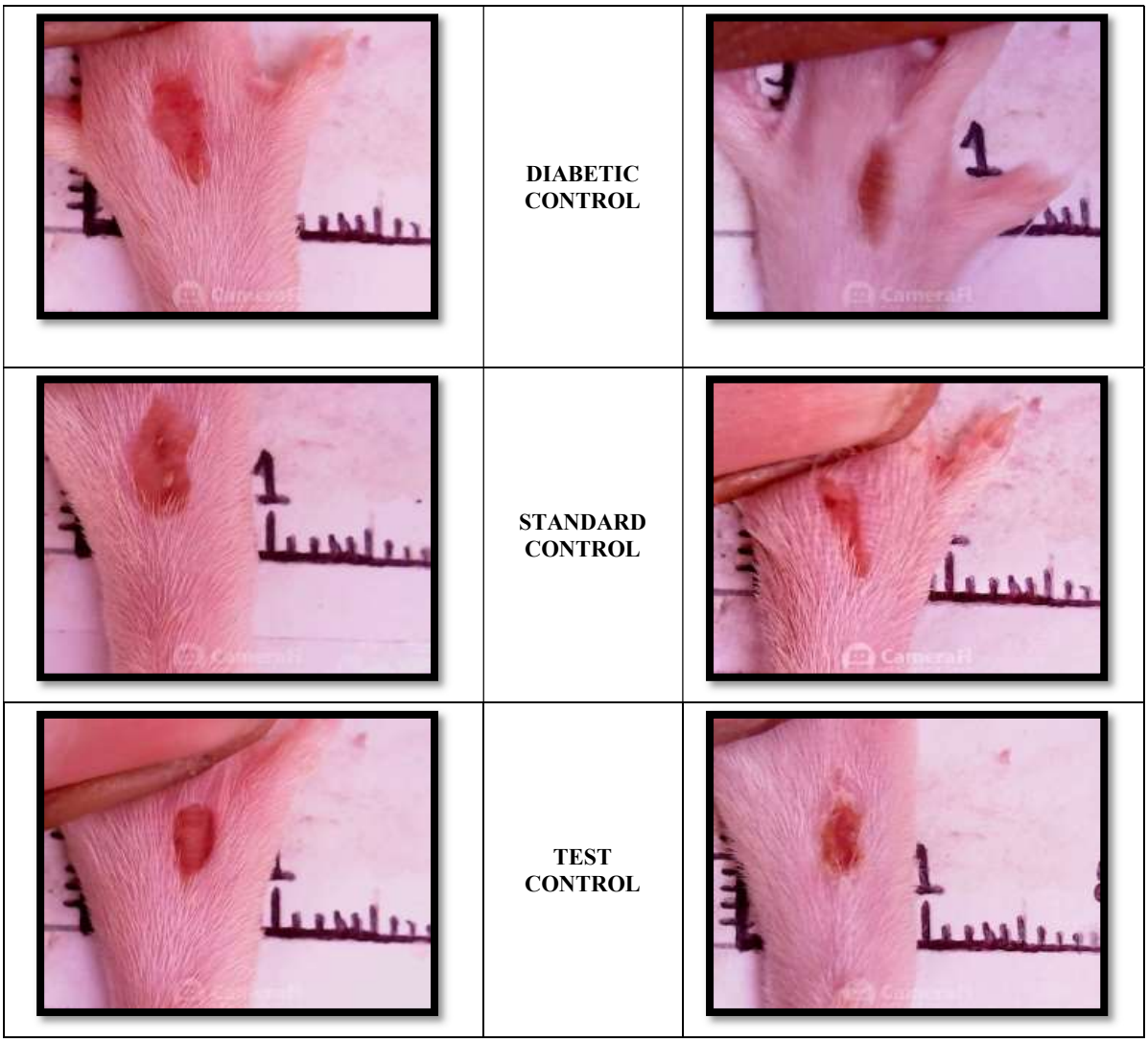


Figure 3.1: Ulcer Area Measurement

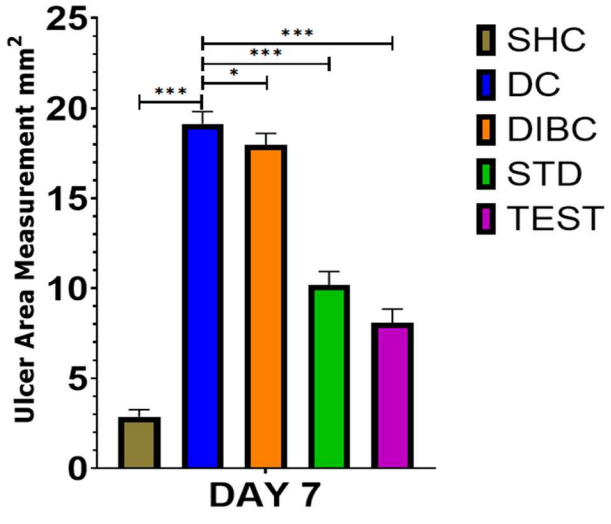


Figure 3.2: Effect of daptomycin on Ulcer Area Measurement in experimentally induce diabetic foot ulcer in Wistar rats

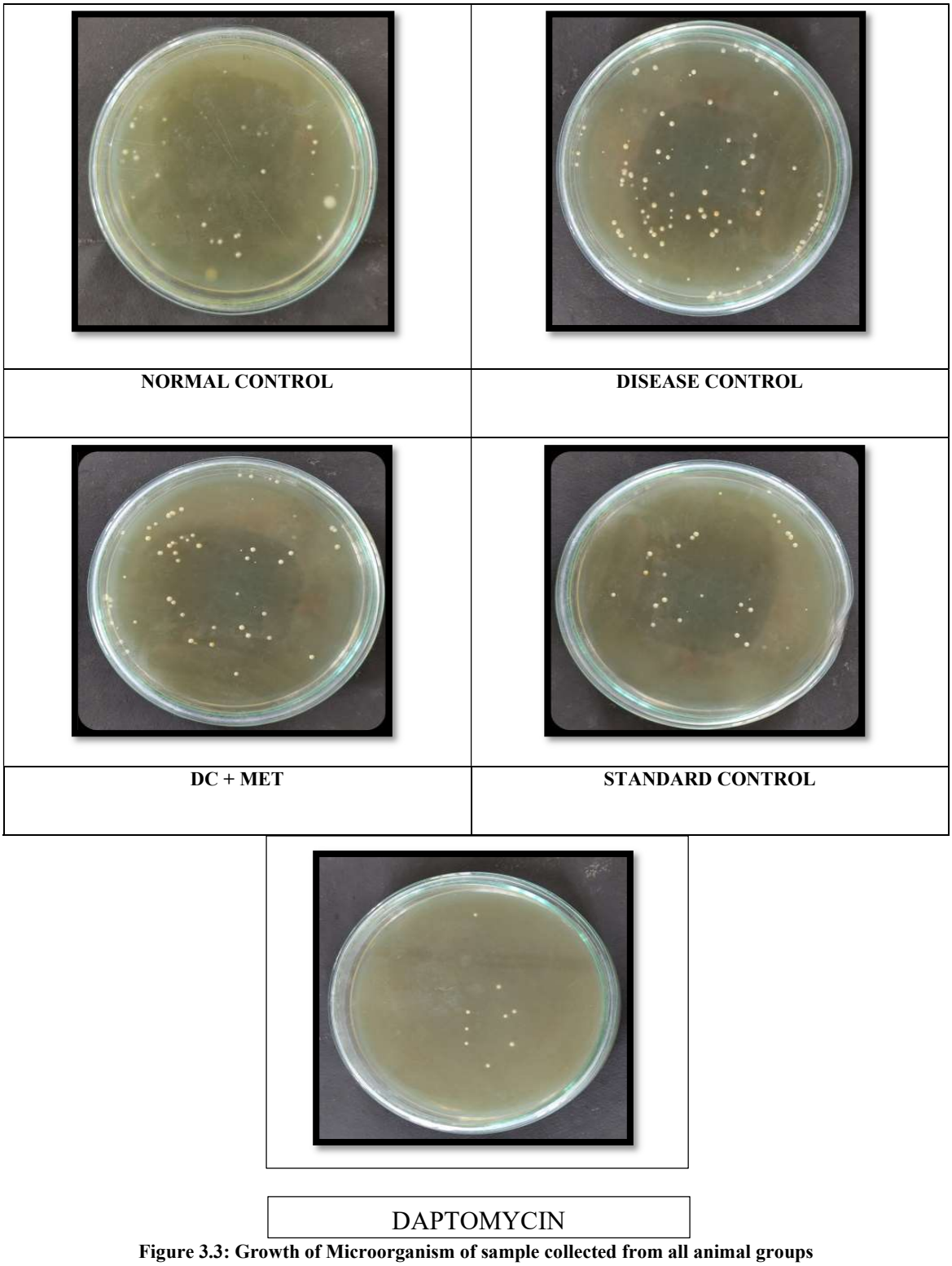
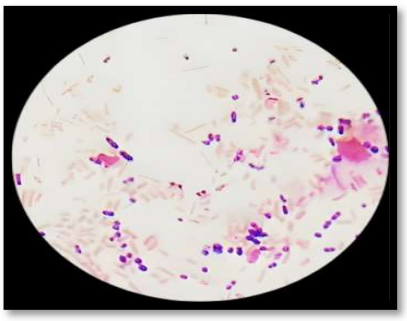
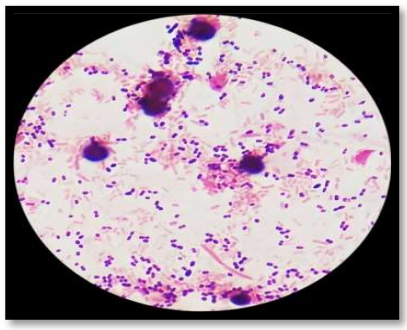


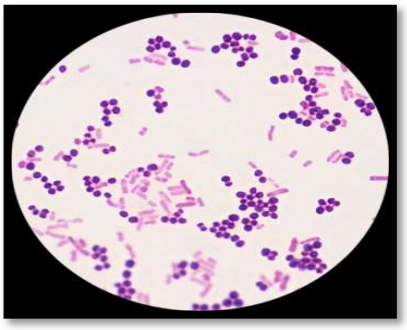
Figure 3.3: Growth of Microorganism of sample collected from all animal groups



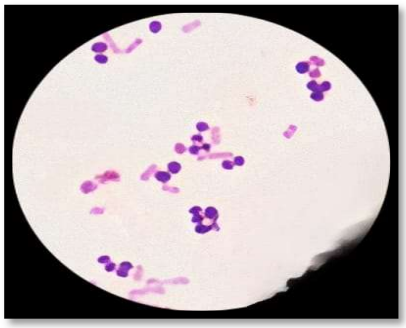
SHAM CONTROL



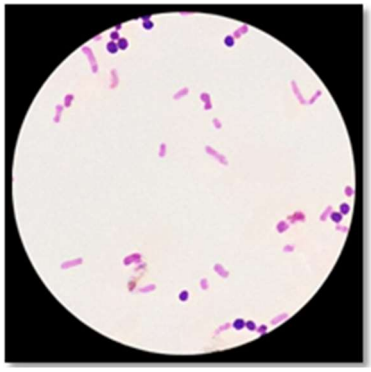
DISEASE CONTROL



DC + MET



STANDARD CONTROL



DAPTOMYCIN

Figure 3.4: Gram staining of sample collected from all animal groups

Table 3.1: Cell Counting of Gram positive and Gram Negative bacteria by ImageJ

	SHAMC	DC	DC + MET	STD	DAPTOMYCIN
Gram Positive Bacteria	49	369	171	25	12
Gram Negative Bacteria	87	407	105	16	25

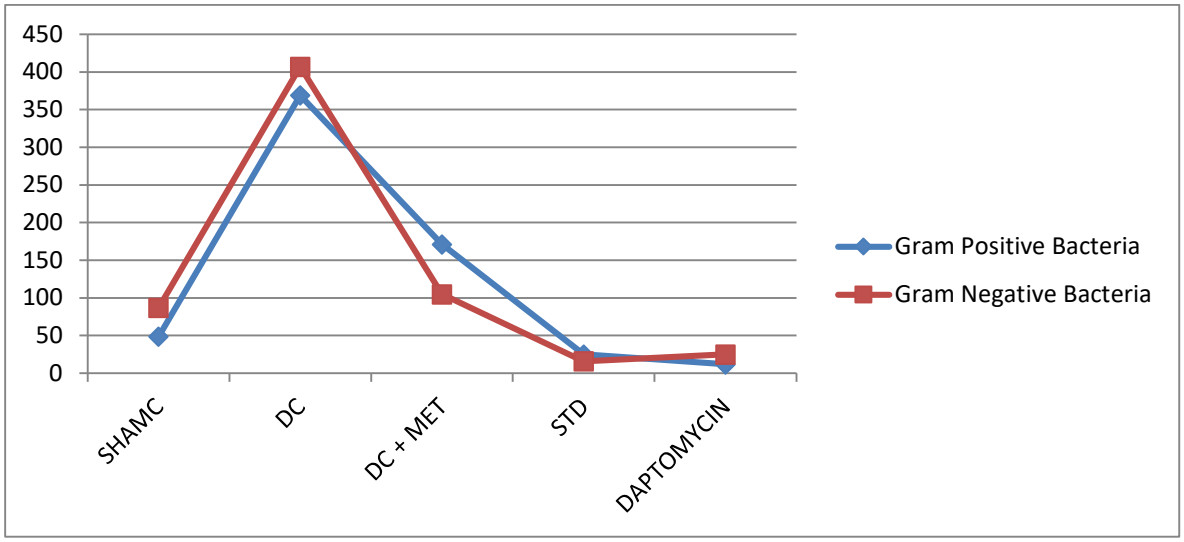


Figure 3.5: Gram staining of sample collected from all animal groups

4. Blood Glucose Level:

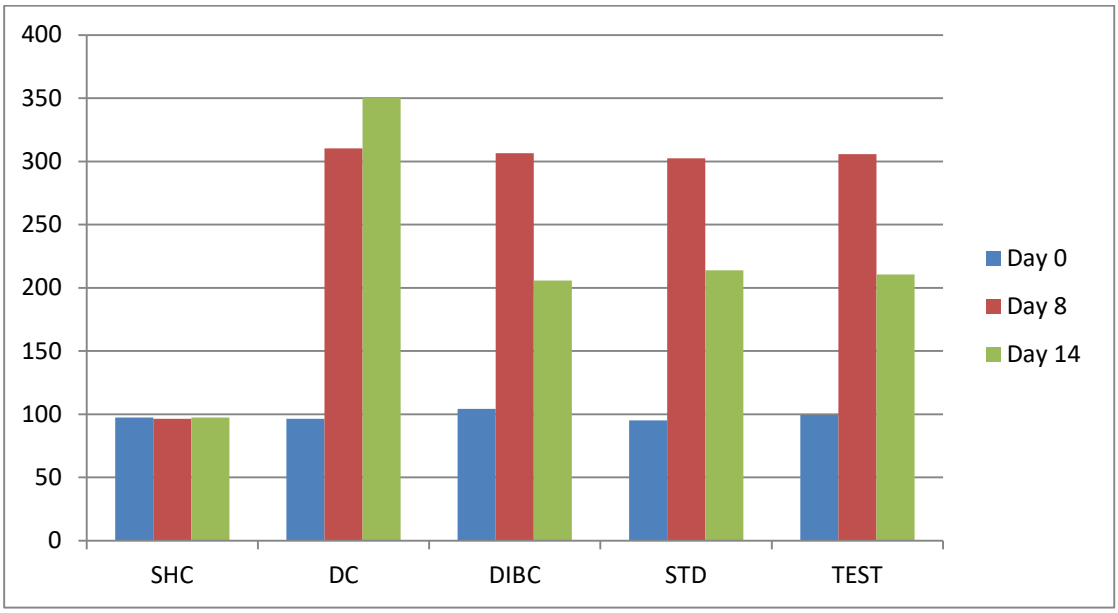
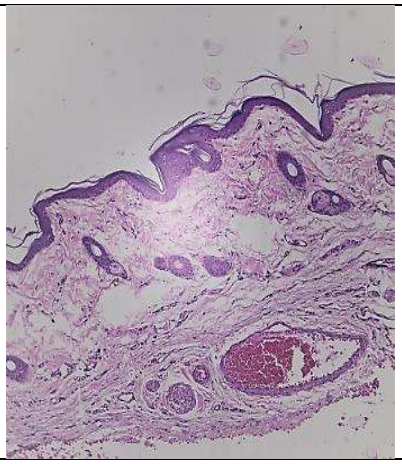
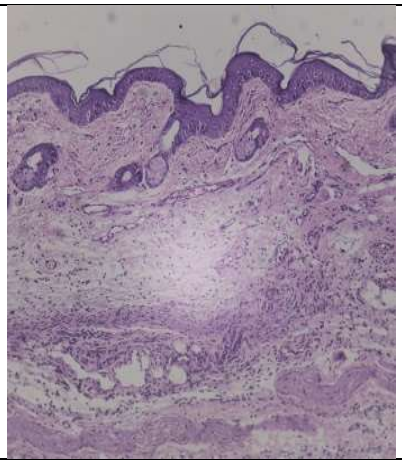


Figure 3.6: Effect of Daptomycin on Blood glucose level in experimentally induced diabetic foot ulcer in rats

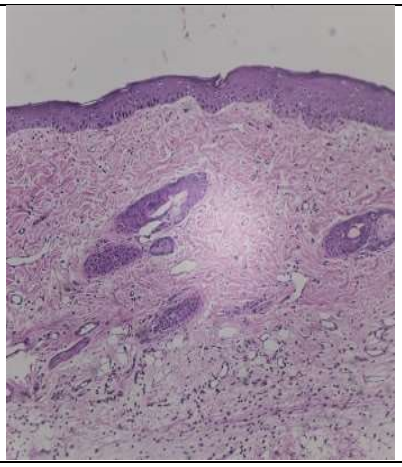
5. Histopathological Evaluation:



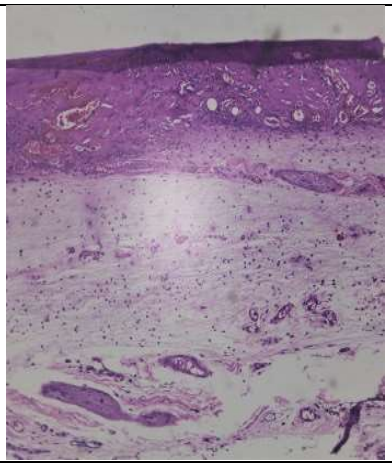
SHAM CONTROL



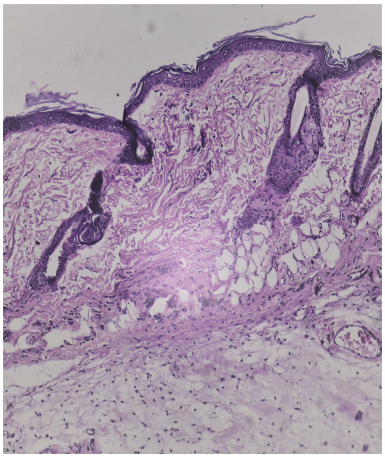
DISEASE CONTROL



DIABETIC CONTROL



STANDARD CONTROL



DAPTOMYCIN TREATED

Figure 3.7: Histopathological investigation

6. DISCUSSION:

Typically, surgical ulcers are induced on the chest or back of rats or mice for the study of wound healing and its measurement method. However, inducing ulcers on the feet for this purpose is very rare. In an attempt to more closely mimic the clinical condition of diabetic foot ulcers, the study induced ulcers on the feet of rats with STZ-induced diabetes **[8-9]**. The ulcer was created by removing the full thickness of the skin, and it should be noted that this artificial ulcer differs from the ulcer in human diabetic feet, which usually develops due to local pressure **[10-11]**.

Ulcer area measurement is a technique used to evaluate the progress of wound healing, and there are various methods available for determining wound area. Some modern methods rely on specialized software to detect the wound area from a manually traced photograph. In the study's animal model, ImageJ a new image processing program based on Java was utilized for this purpose.

The study's sham control group showed a significant decrease in wound size, while the disease control group displayed an increase in wound size and disease progression. The group treated with only metformin had a slightly decreased ulcer area. Ulcer area measurement values were used to determine the additional change in wound area from day 1 to day 7 for the daptomycin and Linezolid treatment groups relative to the disease control group. The results indicated that daptomycin had a greater effect than Linezolid in reducing wound area changes.

The microbial colonization study indicates different animal groups shows difference in no. of colonies grow on culture media plate. In which daptomycin treated group having very few colonies grows.

Cell counting is done by ImageJ program. The result demonstrated that Daptomycin exhibited significantly lower bacterial counts compared to the other groups in the cell counting experiment.

The Histopathological evaluation shows very good wound recovery, high Re-epithelization and Good skin formation. Disease control group shows No wound recovery, No re-epithelization and No skin Formation. The diabetic control, Linezolid treated and Daptomycin treated group show wound recovery, Re-epithelization and skin formation better than disease control but less than that of Sham control group.

6. CONCLUSION:

The results indicated that daptomycin had a greater effect than linezolid in reducing wound area changes. The microbial colonization study indicates the daptomycin-treated group has very few colonies growing.

Cell counting results demonstrated that daptomycin exhibited significantly lower bacterial counts. Based on the results, we conclude that after 7 days of treatment with daptomycin, it demonstrates that daptomycin resists diabetic foot ulcers. We need further study to know the exact mechanism of action of daptomycin on diabetic foot ulcers.

Acknowledgement: First of all, give thanks to the Almighty for everything. I want to convey my sincere thanks to Parul University, my mentor Dr. Jagdish Kakadiya, and my entire family for their advice, passionate support, and insightful criticism of this effort. Last but not least, I want to express my sincere gratitude to my family and parents for their support and encouragement during my studies.

Funding: This work has been partially funded by Parul Institute of Pharmacy & Research, Parul University, Vadodara, Gujarat, India.

Ethics approval: The protocol was conducted in accordance with guidelines of CCSEA.

Protocol Number: PIPR 984/2022/02/02.

Conflict of Interest: There is no conflict of interest between Authors.

REFERENCE:

[1] Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB. The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. Journal of physiology and pathophysiology. 2013;4(4):46-57.

[2] Zaccardi F, Webb DR, Yates T, Davies MJ. Pathophysiology of type 1 and type 2 diabetes mellitus: a 90-year perspective. Postgraduate medical journal. 2016; 92(1084):63-9.

[3] Baynes HW. Classification, pathophysiology, diagnosis and management of diabetes mellitus. J diabetes metab. 2015;6(5):1-9.

[4] Dahiru IL, Amaefule KE, Okpe IO, Ibrahim A, Muazu SB. An overview of diabetic foot disease. Nigerian Journal of Basic and Clinical Sciences. 2016 Jan 1;13(1):1.

[5] Tascini C, Piaggesi A, Tagliaferri E, Iacopi E, Fondelli S, Tedeschi A, Rizzo L, Leonildi A, Menichetti F. Microbiology at first visit of moderate-to-severe diabetic foot infection with antimicrobial activity and a survey of quinolone monotherapy. Diabetes research and clinical practice. 2011 Oct 1;94(1):133-9.

[6] Tedesco KL, Rybak MJ. Daptomycin. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2004 Jan;24(1):41-57.

[7] Joint Formulary Committee. British National Formulary. 50. London: British Medical Association and Royal

Pharmaceutical Society of Great Britain, 2005.

[8] Ahn ST, Mustoe TA. 1990. Effects of ischemia on ulcer wound healing: a new model in the rabbit ear. Ann Plast Surg 24:17–23.

[9] Lau, T.W., Sahota, D.S., Lau, C.H., Chan, C.M., Lam, F.C., Ho, Y.Y., Fung, K.P., Lau, C.B.S., Leung, P.C. 2008. An in vivo Investigation on the Wound-Healing Effect of two Medicinal Herbs Using an Animal Model with Foot Ulcer Eur Surg Res. 41:15-23

[10] Lau, T. W., Lam, F. F. Y., Lau, K. M., Chan, Y. W., Lee, K.M., Sahota, D. S., Ho, Y. Y., Fung, K. P., Leung, P. C., and Lau, C. B. S. 2009. Pharmacological investigation on the wound healing effects of Radix Rehmanniae in an animal model of diabetic foot ulcer. Jo urnal of Ethnopharmacology. 123: 155-162.

[11] Thawer, H. A., Houghton, P. E., Woodbury, M. G., Keast, D., and Campbell, K. 2002. A comparison of computer-assisted and manual wound size measurement, Ostomy Wound Management, 48, 46-53