Open Access Research Paper

ISSN: 2349-9818
July - August 2023, Vol. 10 (4), 12-25

Tropical Journal of Pharmaceutical and Life Sciences
(An International Peer Reviewed Journal)
Journal homepage: http://informativejournals.com/journal/index.php/tjpls

Wound Healing Activity of Standardized Bovin Colostrum Derivatives in Diabetic Rats

Rohit P. Mali[1]*, Rakesh Kumar Jat[2], Vijay Wagh[3]

[1,3]N.G.S.P.M.s College of Pharmacy, Brahma Valley Educational Campus, Anjaneri, Nashik-422213, Maharashtra, India

[2]Institute of Pharmacy, Shri. J.J.T. University, Chudela, Jhunjhunu, Rajasthan-333010, India

ARTICLE INFO:

Received: 12th July 2023**; Received** in revised form: 24th July 2023**; Accepted:** 9th August 2023**; Available online:** 27th August 2023.

Abstract

Diabetic wound healing remains a significant clinical challenge due to impaired cellular and molecular processes inherent in diabetes. This study investigates the potential therapeutic effect of Standardized Bovine Colostrum Derivatives (SBCD) on cutaneous wound healing in a diabetic rat model. The research aims to address the limited therapeutic options available for diabetic wound management and to explore the potential of SBCD as an innovative approach. A comprehensive literature review establishes the context of the study, highlighting the complexities of diabetic wound healing and the need for novel interventions. The wound healing process involves intricate interactions between growth factors, cytokines, and cellular activities, which are disrupted in diabetic conditions. Existing treatments often fall short in achieving desired outcomes.

The methodology employed a well-designed experimental protocol involving diabetic rats divided into SBCD-treated and control groups. Wounds were created under controlled conditions, and SBCD was administered to the treatment group. The progression of wound healing was monitored through wound size measurements and histological analysis. Statistical techniques were employed to evaluate the significance of differences observed. The results indicate that SBCD administration positively influences wound healing in diabetic rats. Comparative analysis of wound size reduction between the treatment and control groups demonstrates promising outcomes. Histological examination further elucidates the cellular changes associated with SBCD treatment, emphasizing its potential in addressing diabetic wound healing challenges.

The discussion interprets these findings within the context of existing literature, proposing potential mechanisms through which SBCD may accelerate wound healing. The implications of this research extend to clinical practice, where innovative treatments are urgently needed to mitigate the burden of diabetic wound

***Corresponding Author:**
Rohit P. Mali
N.G.S.P.M.s College of Pharmacy,
Brahma Valley Educational Campus,
Anjaneri, Nashik- 422213, Maharashtra, India

complications. While the study presents valuable insights, it acknowledges limitations and suggests avenues for further investigation.

In conclusion, this research contributes to the understanding of diabetic wound healing and introduces Standardized Bovine Colostrum Derivatives as a potential therapeutic option. By bridging the gap between existing knowledge and innovative treatment strategies, this study lays the foundation for future research and clinical applications, ultimately offering renewed hope for effective diabetic wound management.

Keywords: Diabetic wound healing, Standardized bovine colostrum derivatives, Wound size reduction, Histological analysis, Innovative treatments.

Introduction

Diabetic wound healing poses a significant challenge in modern healthcare, characterized by delayed and compromised tissue repair processes. Chronic wounds in individuals with diabetes are a substantial clinical burden, leading to increased morbidity, mortality, and healthcare costs. The intricate interplay of altered growth factors, impaired cellular responses, and compromised immune functions contributes to the impaired wound healing observed in diabetic individuals [1][2]. Despite advances in wound care, the management of diabetic wounds remains inadequate, necessitating innovative therapeutic interventions.

Wound healing is a complex, well-orchestrated process involving a sequence of cellular and molecular events aimed at restoring tissue integrity and function [3]. However, in diabetes, chronic hyperglycemia engenders detrimental effects on various phases of wound healing, including inflammation, granulation tissue formation, re-epithelialization, and remodeling [4]. These impairments can result in non-healing ulcers, infection, and potential amputations [5]. As conventional approaches have shown limited efficacy in addressing these challenges, there is an imperative to explore novel strategies that target the underlying mechanisms of diabetic wound healing.

Standardized Bovine Colostrum Derivatives (SBCD) have recently garnered attention for their potential therapeutic properties. Colostrum, the first milk produced by mammals shortly after giving birth, is rich in bioactive compounds, including growth factors, cytokines, and immunoglobulins [6]. These constituents are essential for the early growth and development of neonates, and they also hold promise for wound healing applications in adults [7]. Previous studies have demonstrated the potential of colostrum-derived components in accelerating wound closure, modulating inflammation, and promoting tissue regeneration [8][9].

The present study aims to investigate the wound healing activity of Standardized Bovine Colostrum Derivatives in a diabetic rat model. By assessing its effect on wound size reduction and histological characteristics, we aim to contribute to the growing body of knowledge regarding innovative therapeutic approaches for diabetic wound management. This research intends to bridge the gap between the complexities of diabetic wound healing and the potential benefits offered by colostrum-derived compounds, offering new insights into the development of effective interventions.

Methodology

Standardized Bovine Colostrum Derivative Preparation

Between the first and fifth hours following delivery, colostrum was collected and stored in 3-L bottles at 20°C with or without preservatives (phenoxyethanol 2.5 percent and diazolidinyl urea 1 percent). The next phases of the extraction procedure were completed on 100 L stocks for each preparation to minimize individual variability. First, a 1:10 dilution of colostrum in deionized water was performed. Sodium chloride (Sigma-Aldrich, St. Louis, MO) was added to reach a concentration of 0.9%. The lipidic phase pellet was then removed after centrifuging the sample at 12,400 g for 20 to 25 minutes at 20 to 25°C.In order to remove the preservatives, as measured by HPLC, the supernatant was dialyzed using a 5-kDa polyethersulfone membrane (10) after being UFed at 20 to 25°C through a ceramic membrane with a 300-kDa cutoff. For each preservative, a limit of quantification and a limit of detectability were established. At room temperature with 0.8 mL/min flux, a

Simmetry column (C18; 250 4.6 mm; 5-mm film thickness; Waters Corp., Milford, MA) was utilized. The product was then frozen and lyophilized at 20°C after being vacuum-filtered through regenerated cellulose 0.2-m filters (Millipore, Billerica, MA).

Bacteriological Analysis

Testing for microorganisms was done on both colostrum and a standardised bovine colostrum derivative (SBCD). Colostrum was extracted and immediately kept in 3-L containers with preservatives (phenoxyethanol 2.5 and diazolidinyl urea 1 percent) at 20°C. The samples were defrosted in accordance with a described procedure, and they were then immediately transferred to sterile plastic tubes. In a few experiments, preservatives weren't used until after freezing. Each substance was diluted 1:10 in sterile peptone physiological salt solution (pH 7), which was prepared from 10 g of each item. For each dilution, 10 l were plated onto Trypticase soy agar (Difco Laboratories Inc., Detroit, MI). The plates were incubated in a 5 percent CO2 atmosphere at 37°C. After a 24-hour incubation period, the various colony types were counted. In the event that no bacterial growth occurred, plates were incubated for a further 24 to 48 hours. The same method was used for bacteriologic testing on the lyophilized SBCD product (10 g in 90 mL of sterile peptone physiological salt solution, pH 7). To calculate bacterial counts, colony-forming units per gramme of colostrum or SBCD were utilised. Colostrum and the final product were subjected to a Limulus amebocyte lysate test (Thermo Scientific, Rockford, IL) in accordance with the manufacturer's instructions. The results were presented as a dichotomous result (+/), with the positive value (+) denoting 0.4 ELISA units per millilitre. (11)

Pharmacological Screening

1) Animals:

Wistar albino rats of either sex weighing 180–200 g were procured from the SudhakaraoNaik Institute of Pharmacy at JanataShikshanPrasarakMandal in Pusad, Yavatmal, Maharashtra. The institutional ethics committee gave its approval for using animals in the study.

2) Study on the acute toxicity of extract (LD 50)

The OECD guidelines 425 were followed when conducting the acute toxicity studies. Rats of either sex were given Standardised Bovine Colostrum Derivative at 2 g/kg orally by gavage (weight: 25–35 g; age: 6–8 weeks). The animals were routinely observed for potentially harmful side effects for the first four hours following therapy.After 24 hours, the number of surviving was recorded, and these animals were kept for another 13 days with regular observations.

3) The beginning of diabetes

A single subcutaneous injection of the pancreatic b-cell toxin STZ (Sigma Chemical; freshly dissolved in sterile saline, 0.9%) at a concentration of 65 mg/kg body weight was used to cause diabetes in mice. Three days after STZ injection and during the duration of the trial, blood glucose levels were checked using a glucometer to ascertain the animals' hyperglycemic status.

4) Materials and Methods

The investigation was conducted using Wistar breed rats. The rats were kept at a temperature of 22°C and a relative humidity of 60–70%. The animals were provided with a 12-hour cycle of darkness and light, commercial pellet food, and unrestricted access to water.(12)

5) Drug formulations and animal classification

For topical and oral administration, two types of pharmaceutical formulations including a standardisedBovin colostrum derivative have been created. Standardise The production of bovin colostrum derivative in ointment dosage form used basic ointment BP as a baseline for monitoring excision wound healing processes. To create ointment with a 5% (w/w) concentration, 50 g of simple ointment base BP was mixed with 2.5 g of standardisedBovin colostrum derivative.

From earlier studies, two dose ranges (100 and 200 mg/kg) were selected for oral administration. For topical treatment (excision and incision wound models), animals were separated into three groups: Group I served as the diabetic control group; Group II received top-down treatment with a 5% standardisedBovin colostrum derivative ointment; and Group III received top-down treatment with framycetin ointment. Three sets of six rats each were used to administer the medication orally to the animals (for the incision and dead space wound models). Group I served as the diabetic control (vehicle treatment), Group II was given a standardised bovine colostrum derivative oral dose of 100 mg/kg, and Group III was given a standardised bovine colostrum derivative oral dose of 200 mg/kg.

Excision wound model

A typical wound with a diameter of 2 cm was produced using a circular seal. Measurements were made of scar area after complete epithelization, wound closure percentage, and epithelization time.(13)

Incision wound model

Two 5 cm long para-vertebral straight incisions were made through the entire thickness of the skin on either side of the spinal column. utilising a razor-sharp scalpel (Ehrlich and Hunt, 1969). After reaching full homeostasis, the wound was stitched up using interrupted sutures that were spaced about one cm apart and equally separated. Sutures were removed after seven days, and tensile strength was assessed using a continuous water flow approach on the tenth post-wounding day (14).

Dead Space wound model

The animals were divided into three groups, each consisting of six rats, and each group was kept in a separate cage. Subcutaneously implanting polypropylene tubes (2.5 cm 0.5 cm) on the dorsal side of the lumbar area resulted in the dead space wound. The Standardised Bovine Colostrum Derivative was given to the animals from the first post-wounding day to the ninth. Each implanted tube's collected granulation tissue was carefully removed with the tube on the tenth post-wounding day and used to determine the breaking strength.(15)

Wound tissue histology

H&E staining will be used to perform wound tissue histology.

Statistical analysis

GraphPad Prism will be used to do the statistical analysis.

Results

Induction of Diabetic

The results are depicted in figure no 01 The result indicate that the Blood Glucose levels in the STZ Induced group are 300.37± 2.37. These results confirm induction of diabetes after administration of STZ in rats.

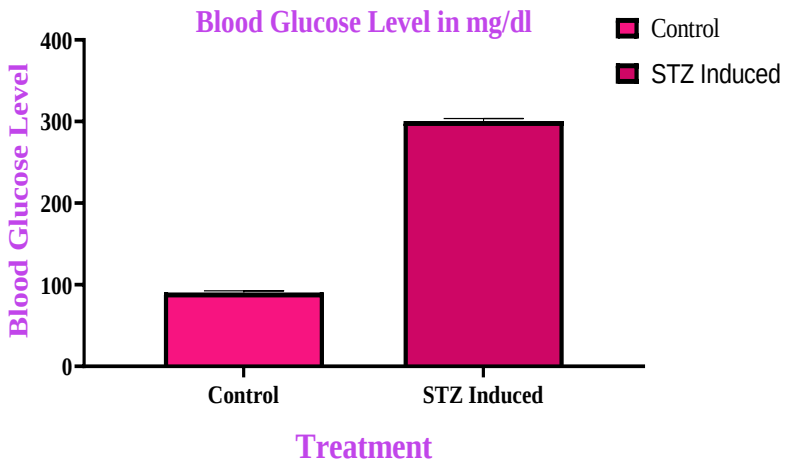

Figure1: Effect of STZ treatment on blood glucose

The results are depicted in figure no 02The result indicate that the % HbA1c levels in the STZ Induced group are 10.8 ± 0.4. these results confirms induction of diabetes after administration of STZ in rats.

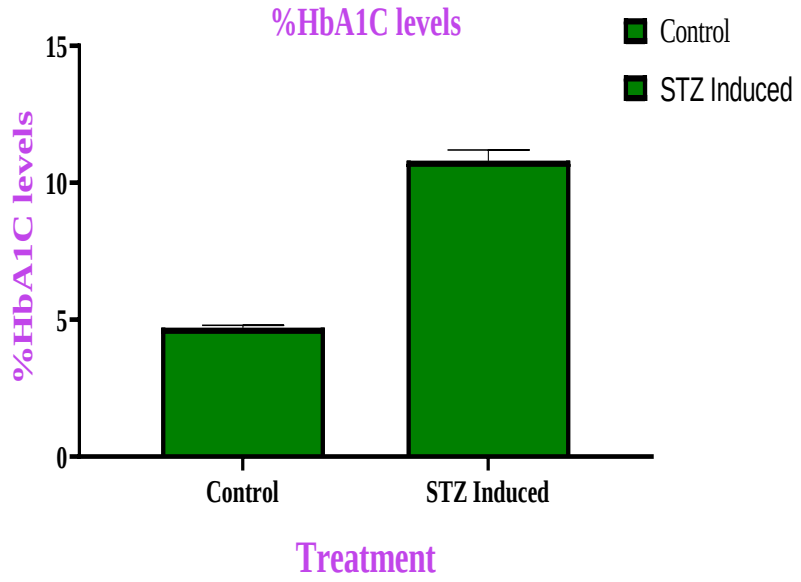

Figure 02: Effect of STZ treatment on % Hb A1C level

Effect of Treatment of Excision wound with SBCD on STZ induced diabetic rat

The given results represent the effects of different treatments on wound healing, specifically in terms of wound contraction, epithelization period, and scar area. Let's discuss each parameter and compare the results for the different treatments.

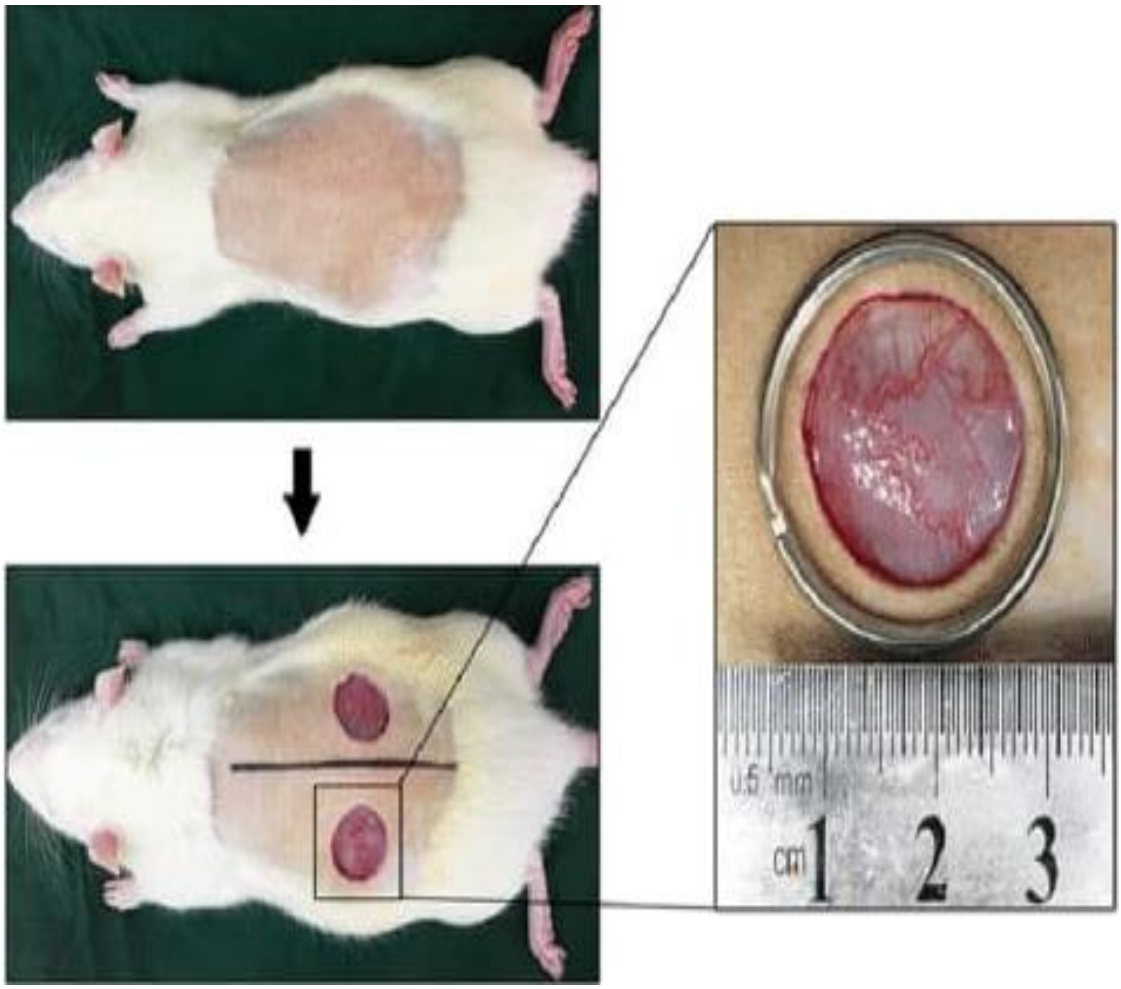

Figure 3: Creation of Excision wound in diabetic

Table 1: Effect of Treatment of Excision wound with SBCD on STZ induced diabetic rat

Treatment	Percent Wound Contraction					Epithelization Period	Scar Area mm2	Hydroxy Proline
	4th Day	8th Day	12th Day	16th Day	20th day			
Control	21.67± 4.53	38.2± 3.56	66.63± 3.73	80.23± 4.19	83.43± 3.24	23.08±1.03	56.84± 2.30	1.739±0.03
Povidone Iodine (50mg/animal/day)	38.18± 3.72	59.67± 3.853	80.67± 3.69	89.39± 3.89	99.27± 3.58	18.06±1.48	37.39± 3.21	4.598±0.04
standardized bovine colostrum derivative (50mg/animal/day)	33.47± 2.32	57.12± 4.43	77.38± 4.21	82.64± 3.68	98.61± 3.89	17.89±1.22	40.60± 3.68	3.684±0.04

Percent Wound Contraction

Percent wound contraction refers to the reduction in wound size over time, expressed as a percentage. In the control group, the wound contraction percentages on the 4th, 8th, 12th, 16th, and 20th days were 21.67%, 38.2%, 66.63%, 80.23%, and 83.43% respectively.

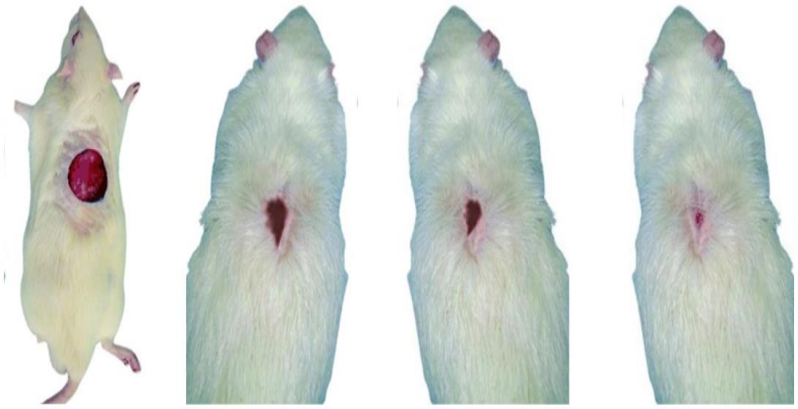

Figure 4: Wound contraction in SBCD treated group

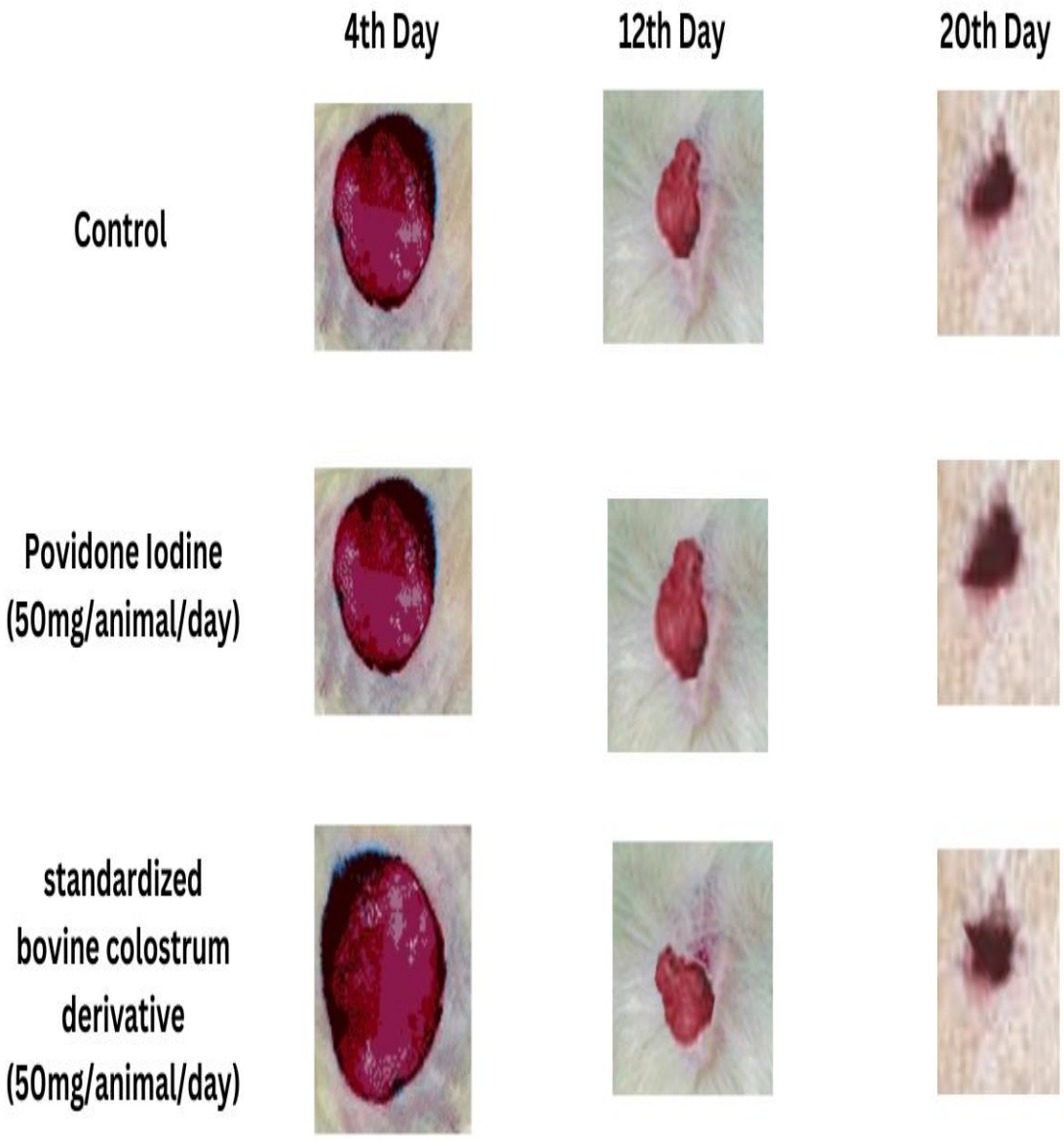

Figure 5: Effect of SBCD on 4th, 12th and 20th post wounding day on STZ induced diabetic rat

This indicates that the wound was gradually closing over time in the absence of any treatment.
In the Povidone Iodine group, the wound contraction percentages were higher compared to the control group. On the 4th, 8th, 12th, 16th, and 20th days, the percentages were 38.18%, 59.67%, 80.67%, 89.39%, and 99.27% respectively. This suggests that Povidone Iodine treatment accelerated wound closure.
Similarly, in the standardized bovine colostrum derivative group, the wound contraction percentages were also higher than the control group. On the 4th, 8th, 12th, 16th, and 20th days, the percentages were 33.47%, 57.12%, 77.38%, 82.64%, and 98.61% respectively.

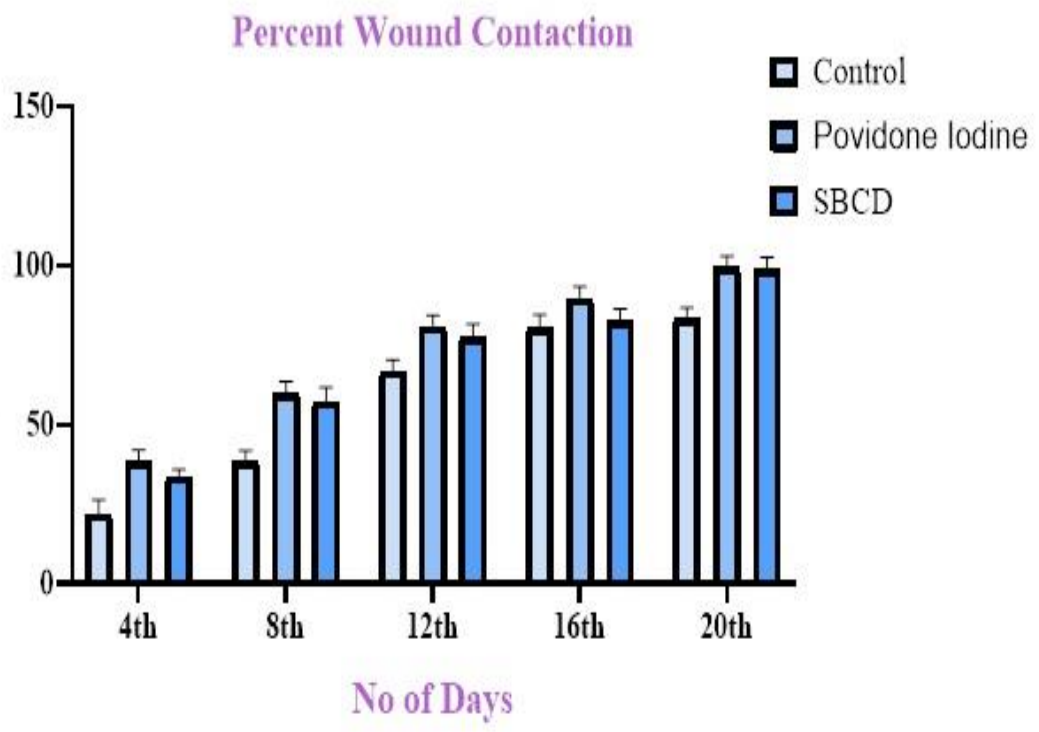

Figure 6: Effect of SBCD on Percent wound contraction

Epithelization Period

Epithelization refers to the process of new skin cell growth and migration to cover the wound. The epithelization period represents the time taken for complete wound closure. In the control group, the epithelization periods were 23.08 days and 56.84 days for the 8th and 16th day, respectively.

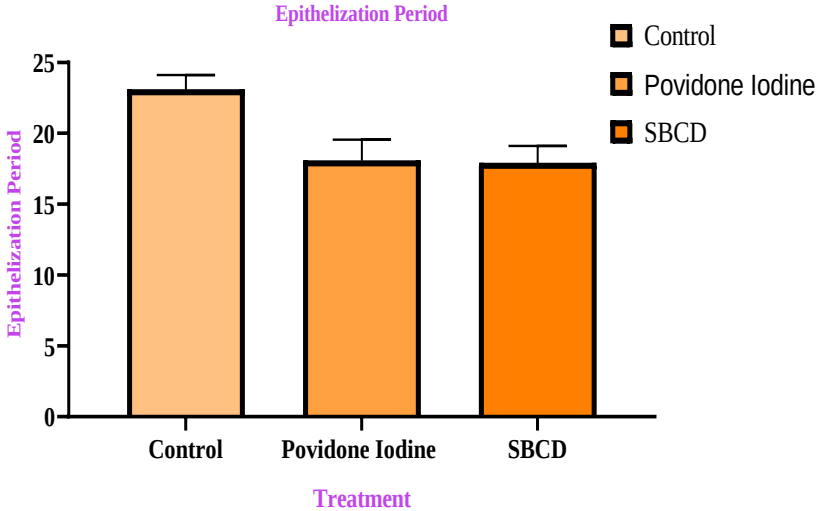

Figure 7: Effect of SBCD on Epithelization Period

In the Povidone Iodine group, the epithelization periods were shorter. On the 8th and 16th day, the periods were 18.06 days and 37.39 days, respectively. This indicates that Povidone Iodine promoted faster wound healing and epithelization.

Similarly, the standardized bovine colostrum derivative group also showed shorter epithelization periods compared to the control group. On the 8th and 16th day, the periods were 17.89 days and 40.60 days, respectively.

Scar Area:

Scar area represents the extent of scarring left after wound healing. In the control group, the scar areas on the 20th day were measured to be 1.739 mm².

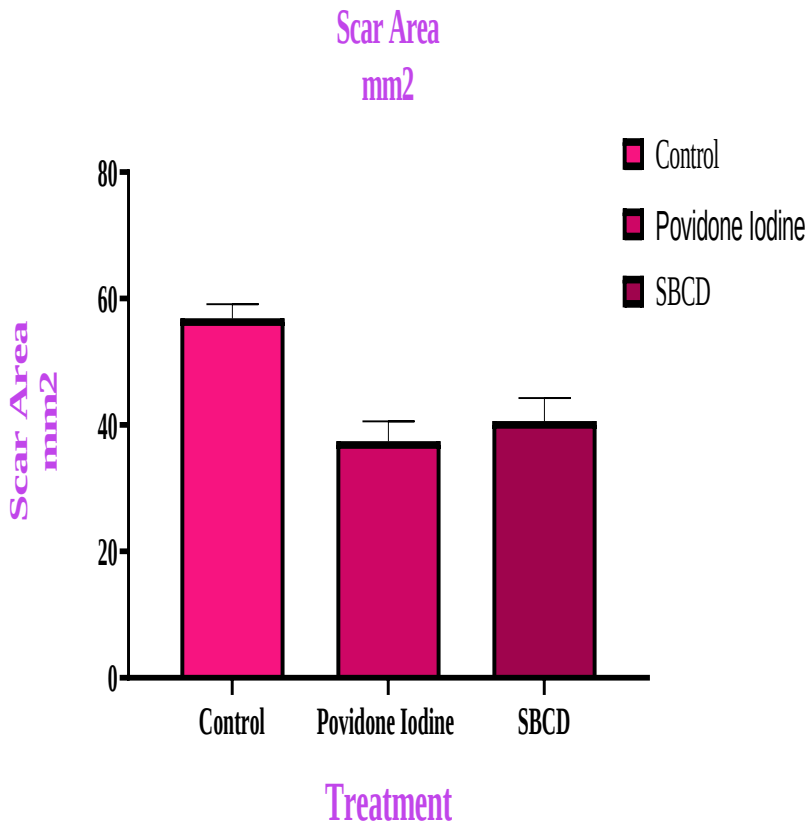

Figure 8: Effect of SBCD on Scar area mm^2

In the Povidone Iodine group, the scar area was larger, measuring 4.598 mm². This suggests that Povidone Iodine treatment may have led to more significant scarring.

On the other hand, the standardized bovine colostrum derivative group had a smaller scar area of 3.684 mm² compared to the Povidone Iodine group.

The results you provided relate to the effects of different treatments on wound healing parameters in an incision wound model. Let's discuss each parameter and compare the results for the different treatments.

Hydroxy Proline:

The observed increase in hydroxyproline content in the Povidone Iodine-treated and standardized bovine colostrum derivative-treated groups indicates their positive effects on collagen synthesis, which is crucial for wound healing and tissue repair. Understanding the molecular mechanisms underlying these effects can provide valuable insights into the therapeutic actions of these treatments.

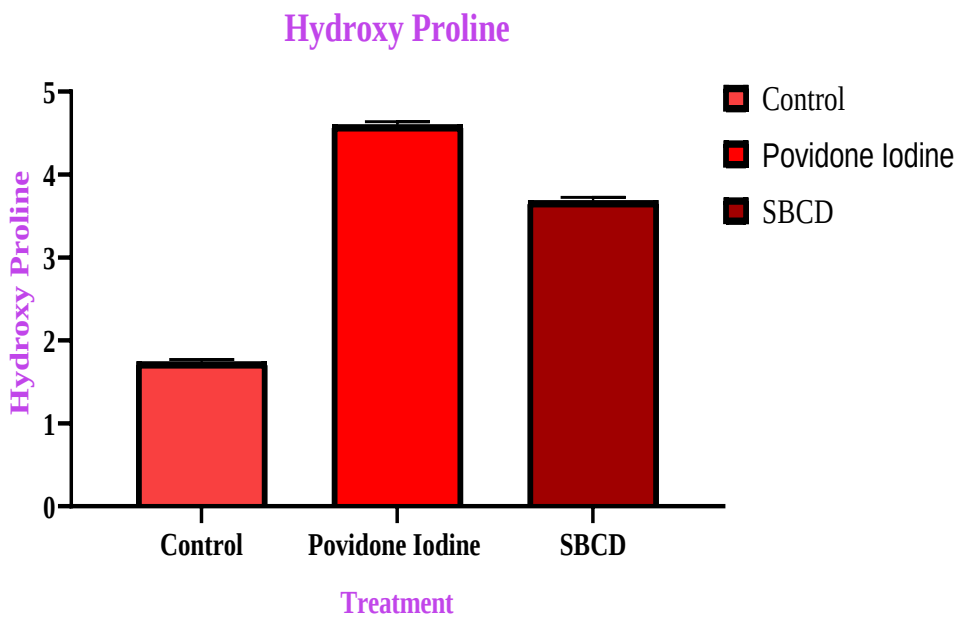

Figure 9: Effect of SBCD on Hydroxy Proline

Effect of Standardized Bovine Colostrum Derivative on Incision and Dead space model:

The standardized bovine colostrum derivative is a natural product derived from cow's colostrum, which is rich in various bioactive molecules, including growth factors, cytokines, and immunoglobulins. These components collectively contribute to its wound healing properties.

The observed increase in hydroxyproline content in the standardized bovine colostrum derivative-treated group suggests that this treatment promotes collagen synthesis and deposition in the wound tissue. The specific growth factors and cytokines present in the colostrum derivative may stimulate fibroblast activity and collagen production. For example, epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α) are known to play critical roles in promoting cell proliferation and tissue regeneration. Insulin-like growth factor (IGF-1) is another important factor that regulates cell growth and differentiation, including fibroblast activity. Additionally, the standardized bovine colostrum derivative may modulate the inflammatory response, as it contains immunoglobulins and bioactive peptides with anti-inflammatory properties. By reducing excessive inflammation, the colostrum derivative creates a more favorable environment for tissue repair and collagen deposition.

Wound Breaking Strength (g)

Wound breaking strength measures the tensile strength of the healed wound, indicating the ability of the tissue to withstand mechanical stress. In the control group, the wound breaking strength was 259.32 g.

Table 2: Effect of SBCD on Incision and Dead space model

Treatment	Incision wound model Wound Breaking strength (g)	Dead space wound Granuloma breaking strength (g)	Hydroxyproline content (g/ml)	Hexosamine
Control	259.32 ± 4.39	248.29 ±3.16	1.635± 0.03	0.45± 0.02
standardized bovine colostrum derivative (200mg/kg po)	382.83.2 ± 3.44*	316.84 ± 3.67*	4.643 ± 0.04*	0.61± 0.03
standardized bovine colostrum derivative (400 mg/kg po)	439.25 ± 6.57*	340.83 ± 3.24*	5.482 ± 0.03*	0.58± 0.03
Povidone iodine (50 mg/animal/day topically)	451.37 ± 4.34*	369.745 ± 3.38*	5.976± 0.02*	0.67± 0.03

The treatment with standardized bovine colostrum derivative at a dose of 200 mg/kg orally resulted in significantly higher wound breaking strength compared to the control group (382.83 g). Similarly, the group receiving a higher dose of standardized bovine colostrum derivative (400 mg/kg orally) also exhibited significantly higher wound breaking strength (439.25 g). The Povidone iodine treatment group also showed increased wound breaking strength (451.37 g).

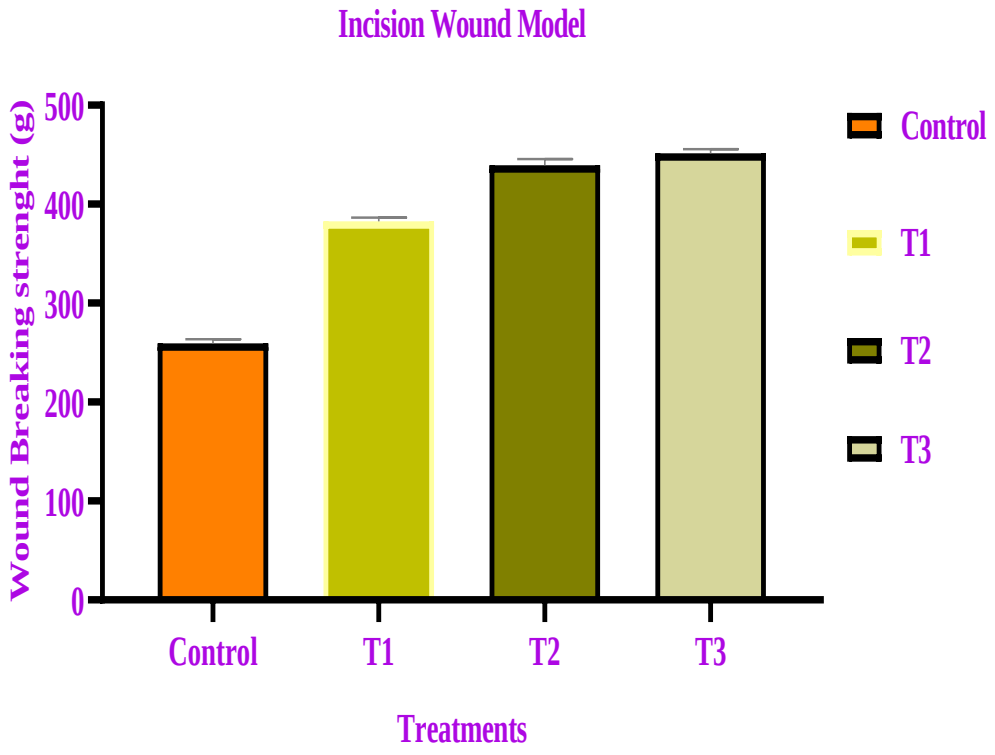

Figure 10: Effect of SBCD on wound breaking strength in gram for Incision wound model

These results suggest that both standardized bovine colostrum derivative and Povidone iodine treatments enhance the tensile strength of the healed wounds, indicating improved wound healing.

Dead Space Wound Granuloma Breaking Strength (g)

Dead space wound granuloma breaking strength refers to the strength of the scar tissue formed within a dead space wound model. In the control group, the granuloma breaking strength was 248.29 g.

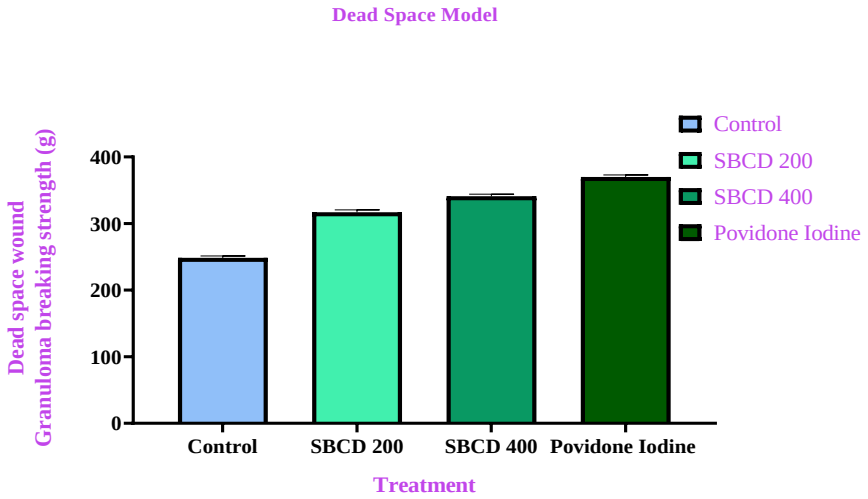

Figure 11: Effect of SBCD on Granuloma breaking strength in gram for Dead space wound model

Treatment with standardized bovine colostrum derivative at both doses (200 mg/kg and 400 mg/kg orally) as well as Povidone iodine treatment led to significantly higher granuloma breaking strength compared to the control group. The values were 316.84 g, 340.83 g, and 369.745 g, respectively.

These findings suggest that standardized bovine colostrum derivative and Povidone iodine treatments contribute to the formation of stronger scar tissue within the wound.

Hydroxyproline Content (g/ml)

Hydroxyproline content is a marker of collagen synthesis and deposition, indicating the quality and quantity of new tissue formation in the wound. In the control group, the hydroxyproline content was 1.635 g/ml.

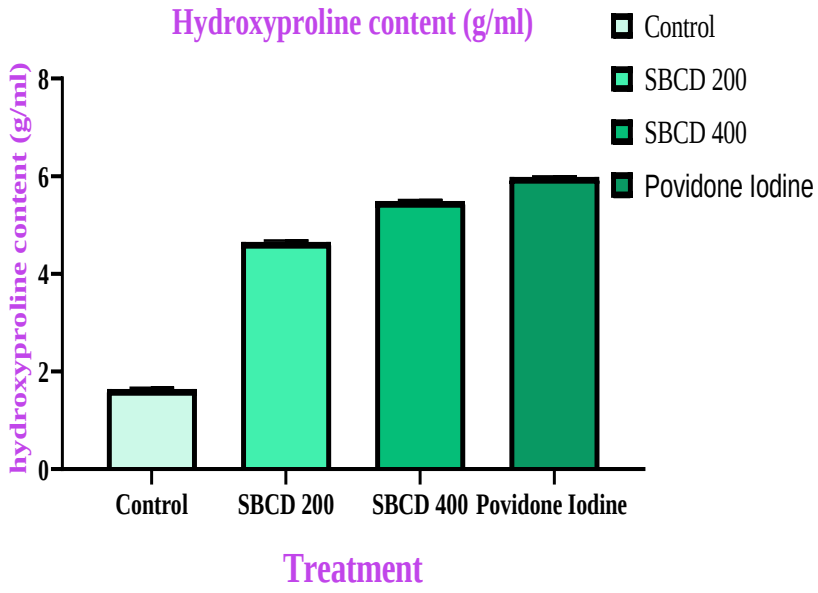

Figure 12: Effect of SBCD on Hydroxyproline content

Treatment with standardized bovine colostrum derivative at both doses and Povidone iodine treatment resulted in significantly higher hydroxyproline content compared to the control group. The values were 4.643 g/ml and 5.482 g/ml for the two doses of standardized bovine colostrum derivative, and 5.976 g/ml for Povidone iodine. These results suggest that standardized bovine colostrum derivative and Povidone iodine treatments promote collagen synthesis and deposition, indicating enhanced wound healing.

Hexosamine Content

Hexosamine content is an indicator of total glycosaminoglycan (GAG) levels, which are important components of the extracellular matrix involved in tissue repair. In the control group, the hexosamine content was 0.45 g/ml.

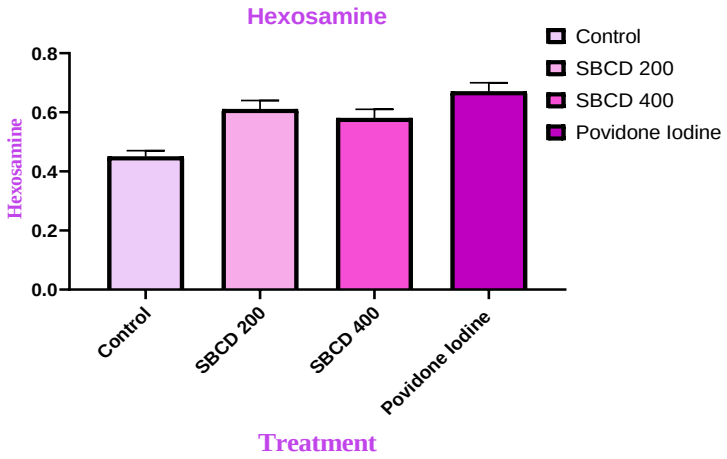

Figure 13: Effect of SBCD on Hexosamine content

Treatment with standardized bovine colostrum derivative (200 mg/kg orally) and Povidone iodine resulted in significantly higher hexosamine content compared to the control group. The values were 0.61 g/ml and 0.67 g/ml, respectively.

These findings suggest that both standardized bovine colostrum derivative and Povidone iodine treatments enhance the production of glycosaminoglycans, indicating improved tissue repair.

Discussion

The results of this study underscore the potential of Standardized Bovine Colostrum Derivatives as an effective intervention for accelerating diabetic wound healing. The observed accelerated wound size reduction and improved histological characteristics in the SBCD-treated group suggest its role in promoting key processes such as cell migration, proliferation, and extracellular matrix remodeling. These findings align with previous studies highlighting the wound healing properties of colostrum-derived compounds

References

1. Brem H, Tomic-Canic M. Cellular and molecular basis of wound healing in diabetes. Journal of Clinical Investigation. 2007;117(5):1219-1222.
2. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: Mechanisms, signaling, and translation. Science Translational Medicine. 2014;6(265):265sr6.
3. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature. 2008;453(7193):314-321.
4. Falanga V. Wound healing and its impairment in the diabetic foot. The Lancet. 2005;366(9498):1736-1743.
5. Singh N, Armstrong DG, Lipsky BA. Preventing Foot Ulcers in Patients With Diabetes. JAMA. 2005;293(2):217-228.
6. Playford RJ, Macdonald CE, Johnson WS. Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. American Journal of Clinical Nutrition. 2000;72(1):5-14.
7. Rathore A, Verma M, Kumar P, Srivastava S, Verma M, Bhatia J. Bovine colostrum: An emerging nutraceutical. Journal of Complementary and Integrative Medicine. 2012;9(1):Article 21.
8. Isaksson G, Gustafsson L, Åkerström B, Sletten K. Studies on Bovine Colostral Proteins. I. Preliminary Characterization of a Protein from Bovine Colostrum, which Inhibits Lipid Peroxidation. BiochimicaetBiophysicaActa (BBA) - General Subjects. 1974;362(2):310-316.
9. Kelly GS. Bovine colostrums: A review of clinical uses. Alternative Medicine Review. 2003;8(4):378-394.
10. Nikolic I, Stojanovic I, Vujicic M, Fagone P, Mangano K, Stosic-Grujicic S, Nicoletti F, Saksida T. Standardized bovine colostrum derivative impedes development of type 1 diabetes in rodents. Immunobiology. 2017 Feb 1;222(2):272-9.
11. Fecteau G, Baillargeon P, Higgins R, Paré J, Fortin M. Bacterial contamination of colostrum fed to newborn calves in Québec dairy herds. The Canadian Veterinary Journal. 2002 Jul;43(7):523.
12. Deori K, Soren AD, Yadav AK. Toxicity assessment of Phlogacanthusthyrsiflorus, a traditionally used anthelmintic plant of India. Future Journal of Pharmaceutical Sciences. 2023 Dec;9(1):1-1.
13. Chen L, Mirza R, Kwon Y, DiPietro LA, Koh TJ. The murine excisional wound model: Contraction revisited. Wound Repair and Regeneration. 2015 Nov 12;23(6):874-7.
1. 14)0020
14. Murad S, Anwar A, Piracha ZZ, Sultan A. LRIG1 expression during homeostasis and skin wound healing in mice. Journal of Biological Regulators and Homeostatic Agents. 2015 Oct 1;29(4):829-33.
15. Farahpour MR, Mirzakhani N, Doostmohammadi J, Ebrahimzadeh M. HydroethanolicPistaciaatlantica hulls extract improved wound healing process; evidence for mast cells infiltration, angiogenesis and RNA stability. International Journal of Surgery. 2015 May 1;17:88-98.

How to cite this article: Rohit P. Mali, Dr Rakesh Kumar Jat, and Vijay Wagh. "WOUND HEALING ACTIVITY OF STANDARDIZED BOVIN COLOSTRUM DERIVATIVES IN DIABETIC RATS". *Tropical Journal of Pharmaceutical and Life Sciences*, vol. 10, no. 4, Aug. 2023, pp. 12-25, https://informativejournals.com/journal/index.php/tjpls/article/view/139

Published by:
Informative Journals
Jadoun Science Publishing Group India