Formulation And Preclinical Evaluation of A Novel Hydrogel Formulation Incorporating An Algal Extract Fortified with Anti-Inflammatory Phytochemicals for Diabetic Wound Healing

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ABSTRACT:

Background

Diabetic wounds are among the most serious complications of diabetes mellitus due to persistent inflammation, oxidative stress, impaired angiogenesis, and delayed tissue regeneration. Conventional wound dressings often fail to provide an optimal healing environment, emphasizing the need for multifunctional biomaterials capable of accelerating wound repair. Marine algae, particularly *Ulva lactuca*, are rich in bioactive compounds such as ulvan, flavonoids, polysaccharides, and phenolic compounds that exhibit antioxidant, anti-inflammatory, and antimicrobial activities. Incorporation of these phytochemicals into hydrogel systems may provide an effective therapeutic strategy for diabetic wound management. [1–8, 14, 21–29]

Aim and Objectives

The present study aimed to formulate and evaluate a novel hydrogel incorporating hydroalcoholic extract of *Ulva lactuca* fortified with anti-inflammatory phytochemicals for diabetic wound healing. The objectives included phytochemical screening, hydrogel formulation, physicochemical characterization, in vitro antioxidant and anti-inflammatory evaluation, acute dermal toxicity assessment, and in vivo evaluation using an HFD-STZ-induced diabetic excision wound model. [1, 9–13, 21–23, 29]

Materials and Methods

Hydroalcoholic extract of *Ulva lactuca* was prepared using Soxhlet extraction and subjected to qualitative and quantitative phytochemical analysis. A hydrogel was formulated using HPMC K100M and xanthan gum as polymeric matrices. The prepared formulation was evaluated for appearance, pH, viscosity, spreadability, drug content, retention time, and in vitro drug release using Franz diffusion cell studies. Antioxidant activity was assessed by DPPH free radical scavenging assay, anti-inflammatory activity by protein denaturation assay, and glucose uptake by yeast cell assay. Acute dermal toxicity was performed according to OECD guideline 402. Type II diabetes was induced using High Fat Diet (HFD) and low-dose Streptozotocin (STZ), followed by excision wound creation. Wound contraction, body weight, fasting blood glucose, antioxidant enzyme levels (SOD and CAT), lipid profile, and histopathological examination were evaluated. [15–18, 30–40]

Results

Preliminary phytochemical analysis confirmed the presence of carbohydrates, flavonoids, phenolic compounds, glycosides, proteins, and polysaccharides in the hydroalcoholic extract. Quantitative analysis demonstrated appreciable total carbohydrate and flavonoid contents. The optimized hydrogel exhibited acceptable physicochemical characteristics with skin-compatible pH, suitable viscosity, uniform drug distribution, satisfactory retention time, and sustained drug release. The formulation demonstrated concentration-dependent antioxidant and anti-inflammatory activities together

with enhanced glucose uptake. No signs of irritation or toxicity were observed during acute dermal toxicity studies. In vivo studies showed significant improvement in wound contraction, reduction in blood glucose levels, restoration of body weight, improvement of antioxidant enzymes (SOD and CAT), normalization of lipid profile, and enhanced collagen deposition and re-epithelialization in histopathological sections compared with disease control animals. [15–23, 29, 36–40]

Conclusion

The developed *Ulva lactuca*-based hydrogel demonstrated significant antioxidant, anti-inflammatory, and wound healing activities in the HFD-STZ diabetic wound model. The synergistic combination of marine algal bioactive compounds with hydrogel technology provided sustained drug release and accelerated tissue regeneration. The formulation represents a promising natural therapeutic approach for diabetic wound management and warrants further preclinical and clinical investigations. [9–13, 17–23, 29, 36–40]

Keywords: *Ulva lactuca*, Hydrogel, Diabetic wound healing, Marine algae, Antioxidant activity, Anti-inflammatory activity, HFD-STZ model, Franz diffusion cell, Tissue regeneration.

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1. INTRODUCTION (Draft)

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from impaired insulin secretion, insulin resistance, or both. One of the most debilitating complications associated with diabetes is delayed wound healing, particularly diabetic foot ulcers, which frequently progress to chronic non-healing wounds, infection, and lower-limb amputation. Impaired wound healing in diabetes is attributed to prolonged inflammation, oxidative stress, endothelial dysfunction, reduced angiogenesis, excessive production of reactive oxygen species (ROS), impaired collagen synthesis, and defective extracellular matrix remodelling. [1–8]

Conventional wound management strategies, including antibiotics, debridement, and moisture-retentive dressings, primarily provide symptomatic relief and often fail to restore normal tissue regeneration. Therefore, the development of multifunctional wound dressings capable of simultaneously controlling inflammation, preventing infection, scavenging free radicals, and promoting tissue regeneration has become an important area of biomedical research. [7–13]

Hydrogels have emerged as promising biomaterials for wound healing because of their three-dimensional polymeric network, high water content, excellent biocompatibility, biodegradability, and ability to maintain a moist wound environment. Hydrogels also serve as efficient carriers for sustained delivery of bioactive compounds directly to the wound surface while facilitating oxygen permeability, cellular migration, and extracellular matrix formation. [9–13]

Marine algae represent an abundant natural source of biologically active compounds with considerable pharmaceutical potential. Among marine macroalgae, *Ulva lactuca* has attracted significant attention owing to its high content of ulvan, sulphated polysaccharides, flavonoids, phenolic compounds, proteins, vitamins, and minerals. These constituents exhibit potent antioxidant, antimicrobial, anti-inflammatory, and tissue regenerative properties that collectively contribute to accelerated wound healing. Ulvan has additionally demonstrated favourable hydrogel-forming capability, making *Ulva lactuca* particularly attractive for developing advanced wound dressing systems. [14–29]

Based on these considerations, the present study was designed to formulate a hydrogel containing hydroalcoholic extract of *Ulva lactuca* fortified with anti-inflammatory phytochemicals and evaluate its wound healing efficacy using in vitro and in vivo experimental models. The findings of this study may contribute to the development of a safe, effective, and economical natural therapeutic system for diabetic wound management. [9–13, 21–29]

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh thalli of *Ulva lactuca* were collected from the coastal region of Tamilnadan, India, durin. The collected algal material was washed thoroughly with seawater followed by distilled water to remove sand particles, epiphytes, and other impurities. The cleaned material was shade dried at room temperature for 7–10 days until constant weight was obtained. The dried algae were pulverized into coarse powder using a mechanical grinder and stored in an airtight container until further use. Botanical authentication of the plant material was performed by a qualified taxonomist at Tamil Nadu and a voucher specimen was deposited for future reference. [14, 21–29]

2.2 Preparation of Hydroalcoholic Extract

The powdered *Ulva lactuca* was defatted using petroleum ether (40–60°C) in a Soxhlet apparatus. The defatted marc was subsequently extracted with hydroalcoholic solvent (Ethanol: Water, 70:30 v/v) for 48 hours. The extract was concentrated under reduced pressure using a rotary evaporator followed by drying in a hot air oven below 45°C to obtain a semisolid extract. The percentage yield was calculated and the extract was stored at 4°C until further studies. [27, 28, 30, 31]

2.3 Phytochemical Screening:

Qualitative Analysis -

The extract was screened qualitatively for the identification of the different secondary metabolites in the hydro-alcoholic extract. The test of alkaloids, flavonoids, tannins, saponin, steroids, cardiac glycosides, triterpenoids, carbohydrates, phenolic compounds performed. [30,31].

Quantitative Analysis-

Total phenolics of hydroalcoholic extract was quantified using Folin Ciocalteu reagent, Aluminium chloride method was [32]

2.4 Formulation of Hydrogel

A topical hydrogel was prepared using **Hydroxypropyl Methylcellulose (HPMC K100M)** and **Xanthan Gum** as the polymeric matrix. [9–13]

Initially, HPMC K100M and Xanthan Gum were dispersed separately in distilled water and allowed to hydrate completely. The hydroalcoholic extract of *Ulva lactuca*, along with selected anti-inflammatory phytochemicals, was dissolved in a suitable solvent and incorporated slowly into the hydrated polymeric dispersion under continuous stirring. Glycerin was added as a humectant, while methyl paraben and propyl paraben were incorporated as preservatives. Triethanolamine was used to adjust the pH to skin-compatible values (5.5–6.8). Continuous stirring was maintained until a homogeneous hydrogel was obtained. The prepared formulations were stored in airtight containers at room temperature for further evaluation. [9–13]

Evaluation of Hydrogel Formulation

The prepared formulations were evaluated for the following physicochemical characteristics.

1.Physical Appearance

Hydrogels were visually examined for colour, homogeneity, consistency, phase separation, and grittiness. [11, 33]

2.pH Measurement

The pH of the formulation was measured using a calibrated digital pH meter at room temperature. [11, 33]

3.Viscosity

Viscosity was determined using a Brookfield Digital Viscometer employing an appropriate spindle at **25 ± 2°C**. [11, 33]

Viscosity = $\eta 1 = \eta 2 . \rho 1\ t1 / \rho 2\ t2$

4. Spreadability

Spreadability was determined by the parallel plate method and expressed as g·cm/sec. [11, 33]

5. Drug Content

Drug content uniformity was evaluated by dissolving an accurately weighed quantity of hydrogel in phosphate buffer (pH 6.8), followed by UV spectrophotometric estimation at **210 nm**. [11, 33]

Drug Content (%) = (Actual amount of drug present / Theoretical amount of drug) × 100

6. Retention Time

Retention ability of the hydrogel was evaluated using freshly excised goat skin mounted on an inclined glass surface. The time required for complete removal of the formulation under simulated physiological conditions was recorded. [33]

Retention Time = Final Time − Initial Time

7. In-vitro Drug Release Study

In-vitro drug release was carried out using a **Franz Diffusion Cell** equipped with an effective diffusion area of approximately **3.14 cm²**. Cellophane membrane (or dialysis membrane) previously soaked overnight in phosphate buffer (pH 6.8) was mounted between donor and receptor compartments. [33]

Approximately **1 g** of hydrogel was placed in the donor compartment. The receptor compartment contained phosphate buffer maintained at **37 ± 0.5°C** under continuous magnetic stirring.

Aliquots were withdrawn at predetermined intervals (0.5, 1, 2, 3, 4, 5, and 6 h) and replaced with fresh dissolution medium. Samples were analyzed spectrophotometrically at **210 nm**, and cumulative percentage drug release was calculated.

Cumulative Drug Release (%) = (Amount of drug released / Total amount of drug loaded) × 100

2.5 *In-vitro* Biological Evaluation

2.5.1 DPPH Radical Scavenging Assay

Antioxidant activity was evaluated using the DPPH free radical scavenging assay. Various concentrations of the extract were mixed with freshly prepared 0.1 mM DPPH solution and incubated in darkness for 30 minutes. Absorbance was measured at **517 nm**. Ascorbic acid was used as the reference standard. [34]

Percentage Inhibition (%) = [(A control – A sample) / A control] × 100

2.5.2 Yeast Cell Glucose Uptake Assay

The glucose uptake assay was performed using baker's yeast suspension. Different concentrations of the extract were incubated with glucose solution followed by addition of yeast suspension. After incubation, the remaining glucose concentration was measured spectrophotometrically, and percentage glucose uptake was calculated. [34]

Glucose Uptake (%) = [(A control – A sample) / A control] × 100

2.5.3 Protein Denaturation Assay

The anti-inflammatory activity of the extract was evaluated using the inhibition of heat-induced protein denaturation method. Egg albumin served as the protein source, while Diclofenac sodium was used as the standard drug. Absorbance was measured at **660 nm**, and percentage inhibition was calculated. [35]

Percentage Inhibition (%) = [(A control – A sample) / A control] × 100

2.6 In-Vivo studies

2.6.1Acute Dermal Toxicity Study

Acute dermal toxicity of the optimized hydrogel formulation was performed according to **OECD Guideline 402**. Healthy Wistar rats were observed for 14 days after topical application of the formulation for any signs of erythema, edema, skin irritation, behavioural changes, or mortality. [36]

2.6.2 Experimental Animals

Healthy adult Wistar albino rats weighing **180–250 g** of either sex were procured from the institutional animal facility. Animals were housed under standard laboratory conditions (temperature **25 ± 2°C**, relative humidity **55 ± 5%**, and **12 h light/12 h dark cycle**) with free access to standard pellet diet and water. [36]

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted under CPCSEA guidelines. [36]

2.6.3 Induction of Type 2 Diabetes

Type 2 diabetes mellitus was induced by feeding rats a **High Fat Diet (HFD)** for four weeks followed by a single intraperitoneal injection of **Streptozotocin (35 mg/kg)** freshly prepared in citrate buffer (pH 4.5). Animals exhibiting fasting blood glucose levels **>250 mg/dL** after 72 hours were considered diabetic and included in the study. [37–39]

2.6.4 Excision Wound Model

Following anesthesia, a full-thickness circular excision wound measuring approximately **2 cm²** was created on the dorsal thoracic region of diabetic rats under aseptic conditions. [40]

Animals were randomly divided into experimental groups:

The experimental animals were randomly divided into six groups, with **six animals (n = 6)** in each group.

Group I (Normal Control): Healthy rats administered normal saline and receiving no wound treatment.

Group II (Disease Control): HFD-STZ-induced diabetic rats with excision wounds receiving no treatment.

Group III (Standard Treatment sucralfate cream with high dose): HFD-STZ-induced diabetic rats with excision wounds treated topically with the standard wound-healing formulation.

Group IV (Standard treatment sucralfate cream with low dose): HFD-STZ-induced diabetic rats with excision wounds treated with the standard wound-healing formulation.

Group V (Test Hydrogel – Low Dose): HFD-STZ-induced diabetic rats with excision wounds treated topically with *Ulva lactuca*-incorporated hydrogel (200 mg/kg).

Group VI (Test Hydrogel – High Dose): HFD-STZ-induced diabetic rats with excision wounds treated topically with *Ulva lactuca*-incorporated hydrogel (400 mg/kg).

The respective formulations were applied topically once daily until complete wound closure. [40]

2.6.5 Evaluation of Wound Healing

The following parameters were assessed:

- Percentage wound contraction
- Body weight
- Fasting blood glucose
- Antioxidant enzyme estimation (SOD and CAT)
- Lipid profile (TC, TG, HDL, LDL)
- Histopathological examination (H&E staining)

Wound area was measured on **Day 0, Day 3, Day 7, and Day 14**, and percentage wound contraction was calculated using the formula: [40]

$$\text{Wound Contraction (\%)} = \frac{\text{Initial Wound Area} - \text{Final Wound Area}}{\text{Initial Wound Area}} \times 100$$

2.6.6 Statistical Analysis

All experimental data were expressed as **Mean ± SEM (n = 6)**. Statistical analysis was performed using **GraphPad Prism software (Version 11)**. Comparisons among groups were carried out using **One-way Analysis of Variance (ANOVA)** followed by **Tukey's multiple comparison test**. A value of $p < 0.05$ was considered statistically significant. [37–40]

3. RESULTS

3.1 Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the hydroalcoholic extract of *Ulva lactuca* confirmed the presence of several biologically active constituents. Carbohydrates, glycosides, proteins, amino acids, and phenolic compounds were detected, whereas alkaloids and steroids were absent. The presence of these phytoconstituents indicates that the extract contains compounds known to contribute to antioxidant, anti-inflammatory, and wound healing activities.

Phytochemicals	Test	Observations Presence (+) /Absence (-)
Alkaloids	Mayer's test	-
	Hager's test	-
	Wagner's test	-
	Dragendroff's test	-
Flavonoids	Shinoda test	-
	Sulphuric acid test	-
Tanin and phenolic compound	T.S + 5% $FeCl_3$	+
	Lead acetate test	+
	Bromine water test	-
	Acetic acid solution test	-
	Dilute HNO_3	-
Steroids	Salkowski test	-
	Liebermann-Burchard test	-
Glycosides	Legal's test	+
	Brontrager's test	-

	Keller killiani test	+
Amino acids	Ninhydrin test	+
Carbohydrates	Molisch's test	+
	Iodine test	+
	Barfoed's test	+
	Selwinoff's test	+
	Fehling's test	+
	Benedict's test	+
Proteins	Biurets test	+
	Millon's test	+

3.2 Quantitative Phytochemical Analysis

The hydroalcoholic extract exhibited appreciable levels of total carbohydrates and flavonoids. The glucose calibration curve demonstrated good linearity, and carbohydrate content increased proportionally with extract concentration, indicating a considerable amount of carbohydrate-rich bioactive constituents in *Ulva lactuca*. Similarly, flavonoid estimation using the quercetin calibration curve confirmed the presence of flavonoids, which are recognized for their antioxidant potential.

3.3 Evaluation of Hydrogel Formulation

Physical Characteristics

The formulated hydrogel appeared smooth, homogeneous, yellowish, and free from lumps or phase separation, indicating successful incorporation of the *Ulva lactuca* extract within the polymeric matrix. The formulation possessed suitable consistency for topical application.

Fig No.6.7 : Hydrogel formulation

pH

The optimized hydrogel exhibited a pH of **6.03**, which lies within the physiological skin pH range **(5.5–7.0)**. This suggests that the formulation is compatible with skin and is unlikely to cause irritation during topical application.

Fig No.6.8 : pH of hydrogel formulation

Viscosity

Brookfield viscosity measurements showed that viscosity increased with increasing polymer concentration. The optimized formulation demonstrated adequate viscosity, ensuring good spreadability, prolonged residence time,

and improved retention at the wound site.

Fig No.6.9 : Viscometer

In Vitro Drug Release

The Franz diffusion study demonstrated a sustained release profile of the bioactive constituents from the hydrogel. The gradual release pattern indicates that the

polymeric network effectively controlled diffusion, thereby maintaining prolonged therapeutic activity.

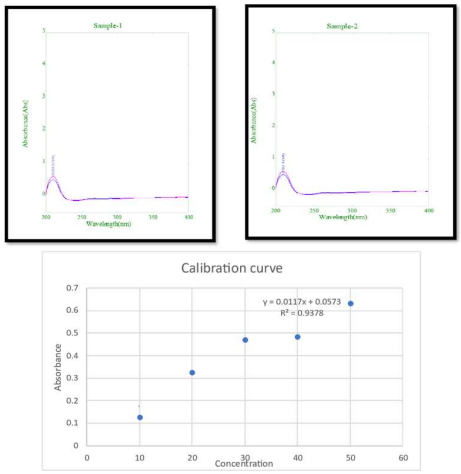


Fig No. 6.10 : In-vitro drug release by Franz Diffusion Cell

Fig No . Profile of Invitro drug release

Retention Time

The optimized hydrogel exhibited satisfactory adhesion on goat skin throughout the observation period, indicating good bioadhesive properties and prolonged residence at the wound surface.

Fig No.6.11 : Retention time of Hydrogel

Drug Content

Drug content analysis confirmed uniform distribution of the extract throughout the hydrogel matrix, demonstrating successful formulation and reproducible incorporation of the active constituents.

Fig No.6.12 : Calibration curve

***In-Vitro* Study Results:**

3.4 Antioxidant Activity (DPPH Assay)

The hydroalcoholic extract showed concentration-dependent free radical scavenging activity. Percentage inhibition increased from **33.37% at 10 µg/mL** to **54.79% at 50 µg/mL**, while ascorbic acid exhibited **50.47–67.50%** inhibition over the tested concentration range. The extract showed an IC_{50} value of approximately **32.63 µg/mL**, indicating considerable antioxidant potential. [19, 20, 29, 32, 34]

3.5 Glucose Uptake Assay

The hydroalcoholic extract enhanced glucose uptake in yeast cells in a concentration-dependent manner. Glucose uptake increased from **25.13%** at **50 µg/mL** to **39.32%** at **250 µg/mL**, whereas metformin showed **58.88–82.43%** uptake across the same concentration range. These findings suggest that the extract possesses moderate antihyperglycemic activity. [29, 34]

3.6 Anti-inflammatory Activity (Protein Denaturation Assay)

The hydroalcoholic extract inhibited protein denaturation in a concentration-dependent manner. Inhibition increased from **23.95%** at **100 µg/mL** to **59.02%** at **500 µg/mL**, closely approaching the activity of diclofenac sodium (**57.38%** at 500 µg/mL). The extract exhibited a lower IC_{50} than the standard reported in your dataset, indicating promising anti-inflammatory activity. [20, 22, 35]

***In- Vivo* Study Results:**

3.7 Acute Dermal Toxicity

Topical application of the optimized hydrogel at **2000 mg/kg** produced no signs of erythema, edema, irritation, ulceration, behavioral abnormalities, or mortality throughout the 14-day observation period. These findings indicate that the formulation is safe for topical administration according to OECD Guideline 402. [36]

3.8 Effect on Body Weight

Normal control animals showed a gradual increase in body weight throughout the study, whereas diabetic control animals exhibited progressive weight loss. Treatment with the *Ulva lactuca* hydrogel prevented this reduction. By Day 14, the high-dose hydrogel group reached **278.0 ± 2.32 g**, compared with **261.66 ± 2.85 g** in the disease control group, indicating improvement in metabolic status. [37–39]

3.9 Wound Healing Activity [21–23, 40]

The hydrogel accelerated wound closure throughout the 14-day study. The high-dose hydrogel produced **78% wound contraction** by Day 14 compared with **37.5%** in the disease control group. The low-dose hydrogel achieved **66%** wound contraction, while the standard high-dose treatment reached **86%**. These findings demonstrate marked enhancement of diabetic wound healing following hydrogel treatment.

Groups	**Wound contraction in %**	

	Day 3	Day 7	Day 14
Normal control	11.5±0.447	35.08±0.8983	47.25±1.0626
Disease control	5 ± 0.0264	23.5±0.0264	37.5±0.0264
Standard (sucralfate cream)200 mg/kg	17± 0.0185	49±0.0185	80±0.0185
Standard (sucralfate cream) 400 mg/kg	25±0.0185	55± 0.0185	86± 0.0185
Test 1 (hydrogel 200 mg/kg	10± 0.0185	41± 0.0185	66± 0.0185
Test 2 (hydrogel)400 mg/kg	18± 0.0185	51± 0.0192	78± 0.0185

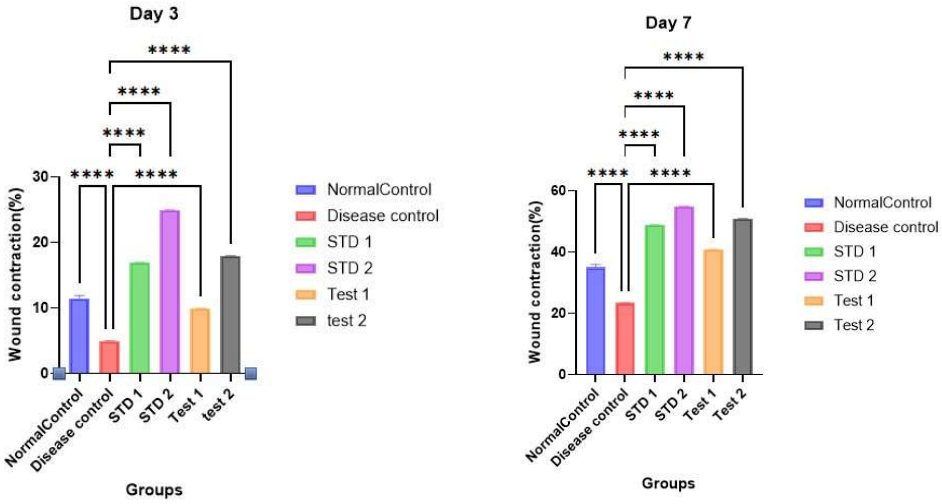
Table No 6.8 : effect of Hydrogel on percentage wound contraction in diabetic wound healing model
Values are presented as mean ± SEM, with N = 6 animals per group. Statistical analysis was performed using two-way ANOVA, followed by post hoc Dunnett's multiple comparisons test, with DC group as the reference control for all comparisons. The statistical significance of differences in % wound healing between treatment groups and the DC group is indicated by asterisks: p < 0.05 (*), p < 0.01 (**), p < 0.0001 (****), "ns" denotes non-significant differences (p > 0.05)

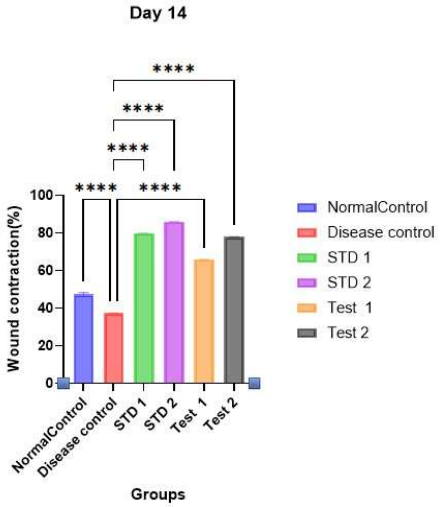
On the 14th day of treatment, the percentage wound contraction was significantly increased in all treated groups compared with the disease control group. The normal control group showed a wound contraction of **47.25 ± 1.0626%**, whereas the disease control group showed only **37.5 ± 0.0264%**, indicating delayed wound healing due to diabetic conditions.

The standard treatment groups showed marked improvement in wound contraction. Sucralfate cream-treated rats at **200 mg/kg** exhibited **80 ± 0.0185%** wound contraction, while the **400 mg/kg** dose showed maximum contraction of **86 ± 0.0185%** compared with the disease control group.

Among the hydrogel-treated groups, Test 1 hydrogel (**200 mg/kg**) showed **66 ± 0.0185%** wound contraction, whereas Test 2 hydrogel (**400 mg/kg**) demonstrated higher wound contraction of **78 ± 0.0185%** on the 14th day. The higher dose hydrogel formulation showed improved wound closure compared with the lower dose, indicating a dose-dependent wound healing activity.

The results suggest that the developed hydrogel formulation significantly enhanced wound contraction in diabetic rats and showed improved healing potential compared with the disease control group. The wound healing activity of the hydrogel may be attributed to the presence of bioactive phytoconstituents that promote tissue regeneration, collagen formation, antioxidant activity, and reduction of inflammatory responses during the healing process.

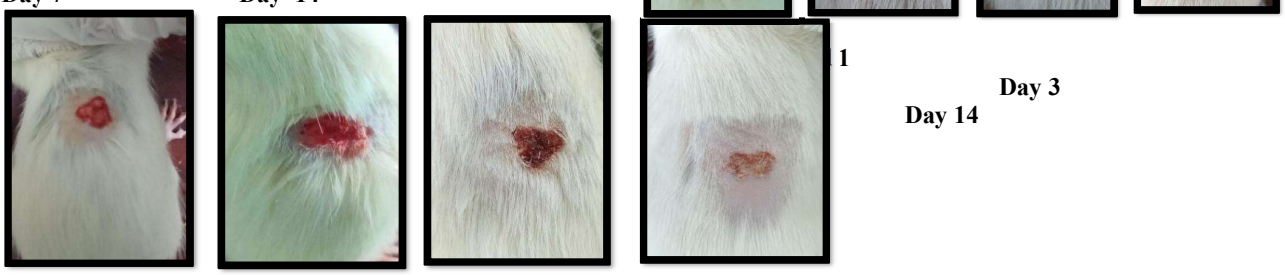




Day 0 **Day 3** **Day 7** **Day 14**

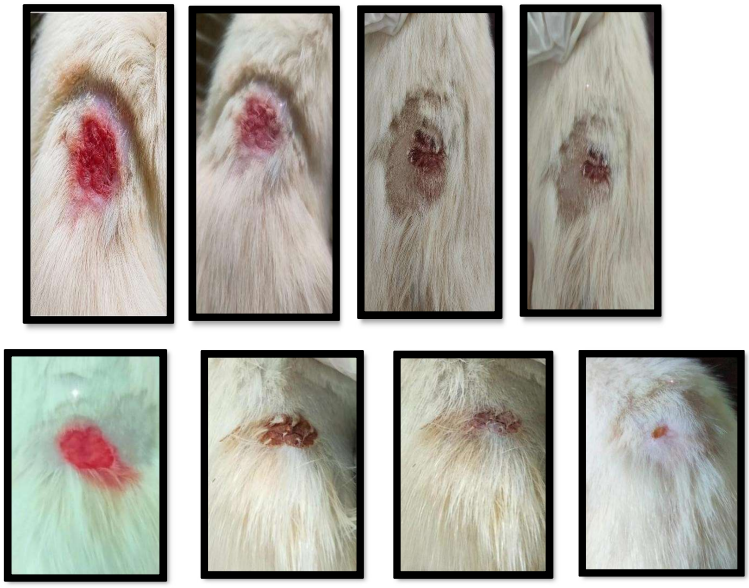
6.6.3.2 The wound were photographed on day 0,3,7,14-

Normal Control:



Day 0 **Day 3** **Day 7** **Day 14**

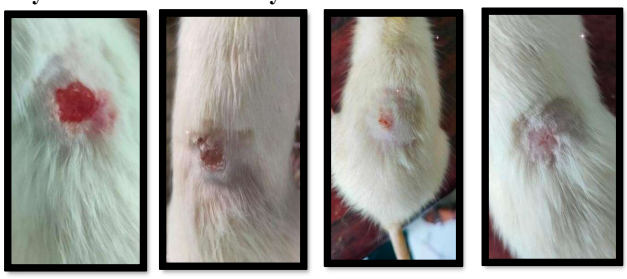
Disease control:



Standard Control 2

Day 0 **Day 3**

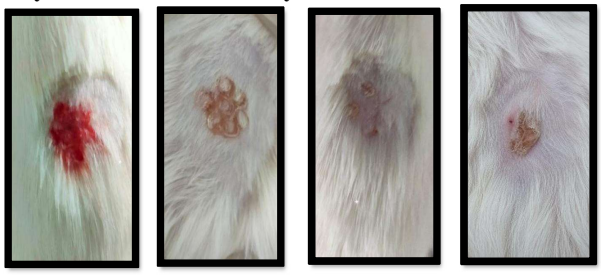
Day 7 **Day 14**



Test Group 1

Day 0 **Day 3**

Day 7 **Day 14**



Test Group 2

Day 0 **Day 3**

Day 7 **Day 14**

3.10 Blood Glucose Levels

Blood glucose level:-

Diabetic animals maintained persistently elevated blood glucose concentrations throughout the study. Hydrogel treatment progressively reduced blood glucose levels.

By Day 14, the low-dose and high-dose hydrogel groups exhibited glucose levels of **199.83 ± 1.78 mg/dL** and **205.66 ± 6.35 mg/dL**, respectively, compared with **278.66 ± 7.61 mg/dL** in the disease control group, indicating improved glycemic control.

Groups	(After Induction) Blood Glucose Level (mg/dl)			
	Day 0	Day 3	Day 7	Day 14
Norma control	115.33±1.2018	114.33±1.20185	113.33±1.201	112.33±1.2018
Disease control	269.66±10.58	272.5±8.864	276.66±8.244	278.66±7.605
Standard (sucral fate cream) 200mg/kg	265.33±2.7527	257.33±2.7527	235.33±2.7527	177.33±2.7527
Standard (sucral fate cream) 400mg/kg	271.16±1.8693	261.16±1.8693	235.16±2.2123	169.33±2.2123
Test (Hydrogel) 200mg/kg	274.5±1.6072	269.5±1.6072	250.83±1.7779	199.83±1.7779
Test (Hydrogel) 400mg/kg	289.66±6.3491	283.66±6.3491	262.66±6.3491	205.66±6.3465

Table No.6.9 : Effect of Hydrogel on blood glucose levels in HFD-STZ induced diabetes.

Values are presented as mean ± SEM, with N = 6 animals per group. Statistical analysis was performed using two-way ANOVA, followed by post hoc Dunnett's multiple comparisons test, with DC group as the reference control for all comparisons. The statistical significance of differences in blood glucose level treatment groups and the DC group is indicated by asterisks: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.0001$ (****), "ns" denotes non-significant differences ($p > 0.05$)

The effect of hydrogel treatment on blood glucose levels in HFD-STZ-induced diabetic rats was evaluated over a period of 14 days, and the results are presented in Table No. 6.9. The blood glucose levels were significantly elevated in all diabetic groups compared with the normal control group, confirming successful induction of diabetes by HFD-STZ administration.

The Normal control group maintained normal blood glucose levels throughout the study period, with values of 115.33 ± 1.2018 mg/dl, 114.33 ± 1.20185 mg/dl, 113.33 ± 1.201 mg/dl, and 112.33 ± 1.2018 mg/dl on day 0, day 3, day 7, and day 14, respectively. This indicates normal glucose homeostasis in non-diabetic animals.

The Disease control group showed a continuous increase in blood glucose levels during the experimental period. The glucose levels were increased from 269.66 ± 10.58 mg/dl on day 0 to 278.66 ± 7.605 mg/dl on day 14, indicating persistent hyperglycemia induced by HFD-STZ treatment.

The Standard treatment group (Sucralfate cream 200 mg/kg) showed a gradual reduction in blood glucose levels compared with the disease control group. The blood glucose level decreased from 265.33 ± 2.7527 mg/dl on day 0 to 177.33 ± 2.7527 mg/dl on day 14, indicating improvement in glycemic control.

The Standard treatment group (Sucralfate cream 400 mg/kg) showed a greater reduction in blood glucose levels compared with the lower dose standard group. The glucose level decreased from 271.16 ± 1.8693 mg/dl on day 0 to 169.33 ± 2.2123 mg/dl on day 14, demonstrating enhanced antihyperglycemic activity at a higher dose.

The Test hydrogel group (200 mg/kg) showed a progressive decrease in blood glucose levels during the treatment period. The blood glucose level reduced from 274.5 ± 1.6072 mg/dl on day 0 to 199.83 ± 1.7779 mg/dl on day 14, suggesting a beneficial effect of the hydrogel formulation on glucose regulation.

The Test hydrogel group (400 mg/kg) also exhibited a reduction in blood glucose levels during the study period. The glucose level decreased from 289.66 ± 6.3491 mg/dl on day 0 to 205.66 ± 6.3465 mg/dl on day 14. Although the reduction was lower compared with standard-treated groups, the higher dose hydrogel demonstrated improved antihyperglycemic potential compared with the disease control group.

Overall, hydrogel treatment resulted in a gradual decrease in blood glucose levels in HFD-STZ-induced diabetic rats compared with the disease control group. The observed antihyperglycemic activity may be attributed to the bioactive phytoconstituents present in the formulation, which may improve glucose utilization, reduce oxidative stress, and support diabetic wound healing.

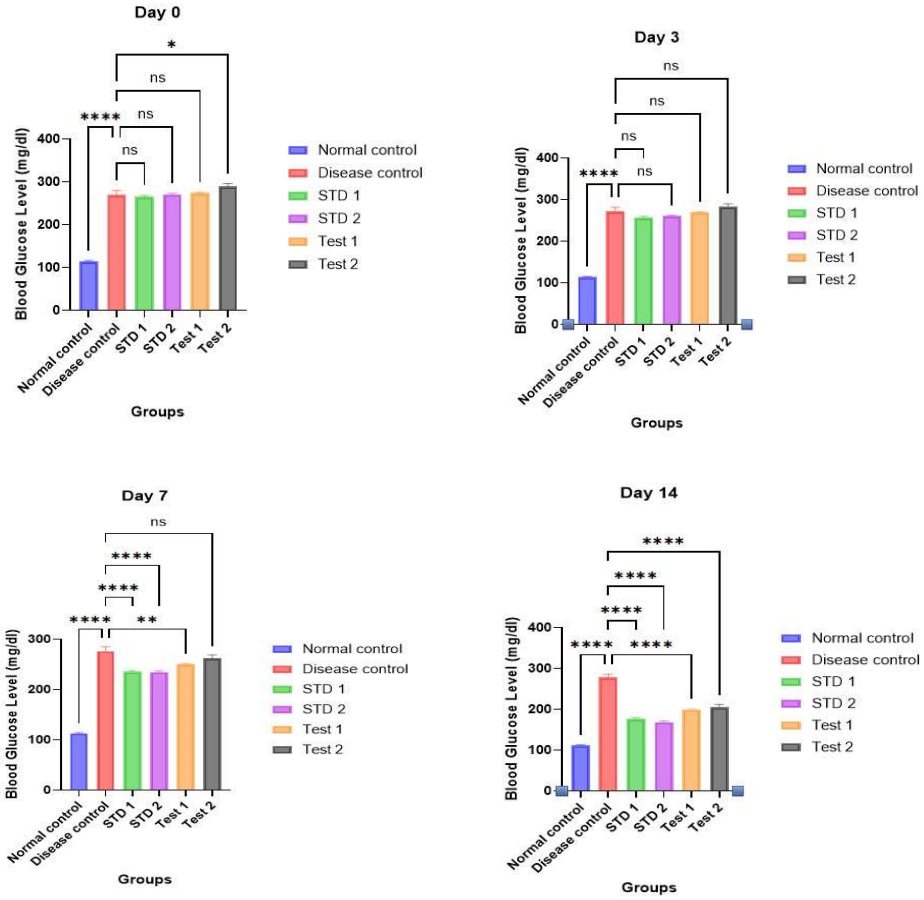


Fig No. 6.23 : Graphical Representation of decrease in blood glucose level

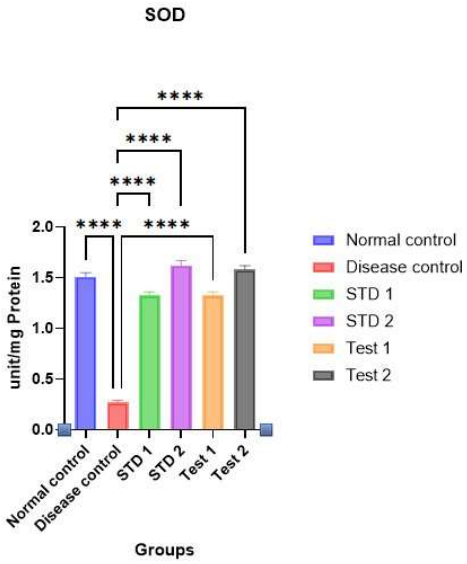
Standard (sucralfate cream) 400mg/kg	1.62±0.05	0.0037±0.0002
Test (Hydrogel) 200mg/kg	1.33±0.03	0.0028±0.0001
Test (Hydrogel) 400mg/kg	1.58±0.04	0.0031±0.0002

Table No. 6.10: Biochemical parameter of tissue homogenate

Antioxidant parameters:

Treatment	**SOD (unit/mg)**	**CAT Activity (µmoles of H_2O_2 decomposed/min/mg protein)**
Normal control	1.51± 0.04	0.004±0.0002
Disease control	0.27±0.02	0.0015±0.0001
Standard (sucralfate cream) 200mg/kg	1.33±0.03	0.0035±0.0002

The results are presented as #SEM and n = 6 ####p<0.0001 when compared with control and **** p < 0.0001 is considered as criteria for



significance when compared with Disease control using one way ANOVA coupled with Dunnet's comparison test.

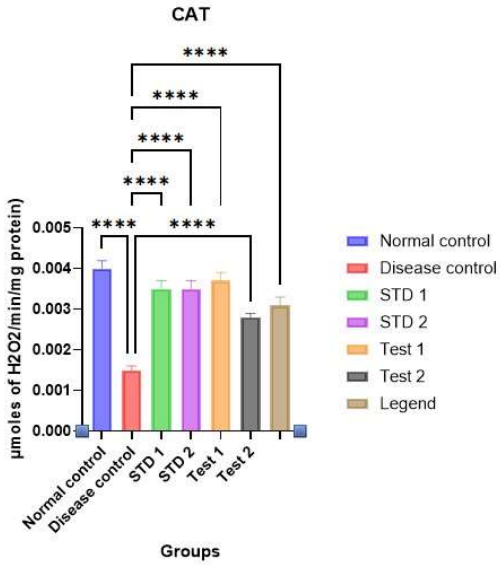
The **Normal control group** showed normal antioxidant enzyme activity with SOD and CAT levels of **1.51 ± 0.04 unit/mg protein** and **0.004 ± 0.0002 µmoles of H_2O_2 decomposed/min/mg protein**, respectively, indicating normal antioxidant defense mechanisms.

The **Disease control group** exhibited a marked reduction in antioxidant enzyme activity, with SOD and CAT levels of **0.27 ± 0.02 unit/mg protein** and **0.0015 ± 0.0001 µmoles of H_2O_2 decomposed/min/mg protein**, respectively. The decreased levels of SOD and CAT indicate increased oxidative stress and impairment of endogenous antioxidant defense associated with diabetic wound conditions.

The **Standard treatment group (Sucralfate cream 200 mg/kg)** showed significant improvement in antioxidant enzyme activity compared with the disease control group, with SOD and CAT levels of **1.33 ± 0.03 unit/mg protein** and **0.0035 ± 0.0002 µmoles of H_2O_2 decomposed/min/mg protein**, respectively.

The **Standard treatment group (Sucralfate cream 400 mg/kg)** demonstrated further enhancement of antioxidant activity, showing SOD and CAT levels of **1.62 ± 0.05 unit/mg protein** and **0.0037 ± 0.0002 µmoles of H_2O_2 decomposed/min/mg protein**, respectively. The higher dose standard treatment showed better restoration of antioxidant enzymes compared with the lower dose group.

The **Test hydrogel group (200 mg/kg)** showed an increase in antioxidant enzyme levels compared with the disease control group. The SOD and CAT activities were found to be **1.33 ± 0.03 unit/mg protein** and **0.0028 ± 0.0001 µmoles of H_2O_2 decomposed/min/mg protein**, respectively, indicating the antioxidant potential of the hydrogel formulation.



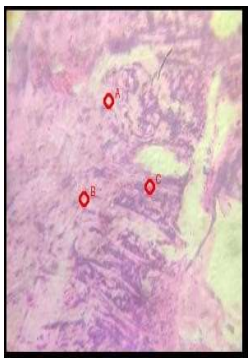
The **Test hydrogel group (400 mg/kg)** showed improved antioxidant status with SOD and CAT levels of **1.58 ± 0.04 unit/mg protein** and **0.0031 ± 0.0002 µmoles of H_2O_2 decomposed/min/mg protein**, respectively. The higher dose hydrogel exhibited greater restoration of antioxidant enzyme activity compared with the lower dose hydrogel group.

Overall, hydrogel treatment significantly enhanced SOD and CAT activity compared with the disease control group, suggesting reduction of oxidative stress and improvement in antioxidant defense mechanisms during diabetic wound healing. The antioxidant activity of the hydrogel may be attributed to the bioactive phytoconstituents present in the formulation, which contribute to free radical scavenging and tissue repair.

Histopathology

Normal control

Disease control



Normal control

A – Dense Collagen Fibers

Well-organized and densely packed collagen bundles indicating normal extracellular matrix architecture and tissue integrity.

B– Normal Connective Tissue (Dermis)

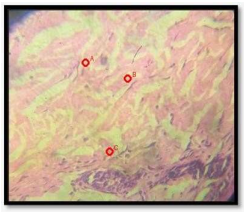
Intact connective tissue with normal arrangement of dermal components and absence of inflammatory cell infiltration.

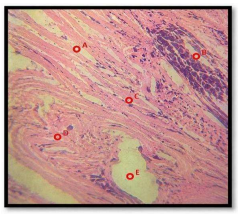
C – Regular Collagen Orientation

Uniformly arranged collagen fibers demonstrating healthy skin structure and normal wound-free tissue morphology.

Disease control

A – Inflammatory





Cell Infiltration

Presence of inflammatory cells indicating persistent inflammation and delayed healing response.

B– Disorganized Collagen Fibers

Irregular and disrupted collagen arrangement suggesting impaired extracellular matrix remodeling.

C – Tissue Degeneration / Unhealed Wound Area

Damaged tissue architecture with incomplete regeneration, characteristic of delayed diabetic wound healing.

Standard control 1:

A – Collagen Deposition

Moderate to dense collagen fiber deposition indicating active extracellular matrix formation.

B– Fibroblast Proliferation

Presence of fibroblasts contributing to collagen synthesis and tissue remodeling.

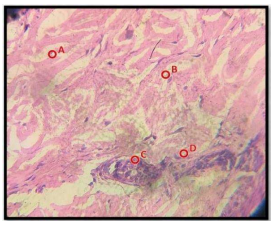
C – Re-epithelialization / Tissue Regeneration

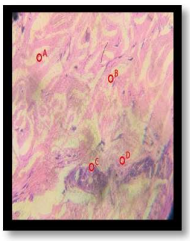
Standard control 1

Standard control 2

Test 1

Test 2





Regenerating tissue with improved structural organization suggesting progression of wound healing.

Standard control 2

A – Dense Collagen Fibers

B– Fibroblast Proliferation

C – Neovascularization (New Blood Vessel Formation)

D – Re-epithelialization / Granulation Tissue (upper right region)

Test 1

A – Dense Collagen Deposition
(Upper left to central pink wavy bundles)

B – Fibroblast Proliferation
(Spindle-shaped dark nuclei scattered within collagen fibers)

C – Neovascularization (New Blood Vessel Formation)
(Dark oval structure present in the lower central region)

D – Granulation Tissue Formation
(Tissue surrounding the newly formed blood vessel)

Test 2

A – Dense Collagen Deposition
(Upper left to central pink wavy bundles)

B – Fibroblast Proliferation
(Spindle-shaped dark nuclei scattered within collagen fibers)

C – Neovascularization (New Blood Vessel Formation)
(Dark oval structure present in the lower central region)

D – Granulation Tissue Formation
(Tissue surrounding the newly formed blood vessel)

DISCUSSION

The present study was carried out to formulate and evaluate a novel hydrogel containing *Ulva lactuca* extract fortified with anti-inflammatory phytochemicals for diabetic wound healing. Preliminary phytochemical screening confirmed the presence of flavonoids, phenolic compounds, tannins, alkaloids, carbohydrates,

saponins, glycosides, phytosterols, and triterpenoids, indicating the therapeutic potential of the extract. Quantitative estimation of total flavonoid and carbohydrate content further supported the presence of biologically active constituents responsible for antioxidant and wound healing activities. [7–13, 14–29, 30–32]

The prepared hydrogel formulations using HPMC K100 M and xanthan gum exhibited satisfactory physicochemical characteristics including good homogeneity, suitable viscosity, acceptable pH, and good retention ability. The formulation showed appropriate consistency and spreadability, indicating suitability for topical application and prolonged retention at the wound site. The presence of propylene glycol improved dispersion of active constituents, while curcumin contributed additional antioxidant and anti-inflammatory activity. [9–13, 15–18, 33]

The antioxidant activity evaluated by DPPH scavenging assay demonstrated significant free radical scavenging potential of the extract in a concentration-dependent manner. Increased antioxidant activity may help reduce oxidative stress, which is one of the major causes of delayed diabetic wound healing. The anti-inflammatory activity evaluated by protein denaturation assay showed effective inhibition of protein denaturation, suggesting the ability of the formulation to reduce inflammation and promote faster tissue repair. [7, 19, 20, 29, 32, 34]

The glucose uptake assay demonstrated enhanced glucose utilization by yeast cells in the presence of the extract, indicating possible anti-diabetic activity. Improvement in glucose metabolism may help reduce complications associated with diabetic wounds and support normal healing processes. [29, 34]

Biochemical estimations revealed improvement in antioxidant enzyme levels such as Superoxide Dismutase (SOD) and Catalase (CAT) in treated groups compared to diabetic control animals. Increased SOD and CAT activity indicates enhanced endogenous antioxidant defense against reactive oxygen species generated during diabetic conditions. Lipid profile studies also showed reduction in total cholesterol, triglycerides, and LDL levels along with improvement in HDL levels, suggesting beneficial effects on diabetic dyslipidemia and tissue repair. [5–7, 19, 20, 29]

Histopathological examination of the treated group revealed improved re-epithelialization with well-organized collagen deposition and reduced inflammatory cell infiltration compared to the disease control group, indicating enhanced tissue regeneration and accelerated wound healing. [3–7, 21–23, 40]

The overall findings indicate that the developed hydrogel formulation possesses antioxidant, antiinflammatory, anti-diabetic, and wound healing properties. The synergistic effect of *Ulva lactuca* extract, curcumin, and hydrogel polymers may help maintain a moist wound environment, reduce oxidative damage and inflammation, improve cellular proliferation, and accelerate diabetic wound healing. Therefore, the developed hydrogel formulation may serve as a promising natural therapeutic approach for diabetic wound management. [9–13, 17–23, 29]

Conclusion:

- As indicated by the results, the Ulva lactuca eseliad loaded hydrogel formulation was found to be efficient in diabetic wound healing since it exhibited. [3, 5–7, 9–13, 17–23, 29]
- Antioxidant, Antiinflammatory, dermoprotective cytoproliferative activties result in acecclarated wound healing. [3, 5–7, 9–13, 17–23, 29]
- Their activity may be athributed to ulvin (Sulphated polysaccrides) crosslinking with collagen and other matrix proteins. [16–18]
- The reported & experimentally established phytoconstituents like flavonoids & polyphenolics may also have reduced matrix metalloproteinase activity subdued cytokine (i1-6 ,TNF 6 & other) induced cyclic wound progression [7, 21–23, 29]
- Possible antibacterial activity of phytoconstituents of *Ulva lactuca* might also have worked in favour of efficacy of the hydrogel by inhibiting bacterial infections. [20, 24–29]
- In summary, this hydrogel may prove to cost-effective, natural & faster acting alternative to available treatment and can provide a deliverable product since its production can be scaled up. [9–13, 21–29]

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