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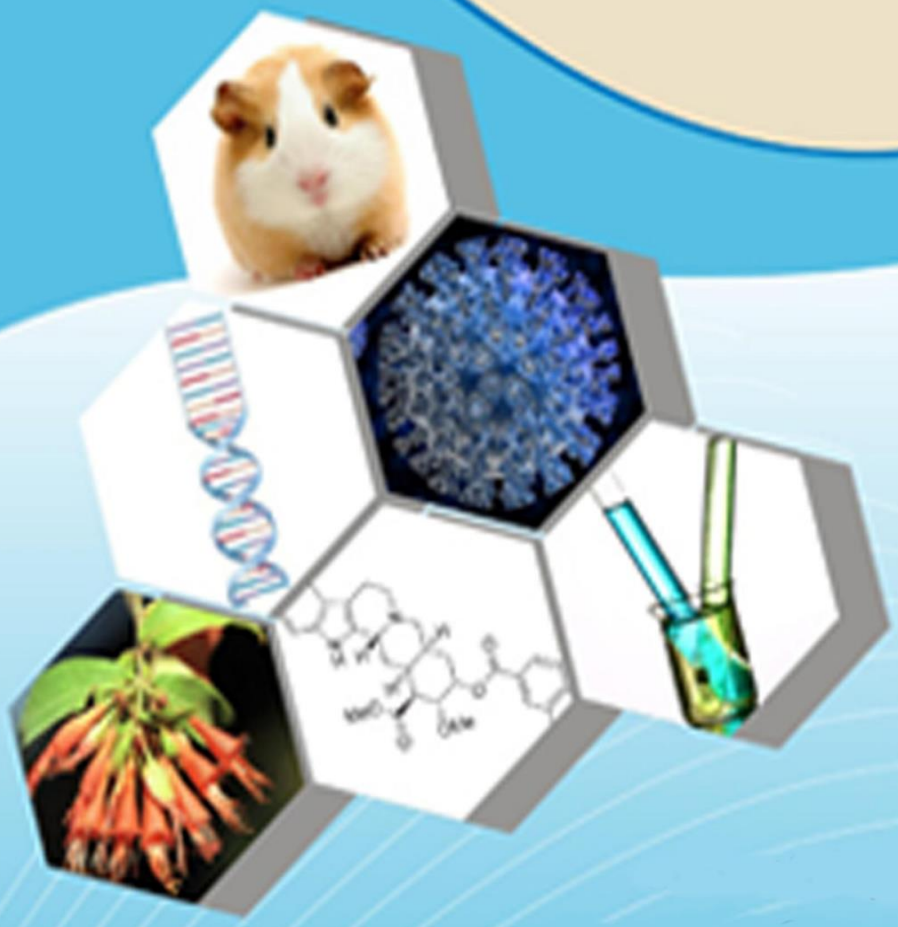

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Assessment of Baicalein-Loaded Hydrogel for Diabetic Wound Healing Management

K.Sujitha [1],N.Rajashekar [2],G.Pratyusha [3],Dodda Neelima [4,]

ABSTRACT

The goal of the current research was to thoroughly investigate the in-vivo wound healing impact of produced baicalein (BCA) loaded hydrogel and compare the results with the commercial formulation. In a prior investigation, prepared hydrogels were previously described and optimized. Glycol chitosan gellan gum polymers were used to create baicalein-loaded hydrogel (GG-GC-HGs). Rats with diabetes wound models (induced by streptozotocin) were used to assess the wound-healing potential of prepared hydrogels. Measurements of wound contraction and biochemical analyses (Hydroxyproline, protein content, and antioxidant test) in the treated wound tissue were used to assess the impact of wound healing. Hematological analysis of the tissue from the wound was done. The study's findings demonstrated that after 10 days of therapy, the percentage of wound contraction in the animal group treated with baicalein loaded GG-GC-HGs decreased significantly ($p < 0.05$), and on day 18, the wounds fully healed. Treatment of baicalein-loaded GG-GC-HGs resulted in a considerable increase in hydroxyproline and protein content; the findings were equivalent to those of the animal reference group (Hydroheal Gel). Following treatment with BCA-loaded GG-GC-HGs, antioxidant status was recovered. These findings were corroborated by histological examination of the wound tissues. In conclusion, baicalein-loaded hydrogel significantly improved diabetic wound healing by promoting fibroblast proliferation, enhancing epithelialization, and lowering oxidative stress.

Introduction

Hydrogels are hydrophilic in nature because of contains some specific hydrophilic functional group (eg. hydroxy, amide and hydrogen sulfite) in the gel form. The presence of polymeric substance in the hydrogel is responsible for greater absorption efficacy. Food and biomaterial scientists refer to polymeric cross-linked network structures as gels or hydrogels interchangeably.[1] Cross linking in hydrogels avoids their crushing during swelling.

Softness, swelling, absorbent property, elasticity, flexibility, and the ability to store water are among the crucial characteristics of hydrogels.[2] As Hydrogels are known for having greater absorption capacity with water that is unique, simulating biological tissue when swollen, since hydrogels characteristics mimic to the real tissues, that's by they have good biocompatibility. and looks like natural living tissue.[3,4] The localized and continuous release of a medicine was discovered to be made easier by hydrogels since they require fewer administrations, don't cause damage, and allow for slightly smaller doses**.**[3]Hydrogels resemble living tissue when swelled and distended because they have low interfacial tension with water and other biological fluids. This characteristic has been successfully applied in the field of tissue engineering. The gel's elastomeric nature also helps lessen mechanical friction between tissues. Because they are non-toxic, biocompatible, biodegradable, readily available, and able to alter the characteristics of an aqueous environment as well as thicken, emulsify, stabilize, encapsulate, and swell as well as form gel films, natural polymers, particularly polysaccharides, have been used to make hydrogel.[5]

Assistant professor [1,2,3,4],
Department of Pharmacy,
Samskruti College of Pharmacy,
Kondapur (V), Ghatkesar (M) Medchal Dist, Telangana, India.

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The biological process of wound healing restores the structural integrity of the damaged skin by causing the skin tissues to grow and regenerate on their own.[6] It often occurs through a series of connected and overlapping processes, including hemostasis, inflammation, angiogenesis, fibroblast proliferation, and tissue remodeling.[7]

Numerous cellular and matrix-building systems cooperate to mend the wound during the repair of wound. In diabetic patients wounds are difficult to repair and becomes as chronic wound. These persistent infections, protracted inflammations, and impaired response of epidermal or dermal cells to stimuli frequently develop in these chronic wounds. Additional anomalies that contribute to the delay in the healing of diabetic wounds include immunopathy, neuropathy, vasculopathy, a lack of growth factors, and the development of biofilms. Additionally, fibroblasts cannot create ECM proteins, and keratinocytes cannot form epithelium. These factors can also cause excessive connective tissue to be deposited, a disease known as fibrosis.[8] Therefore, modified strategies are needed today for diabetic wound healing due to failure of current approaches. Baicalein (BCA) is widely reported for antimicrobial, anti-inflammatory, anticancer, antioxidant, antifungal, anti-fibrosis and protecting to neuronsproperties.[9,10] BCA-based hydrogels could be effectively used in diabetic wound repair due to its antioxidant, antibacterial, antimicrobial and antidiabetic properties. Reactive oxygen species are produced in diabetic wounds to stop bacterial invasion and stop wound infection. In a persistently hyperglycemic microenvironment, reactive oxygen species are released in excessive amounts. Hematopoiesis is hindered as a result, and inflammatory factors are upregulated. Reactive oxygen species overproduction causes diabetic chronic wounds to close more slowly than they should, which is still a problem on a global scale.[11,12] BCA already have been reported as potent antioxidant, anti-inflammatory and antidiabetic agent that work synergistically to repair the chronic wound.[13] Based on these reports, present study was aimed to prepare natural polymer based (glycol chitosan and gellan gum) hydrogel and evaluated for diabetic wound healing effect.

Material and Methods

Materials

Glycol Chitosan was purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Gellan gum powder was obtained from MP Biomedicals, LLC. Baicalein and Calcium chloride was purchased from Sigma Aldrich, Mumbai. All other materials and reagents were used with analytical grade.

Preparation of Hydrogel Formulations

Accurately weighed amount of gellan gum (1% w/v) was dissolved in deionized water. It was continuously stirred for up to 30 minutes until complete hydration. It is required to maintain the temperature of deionized water up to 90°C. CaCl2 solution (0.5% w/v) was added to above gellan gum solution. Three different concentrations of glycol chitosan (0.1, 0.3, 0.5% w/v) were prepared by dissolving glycol chitosan in distilled water. Dropwise addition of gellan gum solution to the glycol chitosan polymer solution with constant stirring at ambient temperature and this is considered as plane hydrogel i.e. hydrogel without drug.

Baicalein-loaded hydrogel was prepared by following same method while initially baicalein was dispersed in distilled water with sonication (Probe Sonicator Advanced, PKS-500F, PCI Analytics, Mumbai, India). Drug dispersion was added into the polymer solution dropwise with continuous stirring. Gellan gum solution was added into drug-containing polymer solution with constant stirring to prepare baicalein loaded glycol chitosan gellan gum hydrogel (GC-GC-HGs).

Characterization of Hydrogel Formulations

Prepared hydrogels were characterized by using different parameters i.e. swelling property, surface morphology, entrapment efficiency, rheology behavior, drug release and stability studies. All these studies were already submitted for publication in another journal and it is under consideration. The optimized formulation was used for further *in-vivo* study for diabetic wound healing effect.

Evaluation of Prepared Hydrogels for Wound Healing Effect

Animals and protocol

All the Inbred house wistar rats weighing 200 to 250 g of either sex were selected for wound healing study and adapted to the hygienic conditions of a laboratory for seven days before to the experiment. A standard laboratory meal for feeding, a commercial pellet diet (Hindustan Lever Pvt, Bangalore, India) and an endless supply of fresh water were used. Animals were divided into three groups each group containing six animals. The control group received plane hydrogel, second group represented as test and received BCA

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loaded hydrogel, while the reference group was given marketed formulation, namely Hydroheal Gel (Dr. Reddy's Laboratories Ltd.) twice daily.

Antioxidants Assay

Antioxidant assay performed by taking a small part of wound tissue collected from the different treatment group of diabetic animals followed by homogenization in phosphate buffer (pH, 7.0), and centrifugation under low temperature under cold. Finally, the antioxidant level was determined in the clear supernatant after centrifugation. The catalase level was estimated following the breakdown of hydrogen peroxide according to the method of Beers and Sizer (1952).[19] Superoxide dismutase (SOD) was assayed according to Misra and Fridovich (1972)[20] based on the enzyme's ability to prevent epinephrine autoxidation. Using the method of Moron *et al*, (1979),[21] reduced glutathione (GSH) concentration was estimated in wound tissue. With 0.1 mL of 25% TCA, tissue homogenates were promptly precipitated, then separated using centrifugation. A UV spectrophotometer was used to measure the amount of free-SH groups in 3 ml of sample by adding 2 mL of 0.6 mM DTNB and 0.9 ml of 0.2 mM sodium phosphate buffer (pH 8.0) to 0.1 mL of the tissue supernatant.

Histological Study

The wound tissue was collected from different treatment groups of diabetic animals and store in 10% formalin. A thick tissue section (6 µm) was cut after the standard processing and stained with haematoxylin and eosin.[22] All selected sections were qualitatively observed under light microscope for presence of fibroblast cells proliferation, collagen fibres and epithelialization of epidermal cells. The observation was recorded in terms of inflammatory cells, fibroblast and collagen fibres, and epithelialization.

Statistical Analysis

Data were represented as mean ± SD for all statistical analysis. Each treatment group contained six animals. All data was analyzed statistically using mean values and ANOVA as well as by the multiple comparisons test (Tukey's). GraphPad Instat software executed a statistical analysis of the results. Presented data were considered statistically significant, if $p < 0.05$.

Results

Diabetes often results in the chronic (delayed-healing) wound, which can harbor infections that can be fatal. More and more data points to the complicated biological processes occurring within the diabetic wound, including persistent inflammation, compromised blood vessels, and improper collagen remodeling, as the cause of refractory healing.[23] Prepared baicalein-loaded Hydrogel (GG-GC HGs) was evaluated for wound healing effect in diabetic wound model. Wound healing effect was observed by observation of wound contraction, biochemical assessment and antioxidant assay in the wound tissue after treatment with GG-GC HGs.

Wound Healing Effect

Wound contraction was measured up to 20 days with the help of transparent paper tracing on each 2 days interval. The treated animal group with baicalein loaded hydrogel (GG-GC-HGs) from 2 to 8 days showed a slight increase in wound contraction compared to the control group that received blank. Compared to the control animal group, a group treatment with baicalein loaded hydrogel caused a significant ($p < 0.05$) difference in the percentage of wound contraction from day 10 to day 18. It has observed that animal treated with baicalein-loaded hydrogel showed 96.81% healing, whereas rats treated with reference hydrogel showed 94.79% healing, were compared to the controls (70.38 %), on the 16th day. On day 18th, neither the animal treated with the baicalein-loaded hydrogel nor reference hydrogel had any scars that had fully healed, which was a sign of full recovery (Table 1 and Fig. 1). The term "epithelialization time" describes the period of time it takes for a wound to seem fully healed, free of moist granulation tissue and coated in fresh epithelium.[24] The epithelialization period for baicalein loaded hydrogel (GG-GC) and standard groups was found 18 days, which was less than the control group.

Effect of Prepared Hydrogels on Protein and Hydroxyproline Content

The granulation tissue's total protein content is a key measure of the degree of protein synthesis as well as cellular proliferation. A rapid increase in protein content was noticed in the case of animals treated with BCA-loaded GG-GC hydrogels as compared to blank group of animals. The observation of BCA-loaded GG-GC hydrogel treated animals suggests that protein content increased due to BCA-loaded GG-GC hydrogel stimulating cellular proliferation through a specific mechanism. The protein content was found to be 72.97 ± 1.71 for animals treated with BCA-loaded GG-GC hydrogel and 73.09 ± 1.67 for reference gel while for animals treated with control group was found to be 47.51 ± 0.83 mg/g of tissue (Table 2 and Fig. 2).

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Hydroxyproline is a key component of collagen and represent as an amino acid necessary for collagen synthesis. Therefore hydroxyproline content measurement is marker of collagen turnover.[25] This improvement in hydroxyproline level showed improved collagen content, since hydroxyproline measurement is the direct marker to estimate collagen synthesis. The level of hydroxyproline content was significantly increased for animals treated with BCA loaded GG-GC hydrogel treated animals compared to the control group. In case of treated animals hydroxyproline content was found to be 68.19 ± 1.46 mg/g of tissue, significantly higher than animals treated with control group i.e. 21.19 ± 0.09 mg/g of tissue.

Antioxidants Level

Baicalein-loaded hydrogel exhibited potent antioxidant activity which can be justified by an increased level of GSH (24.47 ± 0.88 µmol/50 mg tissue), SOD (34.57 ± 0.61 µg/50 mg tissue) a nd c atalase l evel (27.57 ± 0.64 µmol/50 mg tissue) in to the wound tissues collected from different group of diabetic animals during the healing process (Table 3 and Fig. 3). The significant increase was observed in SOD, GSH and CAT level after 16th day in diabetic wounds. Because of reduced activity of SOD and GSH and a higher level of MDA, a lipid peroxidation marker is present that indicates an increase in oxidative stress.[26] Reduced activity of antioxidant substances like SOD results in impairment in the conversion of H2O2 into H2O and O2. It may increase the amount of peroxide-free radicals produced, trigger lipid peroxidation, and start the inflammatory cascade with MDA, causes hindrance in

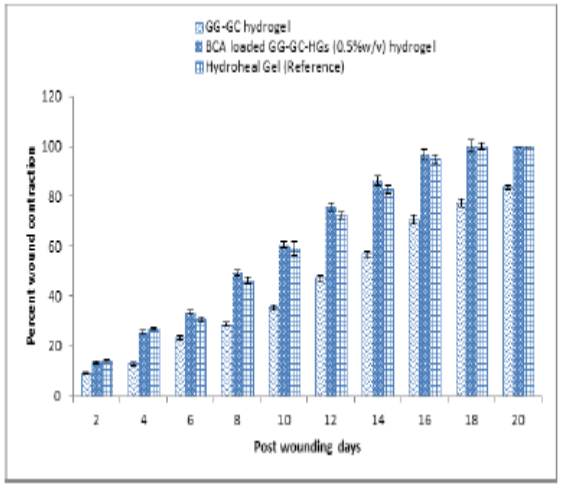

Fig. 1: Effect of prepared baicalein loaded hydrogel (GG-GC-HGs) on wound contraction of diabetic wound animals.

Table 1: Effect of prepared baicalein loaded hydrogel (GG-GC HGs) on wound contraction of diabetic wound animals

Animal Groups	Post wounding days (Percent wound contraction)										Epithelialization period
	2	4	6	8	10	12	14	16	18	20	
GG-GC hydrogel	9.24 ± 0.08	12.62 ± 0.62	23.15 ± 0.77	28.60 ± 0.68	35.27 ± 0.92	47.27 ± 1.05	56.61 ± 1.20	70.38 ± 1.84	77.36 ± 1.67	83.48 ± 1.05	23
BCA loaded GG-GC (0.5% w/v) hydrogel	13.47 ± 0.43	25.39 ± 0.88	33.24 ± 0.82	49.54 ± 1.08	60.59 ± 1.17*	75.71 ± 1.62*	86.34 ± 1.89*	96.81 ± 1.92*	100.00 ± 2.30*	-	18
Hydroheal gel (Reference)	14.08 ± 0.08	26.74 ± 0.37	30.51 ± 0.85	46.37 ± 1.42	58.91 ± 2.71	72.18 ± 1.27	82.53 ± 1.69	94.79 ± 1.90	100.00 ± 1.31	-	18

n = 6 per group, tabulated data represents Mean±S.D. *p < 0.50, when compared each treated group with control group

Table 2: Effect of prepared baicalein loaded hydrogel (GG-GC HGs) on protein content and hydroxyproline content of diabetic wound animals

Animal groups	Protein content (mg/g of tissue)	Hydroxyproline content (mg/g of tissue)
GG-GC (0.5% wv) hydrogel	47.51 ± 0.83	21.19 ± 0.09
BCA loaded GG-GC (0.5% wv) hydrogel	72.97 ± 1.71*	68.19 ± 1.46*
Hydroheal gel (Reference)	73.09 ± 1.67*	70.47 ± 1.27*

n = 6 per group, tabular data represents Mean ± S.D. *p < 0.50, when compared treated group with control group

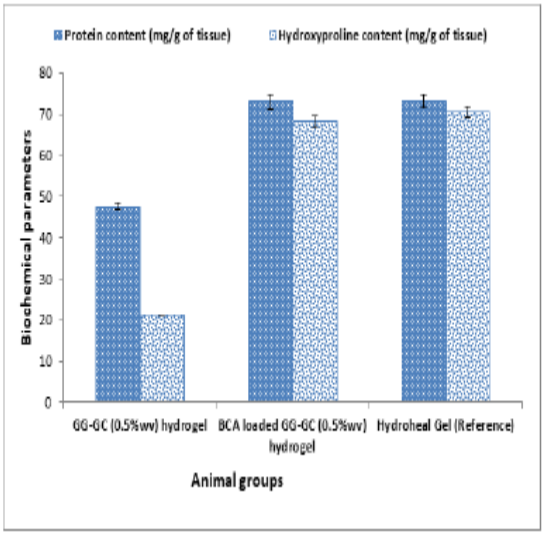

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Fig. 2: Effect of prepared baicalein-loaded hydrogel (GG-GC HGs) on protein content and hydroxyproline content of diabetic wound animals

Table 3: Effect of prepared Baicalein loaded Hydrogel (GG-GC HGs) on antioxidants level in a diabetic wound model

Animal groups	*Content of SOD (µg/50 mg tissue)*	*Content of CAT (µmol/50 mg tissue)*	*Content of GSH (µmol/50 mg tissue)*
GG-GC (0.5% wv) hydrogel	13.09 ± 0.46	11.41 ± 0.71	10.21 ± 0.69
BCA-loaded GG-GC (0.5% wv) hydrogel	34.57 ± 0.61	27.57 ± 0.64*	24.47 ± 0.88*
Hydroheal gel (Reference)	32.19 ± 0.55	25.94 ± 0.43*	25.31 ± 0.48*

n = 6 per group, tabulated data represents as Mean ± S.D. *p < 0.50, when comparing treated group with control group

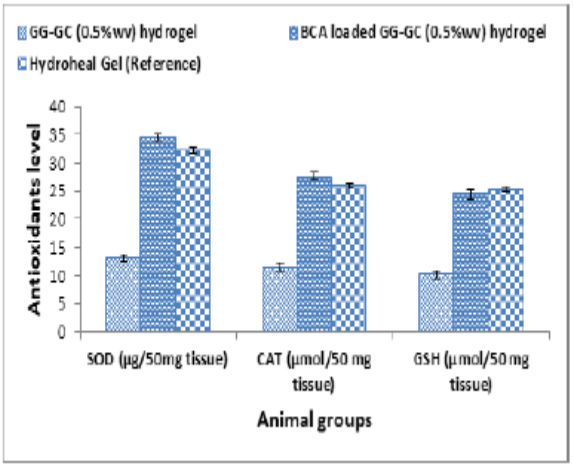

Fig. 3: Effect of prepared baicalein-loaded hydrogel (GG-GC HGs) on antioxidants level of diabetic wound animals

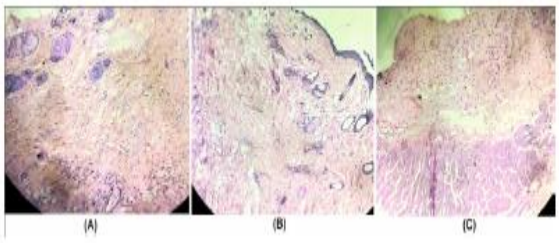

Fig. 4: Photomicrograph of histological observations of different treatment group with prepared hydrogels. (A) Without drug GG-GC hydrogel; (B) BCA loaded GG-GC-HGs; (C) Hydroheal gel (Reference)

wound healing. Additionally, catalase causes degradation of H2O2 into H2O and O2, and GPx acts as a scavenger of cellular peroxides. In brief, an optimum SOD and GSH level accelerates the wound healing process.[27] Baicalein-loaded hydrogel showed significant antioxidant activity and improved wound healing.

Histological Observations

According to histopathological observations, fibroblast cells were dense, well-organized collagen fibers increased, and new blood vessel creation was seen in BCA loaded GG-GC-HGs treated animal group when compared to GG-GC group (Fig. 4). Control group have shown to have some inflammatory cells and edema. In the control group, collagen fibers were not found while these were very prominent in the case of reference and BCA loaded GG-GC-HGs treated group.

Discussion

Results of present study confirmed that, baicalein loaded hydrogel demonstrated strong wound healing potential as measured by a rise in the protein and hydroxyproline levels along with strong antioxidant activity. The healed wounds had tissue regrowth, a noticeable dryness of the wound edges, and a significant reduction in wound area compared to the control group, which confirms the potential of baicalein-loaded hydrogel in wound healing. Healing an injury is a dynamic, complex process. The individual's fluctuating state of health has an impact on the wound environment. Understanding the fundamental concepts of wound healing can be framed by knowing the physiology of the typical wound healing trajectory through the four sequential phases of hemostasis, inflammation, granulation, and maturation.[28] Bacteria and debris are phagocytosed and eliminated during the inflammatory phase, while cytokines and mediators are released, causing cells to migrate and divide during the proliferative phase.[8] Angiogenesis, granulation tissue formation, and epithelization speedup in the proliferative phase correlated by increase in wound contraction with the treatment of baicalein-loaded hydrogel. The epithelial cells crawl across the wound bed during epithelization to cover it.[29] Myofibroblasts, which grab onto the borders of the wound and contract themselves via a mechanism identical to that of smooth muscle cells, work to shrink the wound during wound contraction. Collagen is reshaped and straightened along tension lines during the maturation and remodeling phase, and apoptotic cells that are no longer required are

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eliminated.[30] Hydroxyproline is a marker of collagen biosynthesis. Improved level of hydroxyproline may correlate with the strength and integrity of the tissue matrix. Additionally, hydroxyproline is crucial for homeostasis and epithelialization in the later stages of recovery.

Reactive oxygen species is a key regulator of oxidative stress that causes drop in antioxidant enzyme levels, such as SOD and GSH.[31] Collagen synthesis depends primarily on hydroxyproline, which also speeds up angiogenesis and oxygen transport to the site of the lesion.[32] Treatment with baicalein loaded hydrogel increases the levels of SOD, GSH. This reduced oxidative stress may another factor to improve the healing process. The injured tissue's protein level and cellular proliferation are predicted by its protein concentration.[33] I n t he present study, baicalein loaded hydrogel increased the protein content in the diabetic rats. This would mean that baicalein increases protein synthesis, cellular proliferation, and migration in the tissue of wounds. Increased free radical production and decreased antioxidant activity could worsen things and explain why repairing takes longer in diabetes. In present study, catalase, GSH and SOD levels increased, and oxidative stress at the wound site was diminished when treated with baicalain loaded hydrogel. The delayed healing in diabetic animals has been restored when treated with BCA. Baicalein shows anti-inflammatory and antibacterial activity and exhibits wound healing activity through its antioxidant property. [34, 35]

Conclusion

The outcomes from the present study suggested that BCA loaded hydrogel can accelerate the delayed healing in streptozotocin-induced diabetic animals by increasing the levels of natural antioxidants (via increasing levels of SOD, catalase and GSH) in the wound tissues. BCA loaded hydrogel significantly accelerated the wound contraction process, increasing epithelisation, confirmed by increased protein content and hydroxyproline levels in treated animals. All these biomarkers give the confirmation about improvement in diabetic wound healing of treated animals. Therefore, the prepared baicalein-loaded hydrogel through the anti-inflammatory and antioxidant potential of baicalein could support fast wound healing in diabetic conditions.

References

1. *Varaprasad K, Murali M, Vimala KK, Mohana R. Synthesis and Characterization of Hydrogel-Silver Nanoparticle-Curcumin Composites for Wound Dressing and Antibacterial Application. Colloids Surf B Biointerfaces. 2011;121:784-794. Available from: doi.org/10.1002/app.33508*
2. *Pande PP, Anamica. Polymer Hydrogels and Their Applications. International Journal of Materials Science. 2017; 12: 11-17.*
3. *Buwalda SJ, Boere KW, Dijkstra PJ, Feijen J, Vermonden T, Hennink WE. Hydrogels in a historical perspective from simple networks to smart materials. Journal of Control Release. 2014;03:1-22. Available from: doi.org/10.1016/j.jconrel.2014.03.052*
4. *Peppasa NA, Buresa N, Leobandunga W, Chikawab H. Hydrogels in pharmaceutical formulations. European Journal of Pharmaceutics and Biopharmaceutics. 2000;50:27-46. Available from: doi. org/10.1016/s0939-6411(00)00090-4*
5. *Singh V, Chaubey N. Design and Evaluation of Topical Hydrogel Formulation of Aceclofenacfor Improved Therapy. Journal of Drug Delivery & Therapeutics. 2019; 9(5):118-122. Available from: doi. org/10.22270/jddt.v9i5.3605*
6. *Masood N, Ahmed R, Tariq M, Ahmed Z, Masoud MS, Ali I, Asghar R, Andleeb A, Hasan A. Silver nanoparticle impregnated chitosan- PEG hydrogel enhances wound healing in diabetes induced rabbits. International Journal of Pharmaceutics. 2019;559:23-36. Available from: doi.org/10.1016/j.ijpharm.2019.01.019.*

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