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Original Article

Wound Healing Dressing System for Diabetic Wounds Based on Curcumin and Syringic Acid

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ABSTRACT

Background: This work is based on the development of a wound healing dressing system for diabetic wounds using curcumin and syringic acid. A diabetic wound differs from a normal wound in respect to many pathophysiological changes. Therefore multiple issues like hemostasis, inflammation, cell proliferation and tissue remodeling need to be considered while selecting actives. A combination of curcumin, syringic acid and *Aloe vera* can address such aspects of pathophysiological changes in diabetic wound healing. **Materials and Methods:** Initially curcumin and syringic acid were mixed with *Aloe vera* juice. Carbopol 934 was used as a gelling agent for this mixture. This gel was loaded into sterilized polyurethane foam. The prepared dressing system was evaluated for *in vitro* and *in vivo* performance. **Results:** The dressing system showed excellent folding endurance. *Ex vivo* antibacterial activity was found to be excellent against *Staphylococcus aureus* and *Escherichia coli*. The zone of inhibition of developed foam dressing was found 25 ± 5 mm for *Staphylococcus aureus* and 20 ± 3 mm for *Escherichia coli*. *In vitro* diffusion was found to be 88.40% and 84.65 % for curcumin and syringic acid respectively. Diabetes induced rats were used for evaluating *in vivo* wound healing activity and complete wound healing was observed at the end of 14 days. **Conclusion:** Polyurethane foam dressing system based on curcumin, syringic acid and *Aloe vera* can show promising wound healing in diabetes conditions.

Key words: Polyurethane foam dressing, Curcumin, Syringic acid, *Aloe vera* gel, Diabetic wound.

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INTRODUCTION

Skin is the largest organ of the human body. It performs different functions like forming a protective barrier to the external environment, preventing external noxious agents such as bacteria and viruses and maintaining the internal environment through the regulation of water and electrolyte balance. Skin also plays a role in thermoregulation.[1] A wound is a disruption of the physiological arrangement of skin cells. It also disturbs the normal function of the skin like connecting and protecting underlying tissues and organs. A wound is classified as an acute and chronic wound.[1] The normal wound healing process occurs through phases like hemostasis, inflammation, cell proliferation and tissue remodeling.[2]

Diabetic wounds begin as acute wounds, but the healing process is interrupted and trapped in different phases of wound healing. Thus acute wound fails to repair and becomes more chronic. In diabetic patients, the wound healing process is affected by hyperglycemia, chronic inflammation, micro and macro-circulatory dysfunction, hypoxia, autonomic and sensory neuropathy and impaired neuropeptide signaling.[3] In diabetes, a prolonged inflammatory response after the injury is one of the reasons for delayed wound closure.[3]

The current work describes the development of a polyurethane foam-based dressing system using curcumin, syringic acid and *Aloe vera* gel. The foam dressing absorbs exudates from the wound, provides moisture to the wound helping in the epithelialization.[4] Curcumin, syringic acid and *Aloe vera* have a diabetic wound healing potential.[5,6] The combination of curcumin, syringic acid and *Aloe vera* gives synergistic action in diabetic wound healing. This study suggests that developed foam dressing is a promising treatment for non-healing diabetic wounds.

MATERIALS AND METHODS

Drugs and Chemicals

Polyurethane foam (Bombay Rexine, Pune), Curcumin (Loba Chemie), Syringic acid (P C Chemicals), *Aloe vera* leaves (Institutional botanical garden), Carbopol 934 (Loba Chemie), Sodium benzoate (Loba Chemie), Sodium metabisulphite (Loba Chemie), Glycerin (Loba Chemie), Triethanolamine (Loba Chemie), Alloxan monohydrate (Loba Chemie).

Animals

Adult male Wistar rats, weighing from 250-280 g were procured from the Central Animal House of Institute. The research proposal was approved by The Institutional Animal Ethical Committee (IAEC). *In vivo* study was carried out by following CPCSEA guidelines for the use and care of experimental animals.

Characterization of Polyurethane foam

Density

The dimension (length, width and thickness) of foams were measured using a vernier caliper in mm. The foams were weighed. The density of six samples of foam was calculated and the average density was reported as g/cm^3.[4,7]

Formulation and Development of Dressing System

Fresh *Aloe vera* leaves were collected from the institutional botanical garden and washed. *Aloe vera* pulp was collected and homogenized. The obtained *Aloe vera* juice was filtered through a muslin cloth and observed against a white background to see any particulate matter.[8] *Aloe vera* gel formulation was prepared with different concentrations of carbopol 934. Carbopol was soaked overnight in water and stirred with a mechanical stirrer for 30 min.[9] *Aloe vera* juice was mixed with carbopol dispersion and again stirred continuously for 30 min.[10] Ascorbic acid as an antioxidant and sodium benzoate as a preservative were added in formulation II. Sodium metabisulphite, sodium benzoate and glycerin were added in formulation III and stirred for 30 min.[10] Curcumin and syringic acid actives were added in formulation IV and stirred for 30 min. Triethanolamine was added slowly in all formulations and stirred continuously for 30 min with a mechanical stirrer.[9] The prepared formulations were used for the development of the dressing system. The polyurethane foam was sterilized using gaseous sterilization. The dressing systems were prepared under an aseptic condition. The polyurethane foam was dipped in the formulation of curcumin, syringic acid and *Aloe vera* gel. The developed polyurethane foam-based dressing system was subjected to *in vitro* and *in vivo* performance. Formulation composition was presented in Table 1.

Evaluation of the Dressing System

pH

The pH of the dressing was measured using a pH meter.

Folding Endurance

The folding endurance of foam dressing was determined by repeatedly folding foam at the same point until it breaks.[11] The number of times foam could be folded is its folding endurance value.

Ex vivo Antibacterial Activity

The Agar diffusion method was used to study *ex vivo* antibacterial activity.[4] *Staphylococcus aureus* and *Escherichia coli* strains were used. The bacteria were grown in a culture slant kept for overnight incubation and suspended in the culture broth. The 100 µl broth was used and streaked on the agar plate. The dressing was placed on the center of the agar plate and placed in an incubator at 37°C for 24 hr. The zone of inhibition is a clear zone of the surrounding of test dressing and reported in millimeter.

In vitro Drug Diffusion Study

In vitro drug diffusion study was carried out using Franz diffusion cell.[12] The foam dressings were cut into 1 × 1 cm squares and loaded with curcumin, syringic acid and *Aloe vera* gel formulation. The dressing was placed on top of the cellulose acetate membrane. The receptor compartment was filled with ethanolic phosphate buffer pH 6.8. During the diffusion study, the medium was continuously stirred with a magnetic bar. The receptor compartment temperature was kept at 37°C. Samples were withdrawn for 6 hr at different time intervals. Sink conditions were maintained by replacing fresh ethanolic phosphate buffer pH 6.8.[4] The released curcumin and syringic acid concentration were determined by a UV-visible spectrophotometer by quantification of the absorption band at 423 nm and 276 nm respectively.

Table 1: Formulation composition.

Ingredients	Formulation composition			
	I	II	III	IV
Curcumin (g)	-	-	-	1
Syringic acid (g)	-	-	-	1
Aloe vera juice (ml)	20	20	20	20
Carbopol 934 (g)	1	1.5	1.5	1.5
Ascorbic acid (g)	-	1	-	-
Sodium metabisulphite (g)	-	-	1	1
Sodium Benzoate (g)	-	0.5	0.5	0.5
Glycerin (ml)	-	-	5	5
Triethanolamine (ml)	qs	qs	qs	qs
Water (ml)	qs to 100	qs to100	qs to100	qs to 100

Skin Irritation Test

A skin irritation test was performed on rat skin. The back skin of the rat was shaved. The methylated spirit was used as an antiseptic to the shaved region. Foam dressing was applied on the shaved rat skin of the test group and distilled water was applied to the control group of rats. Skin sites were observed daily for 7 days for any type of dermal irritation.[4,13]

In vivo Study

Animal Housing

The animal studies were carried out with the approval of the Institutional Animal Ethics Committee. The animals were procured from the institutional animal house. The male albino Wistar rats weighing from 200-250 gm. were selected for the study. The rats were accommodated in propylene cages under standard laboratory conditions with a 12-hr light-dark temperature (25 ± 2°C) and relative room humidity (55%).[13,14]

The animals were provided with standard feeding and water for the entire study period. In this study, animals were divided into three groups each consisting of six animals.

Induction of Diabetes

Alloxan monohydrate injection was used to induce diabetes in the rat. Male albino Wistar rats were kept for overnight fasting. On the next day, a single injection of alloxan monohydrate 120 mg/kg was freshly prepared in a cold citrate buffer pH 4.5 and given by the intraperitoneal route.[13] After the three days of induction, blood samples were withdrawn from the vein of the rat's tail using a lancet. Blood glucose levels were estimated by a glucometer (Contour T S) to check the development of the hyperglycemic condition.[13,15] Rats with blood glucose levels of more than 250 mg/dl were selected for the study.

Animal Model

The wound healing potential of *Aloe vera* gel containing curcumin and syringic acid was studied using a rat wound healing model. The incisional animal model was used.[2] Rats in each group were anesthetized using ketamine (100 mg/kg) and xylazine hydrochloride (10 mg/kg) injection. The back skin of each rat was shaved and a 3 cm long incision was made through the skin and subcutaneous tissue using a surgical scalpel. After the incision was made, the parted skin was stitched using a surgical thread.[16] The wound was left undressed for the next 24 hr and rats were treated as per groups described in Table 2.

The rate of wound closure of rats was measured daily. A visual comparison of skin recovery was recorded photographically.

Table 2: *In vivo* study group.

Group (*n*=6)	Formulation composition
Standard	Marketed Formulation Povidone iodine ointment
Diabetic Test	Topical polyurethane foam dressing system of Aloe vera gel containing syringic acid (1%) and curcumin (1%)
Control	Undressed wound

Evaluation Parameters for Wound Healing

Tensile Strength

The tensile strength of healed wound was measured using a tensometer on day 14.[16] It was measured as maximum stretching force per unit of the cross sectional area of healed tissue.

RESULTS

Characterization of Polyurethane Foam

Density

The density of the polyurethane foams is summarized in Table 3. The average density of polyurethane foam was found to be 0.0255 g/cm^3. This polyurethane foam was used for the development of a dressing system. The polyurethane foam holds the formulation of *Aloe vera* gel containing curcumin and syringic acid. Generally, as the density of polyurethane foam increases, porosity decreases.

Formulation and Development of Dressing System

In formulation 1 color change and fungus growth were observed after 24 hrs as shown in Figure 2. The color change and fungus growth were also observed in polyurethane foam impregnated with the same formulation. The fungus growth was observed due to the absence of preservatives in the formulation. It is depicted in Figure 1. Formulation 1 of gel was dripping from the polyurethane foam. The concentration of carbopol 934 was 1 %. The viscosity of the gel was 3.5 cps. The dripping of the gel from foam was due to the lower viscosity of the gel.

In formulation II, carbopol 1.5 % was used to increase the viscosity of the gel. As viscosity increased, it did not drip out from polyurethane foam. After 15 days, the color change from white to brownish was observed in formulation II. The color change of formulation before and after is shown in Figure 3 and Figure 4 respectively. The drying of gel was observed in the polyurethane foam loaded with formulation II. It is illustrated in Figure 5.

In formulation III, glycerin was added to overcome the problem of drying of gel. Glycerin acts as a humectant. It holds the water present in the gel and prevents the drying of the gel. In the same formulation, sodium metabisulphite was used as an antioxidant instead of ascorbic acid. It was found that sodium metabisulphite prevents oxidation and

Table 3: Density of polyurethane foam.

Polyurethane foam sample	Density (g/cm^3)
1	0.024
2	0.029
3	0.025
4	0.025
5	0.026
6	0.024
Average density	0.0255 ± 0.01

± denotes the standard deviation

Figure 1: Color change and fungus growth in *Aloe vera* gel.

Figure 2: Fungus growth in polyurethane foam loaded with A*loe vera* gel.

Figure 3: *Aloe vera* gel before color change.

Figure 4: *Aloe vera* gel after color change.

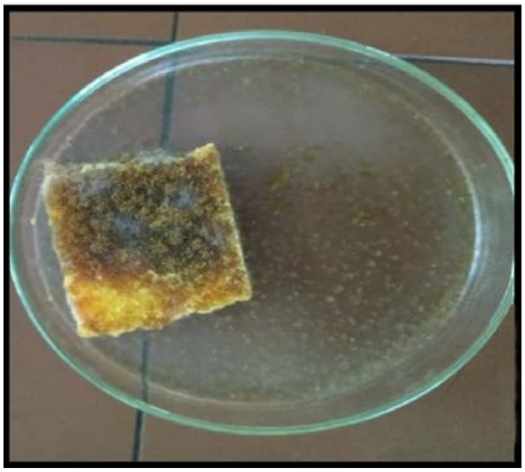

Figure 5: The drying of the gel in polyurethane foam.

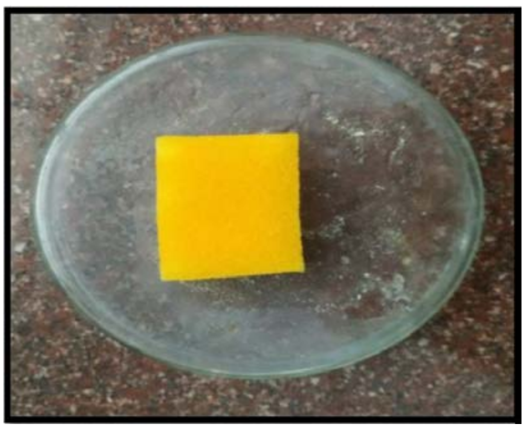

Figure 6: Developed polyurethane foam dressing impregnated with *Aloe vera* gel containing curcumin and syringic acid.

color change was not observed after 30 days. The polyurethane foam loaded with formulation was depicted in Figure 6. This developed foam dressing was used for *in vitro* and *in vivo* testing.

Evaluation of the Dressing System

pH

The pH of the dressing system was measured using a pH meter. It was found to be in the range of 6-7.

Folding Endurance

The folding endurance of the dressing system was found to be more than 150. During the handling of foam dressing, it could not break. It has sufficient strength and elastomeric properties. The flexibility of the foam is important. It is required for the comfortable and safe application of foam on the wound.

Ex vivo Antibacterial Activity

The antibacterial activity of polyurethane foam dressing containing curcumin and syringic acid was measured against bacterial strains of *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* is a gram positive bacteria and *Escherichia coli* is a gram negative bacteria commonly present at the wound site. The presence of pathogenic bacteria or wound bioburden affects the wound healing process. It delays the wound healing process.

The antibacterial activity results revealed that the antibacterial capabilities of polyurethane foam dressing containing curcumin, syringic acid were significantly greater than blank polyurethane foam. The zone of inhibition of developed polyurethane foam dressing was found to be 25 ± 5 mm for *Staphylococcus aureus* and 20 ± 3 mm for *Escherichia coli*. In the case of blank polyurethane foam, the zone of inhibition was found to be 5 ± 1 mm for *Staphylococcus aureus* and 3 ± 1 for *Escherichia coli*. The result showed that developed polyurethane foam dressing is effective as far as their antimicrobial potential is concerned (Table 4).

In vitro Drug Diffusion Study

In vitro drug diffusion study, the slow release of curcumin and syringic acid were observed from polyurethane foam dressing. The concentration of curcumin and syringic acid released at any time was calculated from the calibration curve. At 6 hr, the percentage cumulative drug permeation of curcumin was 88.40 and the percentage cumulative drug permeation of syringic acid was 84.65. There is no burst release of curcumin and syringic acid and released slowly for 6 hr. The data is mentioned in Table 5.

Skin Irritation Test

It was observed that there was no redness and swelling in the tested area as compared to the control group. The test group didn't show any infection. This result revealed that developed polyurethane foam dressing impregnated with *Aloe vera* gel, curcumin, and syringic acid was safe to apply on the skin.

In vivo Study

The wound contraction was better in the animals treated with the developed polyurethane foam dressing. The test group showed a fast

Table 4: **Zone of inhibition.**

Sample	Zone of inhibition (mm)	
	Staphylococcus aureus	*Escherichia coli*
Blank Polyurethane foam	5 ± 1	4 ± 1
Polyurethane foam loaded with curcumin and syringic acid	25 ± 3	20 ± 3

Table 5: **% cumulative drug permeation of curcumin and syringic acid.**

Time (minutes)	% cumulative drug permeation of curcumin	% cumulative drug permeation of syringic acid
30	13.30	10.25
60	21.36	18.36
90	27.65	26.35
120	32.45	34.50
150	35.55	38.68
180	42.75	43.75
210	47.50	50.27
240	53.25	55.65
270	62.59	63.59
300	70.25	68.76
330	78.65	75.55
360	88.40	84.65

Table 6: Tensile strength of the wound.

Groups	Tensile strength of wound at day 14 (*n*=6) Mean ± SD
Control	90 ± 5
Standard	115 ± 5
Diabetic Test	140 ± 5

rate of recovery. The wound tensile strength of the test group of animals was greater than the control and standard group of animals. The wound tensile strength of all groups is shown in Table 6. On day 14, treatment groups showed smaller wound areas as compared to other groups.

The test group showed greater wound strength may be due to wound healing activity of curcumin, syringic acid, and *Aloe vera* gel which was loaded into the polyurethane foam. These results revealed that developed polyurethane foam dressing promotes diabetic wound healing.

DISCUSSION

Diabetic wound healing is a complex process and is affected by different pathophysiological factors. The present study investigated the diabetic wound healing activity of polyurethane foam based dressing system using curcumin, syringic acid and *Aloe vera* gel. Curcumin, syringic acid and *Aloe vera* gel formulation successfully developed and impregnated into the polyurethane foam. Polyurethane foam maintains a moist environment at the wound site and has high moisture permeability.[17]

Curcumin and syringic acid has well known antibacterial activity.[18,19] It shows antibacterial activity against gram positive as well as gram negative bacteria. Developed polyurethane foam dressing containing curcumin and syringic acid showed antibacterial activity against strains of *Staphylococcus aureus* and *Escherichia coli*. It helps in reducing the wound bioburden and faster wound closure. *In vitro* drug diffusion study of dressing system, curcumin and syringic acid releases slowly up to 6 hr. The skin irritation study showed the safe application of dressing on the wound. It did not produce any type of dermal irritation and is comfortable to use on the wound area.

In vivo study, wound healing activity of dressing system was performed against control, test and a standard group of animals. The rate of wound closure was found faster in the diabetic test group as compared to the control and standard group. The tensile strength of the wound was found to be higher in the diabetic group. Thus developed polyurethane foam dressing was found to be very effective for non-healing diabetic wounds.

CONCLUSION

The polyurethane foam dressings were successfully developed using curcumin, syringic acid and *Aloe vera* gel. The developed polyurethane foam dressing did not induce skin irritation and efficiently stimulated wound healing activity by modulating some aspects of inflammatory, proliferative and tissue remodeling phases. *In vivo* study, wound closure of the diabetes test group was significantly faster as compared to the standard and control groups. The diabetic wound healing activity was mainly due to the combined effect of curcumin, syringic acid and *Aloe vera* gel. This combination accelerates the proliferation and differentiation of cells, which resulted in the effective diabetic wound healing of the test group. These results imply that the developed polyurethane foam based dressing system significantly regulates wound healing in diabetic conditions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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