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WOUND HEALING EFFICACY OF POLYHERBAL OINTMENT: IN VIVO EVALUATION IN RATS USING EXCISION WOUND MODEL

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ABSTRACT

Background: Wound healing is a complex physiological process involving haemostasis, inflammation, proliferation and remodelling. Diabetes mellitus presents a major challenge to wound healing primarily due to impaired angiogenesis, reduced collagen synthesis and prolonged inflammation.

Objectives: The purpose of present study was to evaluate the in vivo wound healing potential of polyherbal ointment in alloxan-induced diabetic rats using an excision wound model.

Materials and methods: In order to evaluate the in vivo wound healing potential of polyherbal ointment in alloxan-induced diabetic rats using an excision wound model, the polyherbal formulation was prepared using selected medicinal plants having antimicrobial, anti-inflammatory and antioxidant properties then the excision wounds of uniform size were created under aseptic conditions and healthy albino rats were divided into different groups such as control, standard and test groups. The animals of the test group received topical application of the polyherbal ointment while the standard group was treated with a conventional wound healing agent.

Results: Wound healing was assessed by measuring the percentage of wound contraction, epithelialization period and biochemical parameters such as hydroxyproline content. The histopathological analysis evaluated collagen formation, fibroblast proliferation and neovascularization.

Conclusion: The enhanced wound healing results from these parameters suggested that the polyherbal ointment possesses significant wound healing activity in diabetic conditions, likely due to the synergistic effect of its phytoconstituents highlighting the therapeutic potential of polyherbal formulations as effective and safe alternative in managing diabetic wounds.

KEYWORDS: Excision wound model, Albino rats, Polyherbal ointment, Diabetic wound healing, Epithelialization, In vivo, Hyperglycemia

INTRODUCTION

Wound is nothing but the destruction of living tissue produced by cut, blow or break[1] .Wound healing is defined as a natural process by which wounds return to their normal state with the support of complicated cellular and biomolecular processes (haemostasis, inflammation, proliferation, and remodelling) that restore damaged wound tissue into its original state [2,3].However diabetic wounds are skin and tissue damage caused by prolonged hyperglycemia that hinders the normal wound healing process due to disruption in skin regeneration which prevents normal wound healing. If left untreated and unmanaged more than half of diabetic wounds develop into chronic wounds with increased risks of amputation and death [4].

Basically, wounds are classified as external origin and internal origin on the basis of origin and acute or chronic depending on the healing potential or pathogenesis of wound [5]. Diabetic wound falls under the category of chronic wound which are difficult to heal [6] and the reason for problematic healing of wound are discussed below

1.Hyperglycemia: the persistent chronic high blood glucose level can constrict and harden blood vessels leading to reduced blood, oxygen and nutrient supply to the injured site thus affecting wound repair.

2.Neuropathy: diabetic neuropathy affects the sensory nerve and autonomic nervous system leading to loss of sensation as a result patient becomes unaware of injuries and treatment gets delayed.

3.Microvascular disease: poor circulation in diabetic patients make them more prone to microvascular disease which damages the microcirculation around the wound causing insufficient blood supply.

4.Impaired immune response: diabetes impairs the immune response leading to decreased patients' resistance in fighting infections, making them more prone to infections which further complicates wound healing.

5.Altered inflammatory response and other complications: diabetic wounds have chronic inflammation which disrupts normal healing process and prolongs the inflammatory phase. Besides presence of other complications such as hypertension and arteriosclerosis further impairs wound healing process. [3,7].

MATERIAL & METHODS

The pharmacognostic investigations were carried out on the polyherbal formulation of

Acacia nilotica	Family: Fabaceae[8]
Adrographis echioides	Family: Acanthaceae[9]
Canna indica	Family: Cannaceae[10]

Plant collection and Authentication:

The leaves of Acacia nilotica, Andrographis echioides and Canna indica were collected from the local area of Hyderabad then washed with tap water to remove dust, soil and other impurities. The collected leaves were then shade dried, powdered and sieved through mesh No.40 and stored in closed container. The specimen was identified and authenticated by Dr. L. Rasingam, Scientist -In- charge, Botanical survey of India, Deccan regional centre, Hyderabad, Telangana state, India.

Extraction procedure [11]

Preparation of plant extract: The leaves of Acacia nilotica, Andrographis echioides, and Canna indica were collected and shade dried. Later it was powdered in a mixer to required particle size and sieved through sieve no 40.

About 500 gm of powder material was extracted in Soxhlet apparatus using successive solvents of increasing polarity such as petroleum ether, chloroform, ethanol, and water for 48 hours. The filtrates were collected and evaporated to dryness under reduced pressure using rotary flash evaporator. The extracts obtained from different solvents were weighed and its percentage was calculated in terms of air-dried weight of plant material. Then the dried extracts were preserved at 4° C in small sterilized containers.

i. Preparation of petroleum ether extract

The powder was packed in Soxhlet apparatus and extracted with petroleum ether (60-80°C) for 48 hours. The extract was transferred to china dish and evaporated to thick paste on water bath maintained at 50°C to get the petroleum ether extract. The marc was then air dried thoroughly to remove the solvent before it was taken for further extraction with next solvent.

ii. Preparation of chloroform extract

The air-dried powder from the above process was extracted successively with chloroform to get chloroform extract. The marc was then collected, dried and used for preparing further extraction with ethanol.

iii. Preparation of ethanol extract

The dried chloroform marc was successively extracted with ethanol to get ethanolic extract. The marc obtained from this step was collected, dried and used for further investigation.

iv. Preparation of aqueous extract

2000 gm of powder was taken in 5000ml beaker and macerated with 3000ml of distilled water and 100ml of chloroform (as preservative) for 7 days. The marc from this step was concentrated at 50° C on water bath to get semi solid mass.

The percentage yield from all the extracts was calculated and tabulated.

Preparation of ointment:[12]

The simple ointment base was first prepared using wool fat, hard paraffin, cetostearyl alcohol, white soft paraffin. All the ingredients were mixed with gentle heating and stirring. Then the ointment base was cooled and packed in wide mouthed air tight container. The herbal extract was then added to the ointment base in a concentration of 1% w/w slowly with constant stirring until the mass cools down and a homogenous product is obtained. The polyherbal ointment was then packed in air tight, light resistant container.

Formula for the preparation of simple ointment [13]

Ingredients	Quantity
Wool fat	2gm
Hard paraffin	2gm
Cetostearyl alcohol	2gm
White soft paraffin	34gm

Formula for the preparation of Polyherbal ointment

Ingredients(%w/w)	Medicated formulation
Acacia nilotica	1
Andrographis echioides	1
Canna indica	1
Simple ointment base	q.s

Induction of diabetes in rats:

The animals were fasted overnight and were made diabetic with administration of single dose of intraperitoneal injection of alloxan mono hydrate at a dose of 150 mg/kg dissolved in normal saline. As alloxan is capable of producing fatal hypoglycemia, soon after 2 hours of its administration rats were then treated with 0.5ml of 25% dextrose solution followed by oral administration of 5
% glucose solution (kept in bottles in their cages) ad libitum for next 24 hours. Hyperglycemia was confirmed on the third day after alloxan injection by estimating the blood glucose level with the help of glucometer. Only those rats having blood glucose level of 300 mg/dl were used for the study. [13,14]

Excision wound model:
The excision wound model gives the study of wound contraction rate and period of epithelialization. In this model animals were first randomly divided into 6 groups containing 6 animals each. The grouping of animals was done as follows:
Group-I: Control group
Group-II: Test group treated with Acacia nilotica ointment (1% w/w formulation)- Formulation-I
Group-III: Test group treated with Andrographis echioides ointment (1% w/w formulation)- Formulation-II

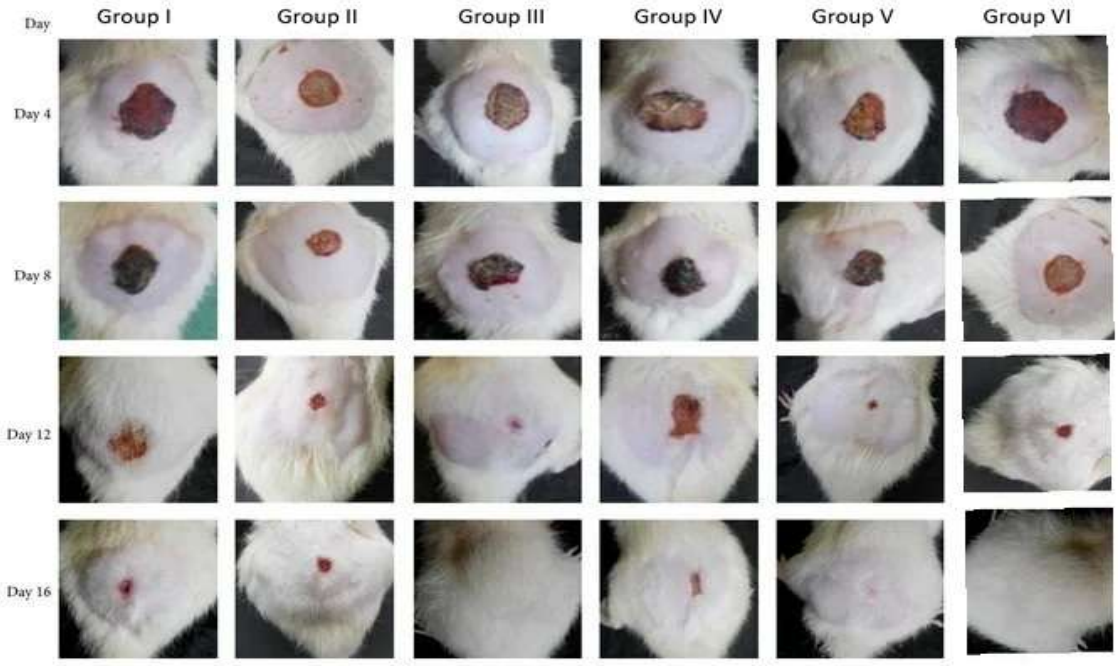

Figure: Photograph showing day wise Excision wounds in Diabetic rats of Control (Group-I), Formulation- I (Group-II), Formulation- II (Group-III), Formulation-III (Group-IV), Formulation- IV (Group-V), Standard (Group-VI)

Group-IV: Test group treated with Canna indica ointment (1% w/w formulation)- Formulation-III
Group-V: Test group treated with Polyherbal ointment (1% w/w formulation)- Formulation-IV
Group-VI: Standard group (Treated with a reference standard silver sulphadiazine 1% w/w)
After grouping the animals were anaesthetized with ketamine hydrochloride (100mg/kg, i.m.) and the dorsal skin near the neck region of each animal was shaved and prepared for excision after washing with spirit. Then a cutaneous wound of about size 2 sq.cm and depth 2mm was created by marking the area using methylene blue on the shaved area of animals with the help of sterile surgical blade and scissors under the influence of anaesthesia. The ointments were then applied once daily to the excised wound regions of the respective groups for a period of 21days. The wound closure rate was evaluated by tracing the wound area on day 1, followed by measurement on every 4th day till 16th day [13,15].

Histopathology:
At the end of experimental period animals were anaesthetized and wound tissue samples were excised for histopathological evaluation. The collected skin specimens were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin. The sections were then stained using hematoxylin and eosin (H&E).
The histological assessment of wound healing was carried out by examining the degree of dermal inflammation, extent of angiogenesis, granulation tissue formation and re-epithelialization[16].

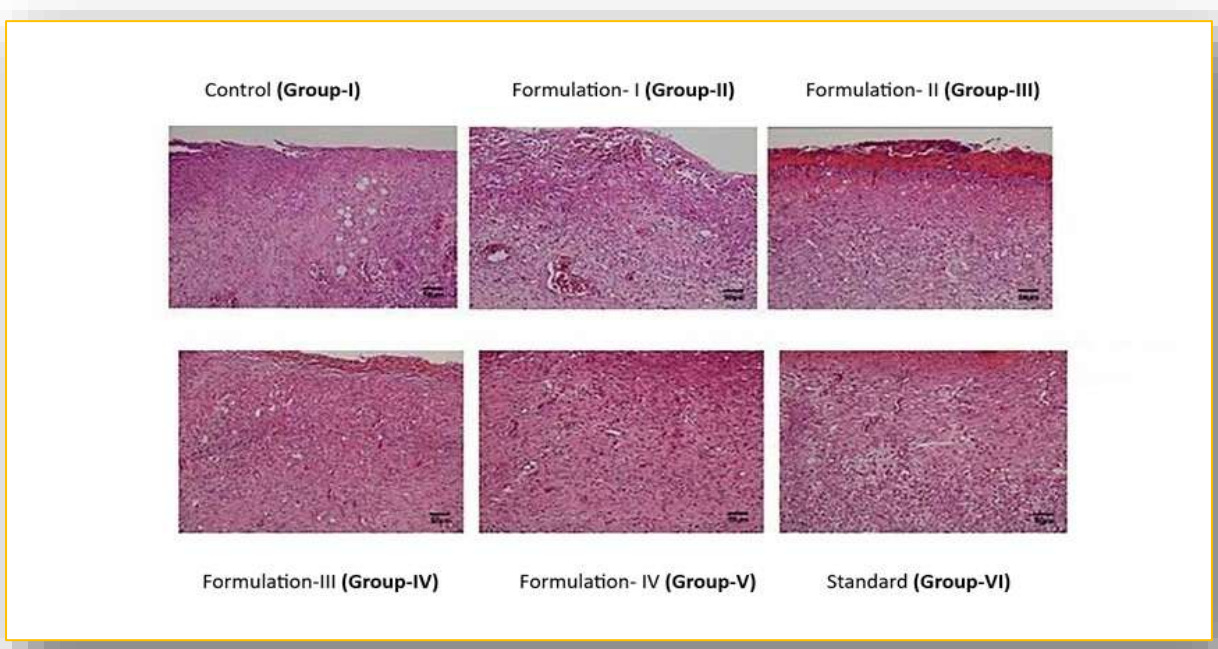

Figure: Histopathology images showing HE-stained skin tissue of diabetic rats in different treatment groups

RESULTS

Table: Effect of petroleum ether extract on diabetic wounds using rat excision wound model

GROUP	PERCENTAGE WOUND CONTRACTION (%)				EPITHELIZATION PERIOD
	4TH DAY	8TH DAY	12TH DAY	16TH DAY	
Control	17.50±0.31	29.345±1.33	43.10±1.20	48.50±0.67	30.12±0.60
Formultion – I	22.52±0.80	43.27±0.10	68.90±1.23	69.28±0.73	25.35±0.25
Formultion – II	21.25±8.0	41.72±1.0	64.09±2.31	63.82±7.30	26.52±5.30
Formultion – III	23.10±2.32	45.88±4.71	72.2±3.97	74.74±7.60	24.23±1.02
Formultion – IV	29.22±3.22	52.99±7.14	83.4±9.73	90.47±6.07	21.70±2.29
Silver Sulphadiazine (Standard)	38.08±1.62	57.72±1.94	86.40±0.77	97.84±0.29	18.00±0.87

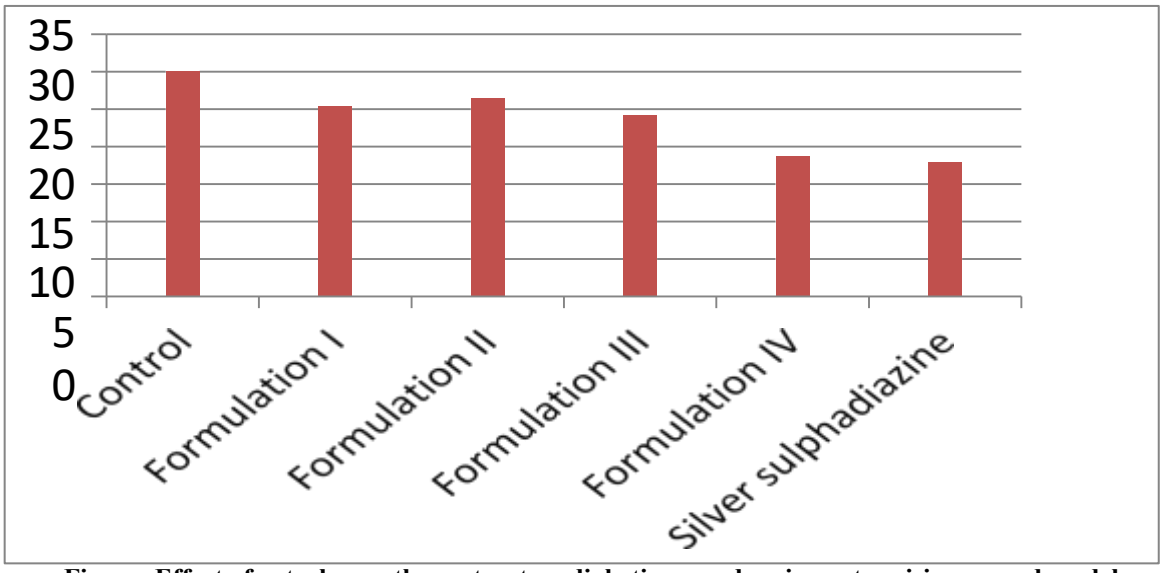

Figure: Effect of petroleum ether extract on diabetic wounds using rat excision wound model

Table: Effect of chloroform extract on diabetic wounds using rat excision wound model

Group	PERCENTAGE WOUND CONTRACTION (%)				EPITHELIZATION TIME (DAYS)
	4TH DAY	8TH DAY	12TH DAY	16TH DAY	
Control	17.50±0.32	29.345±1.32	43.10±1.20	48.50±0.67	30.12±0.60
Formultion – I	30.34±1.12	51.45±0.56	80.69±0.15	85.57±0.65	21.91±0.78
Formultion – II	26.30±0.08	47.41±0.52	76.65±0.11	81.53±0.61	25.87±0.74
Formultion – III	31.32±0.10	51.43±0.54	80.67±0.13	85.55±0.63	20.25±2.76
Formultion – IV	31.21±8.22	50.90±8.14	81.40±9.10	89.97±9.01	20.00±2.29
Silver sulphadiazine (Standard)	38.08±1.62	57.72±1.94	86.40±0.77	97.84±0.29	18.00±0.87

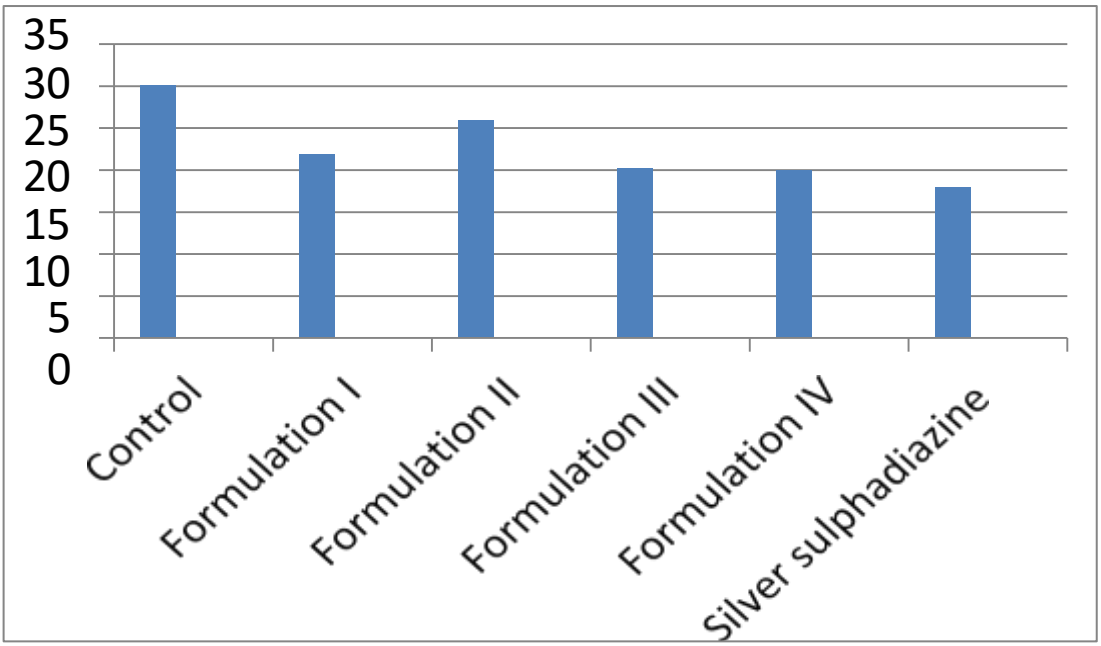

Figure: Effect of chloroform extract on diabetic wounds using rat excision wound model

Table: Effect of ethanol extract on diabetic wounds using rat excision wound model

GROUP	PERCENTAGE WOUND CONTRACTION (%)				EPITHELIZATION TIME (DAYS)
	4TH DAY	8TH DAY	12TH DAY	16TH DAY	
Control	17.50±0.32	29.345±1.32	43.10±1.20	48.50±0.67	30.12±0.60
Formultion – I	30.34±1.12	51.45±0.56	80.69±0.15	85.57±0.65	21.91±0.78
Formultion – II	27.50±0.18	48.31±0.92	77.65±0.61	82.93±0.11	25.87±0.74
Formultion – III	30.22±0.10	51.23±0.52	80.27±0.23	85.52±0.33	21.25±2.76
Formultion – IV	39.25±8.25	56.95±8.15	86.44±9.11	96.77±9.21	18.30±2.29
Silver Sulphadiazine (Standard)	38.08±1.62	57.72±1.94	86.40±0.77	97.84±0.29	18.00±0.87

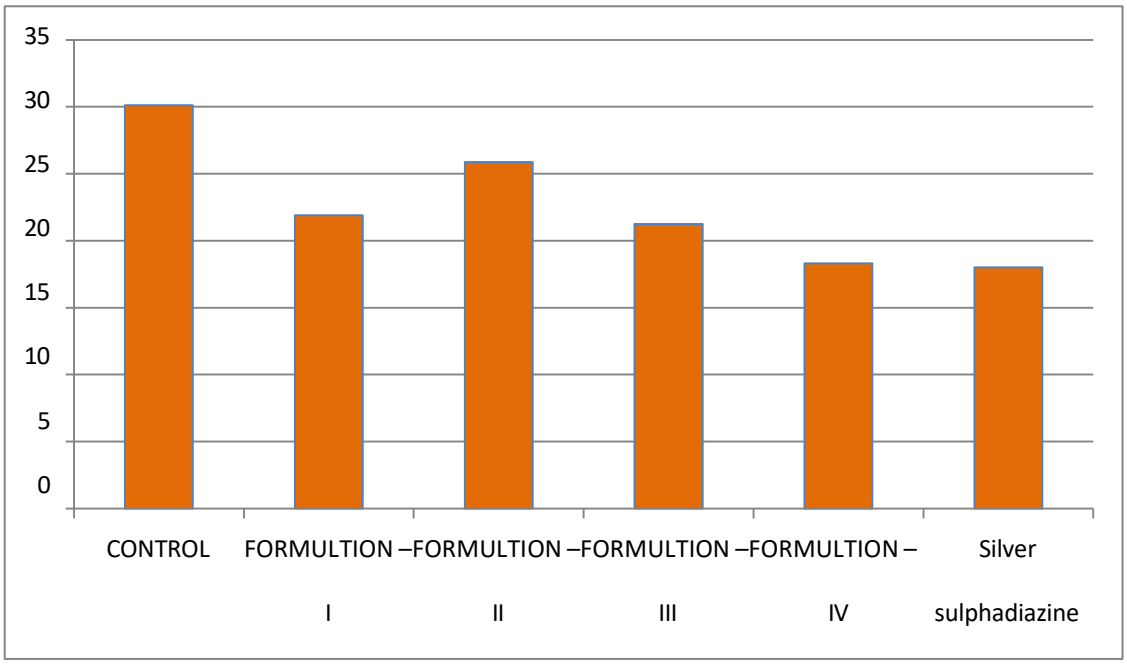

Figure: Effect of ethanol extract on diabetic wounds using rat excision wound model

Table: Effect of aqueous extract on diabetic wounds using rat excision wound model

Group	PERCENTAGE WOUND CONTRACTION (%)				EPITHELIZATION TIME (DAYS)
	4TH DAY	8TH DAY	12TH DAY	16TH DAY	
Control	17.50±0.32	29.345±1.32	43.10±1.20	48.50±0.67	30.12±0.60
Formultion – I	30.34±1.12	51.45±0.56	80.69±0.15	85.57±0.65	21.91±0.78
Formultion – II	26.30±0.08	47.41±0.52	76.65±0.11	81.53±0.61	25.87±0.74

Formultion – III	31.02±0.81	49.40±0.50	70.90±0.63	79.15±0.33	23.05±9.75
Formultion – IV	34.25±8.72	54.47±8.04	82.40±9.60	91.07±9.31	19.45±2.89
Silver Sulphadiazine (Standard)	38.08±1.62	57.72±1.94	86.40±0.77	97.84±0.29	18.00±0.87

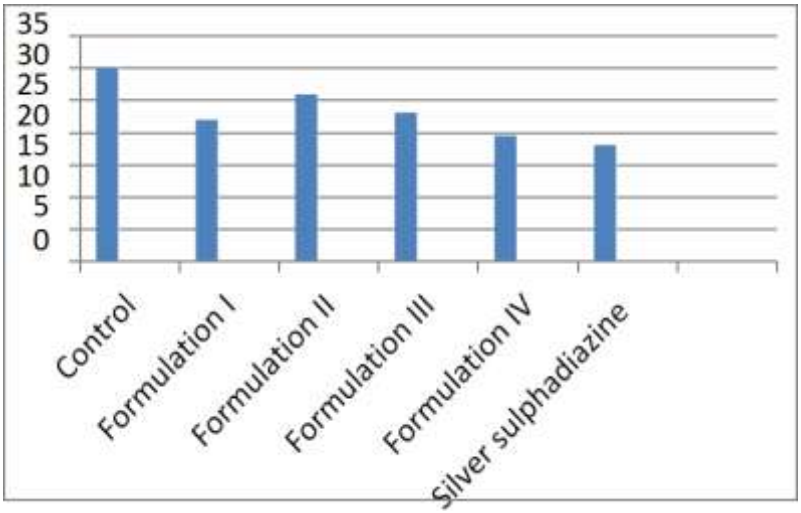

Figure: Effect of aqueous extract on diabetic wounds using rat excision wound model

Table: Histological comparison of Excision wound values per treatment

Treatments	Dermal inflammation	Neovascularization (Angiogenesis)	Re-epithelialization	Granulation
Control	+++	+	-	+
Formulation-I	++	++	-	+
Formulation-II	++	+	-	+
Formulation-III	+	++	-	+
Formulation-IV	+	+++	+	++
Standard	++	++	-	++

For dermal inflammation
- = no dermal inflammation, angiogenesis
+ = mild dermal inflammation, angiogenesis
++ = moderate dermal inflammation, angiogenesis
+++ = marked dermal inflammation, angiogenesis

For granulation tissue thickness
- = none
+ = scant
++ = moderate
+++ = marked

For re-epithelialization
- = no re-epithelialization
+ = < 50% of re-epithelialization
++ = > 50% of re-epithelialization
+++ = complete re-epithelialization

DISCUSSION

Impaired wound healing due to persistent hyperglycemia, oxidative stress, and dysregulation of cellular mechanisms is a major complication of diabetes mellitus. In the present study, the excision wound model was employed to assess the wound healing in diabetes using alloxan as diabetogenic agent which selectively destroys pancreatic β- cells resulting in insulin deficiency and sustained hyperglycemia. This hyperglycemic state delays the normal phases of wound healing namely inflammation, proliferation, and remodelling leading to prolonged wound closure time.

Further from excision wound model different parameters such as percentage wound contraction, epithelialization period evaluated shows delayed wound contraction in diabetic control rats whereas treatment groups showed improved wound contraction. This may be attributed to increased collagen synthesis, and angiogenesis.

Histopathological observations support these observations and revealed better collagen alignment, increased fibroblast density, and neovascularization in treated groups compared to diabetic control group.

Collectively, these findings indicate that the treatment with polyherbal formulation significantly accelerates wound healing in excision model of alloxan-induced diabetic rats thereby supporting the potential therapeutic application of the treatment in managing diabetic wounds.

CONCLUSION

In conclusion the polyherbal ointment demonstrated significant wound healing activity in the excision model in rats. The formulation effectively enhanced wound contraction, shortened the epithelialization period, and improved the quality of life by improved rate of wound healing.

These observed effects may be due to the combined action of bioactive phytoconstituents that promotes tissue regeneration, reduce inflammation and prevents microbial infection. The findings support the traditional use of herbal medicines in wound care and highlight the potential of polyherbal formulation as safe and effective alternative to conventional treatment.

However, further studies are recommended to isolate and characterize the active phytoconstituents, understand the precise mechanism of action, and validate the efficacy through clinical trials in humans.

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