

Evaluation of the Wound Healing Potential of a Polyherbal Formulation in Diabetic Rats Using Incision and Burn Wound Models

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ABSTRACT

Background: Diabetes mellitus is a chronic metabolic disorder associated with impaired wound healing, prolonged inflammation, reduced collagen synthesis, and delayed tissue regeneration. Experimental diabetic wound models are therefore important for understanding the mechanisms underlying delayed healing and for evaluating potential therapeutic interventions. **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 incision wound model and burn wound model. **Materials and Methods:** Experimental diabetes was induced using alloxan. After confirmation of the diabetic state incision and burn wound were created under suitable experimental and ethical conditions. Then the polyherbal formulation was prepared and applied using selected medicinal plants having antimicrobial, anti-inflammatory and antioxidant properties. The animals were monitored throughout the study for changes in wound size, percentage of wound contraction, wound breaking strength, epithelialization period and general healing characteristics. **Results:** Diabetic rats showed delayed wound healing compared with non-diabetic as reflected by slower wound contraction, prolonged epithelialization, and delayed restoration of normal tissue architecture. **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. In addition, the established incision and burn wound model in diabetic rats provides useful experimental models for studying the pathophysiology of diabetic wounds.

Keywords: Incision wound model, Burn wound model, Albino rats, Polyherbal ointment, Diabetic wound healing, Epithelialization, In vivo, Hyperglycemia, Collagen, Wound contraction, Wound breaking strength.

INTRODUCTION

Diabetes mellitus is a chronic metabolic illness which is due to irregular insulin production or defects in insulin secretion or action and or glucose sensing. Basically, it is of two types, type-I diabetes mellitus(T1DM): due to auto immune mediated cell destruction and type-II (T2DM): because of insufficient peripheral insulin resistance compensation. The characteristic classical symptom is three polyps and one loss i.e., polydipsia, polyphagia, polyuria and weight loss^[1,2].

Of these two prevalent types 10-15% of diabetics suffer from T1DM and the rest 85-90% suffers from T2DM^[3]. Despite variations in pathogenesis among

these sub types the underlying pathological basis remains the body's inability to properly handle and utilize glucose resulting in elevated blood glucose levels^[2] (hyperglycemia) a characteristic feature of diabetes mellitus has detrimental impact on long-term health of an individual^[4] as it leads to a serious complication-poor wound healing which presents a significant challenge for individuals with diabetes. In recent estimates 20% diabetic patients suffer from poor wound healing worldwide^[5].

Wound is nothing but the destruction of living tissue produced by cut, blow or break^[6]. 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 [7,8]. 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 [5].

Traditionally different parts of plants were used to cure cuts, chronic wounds and burns. These medicinal plants contain a number of bioactive constituents which have proliferative, migratory, anti-oxidative, anti-bacterial, pro-angiogenic, antiseptic and immune modulatory properties that promotes wound healing, can help even treat ulcers, skin infections, scabies leprosy, and venereal diseases. This may be due to the synergism (positive herb-herb interaction) of primary active component with other components present [9,10,11,12]. Nowadays health care professionals around the world are using numerous plants and herb species in the treatment of both diabetic and non-diabetic wounds because of the assumption that the ingredients from medicinal plants are less toxic and have fewer side effects compared to the therapeutic agents [6].

Signs and symptoms of diabetic wound

1. Chronic pain or completely painless
2. Signs of inflammation (swelling, redness, heat, pain and loss of function)
3. Signs of infection (pus drainage, discharge, bad odour and dead tissue)
4. Signs of nerve damage (numbness and dullness)
5. Signs of progressively worsening infection (fever and or chills)

Classification of Diabetic wounds ^{6,7}:

Generally diabetic wounds are of two types on the basis of origin

1. External origin wounds

Because of peripheral neuropathy in diabetic patients this type of wound generally goes unnoticed.

Ex: cuts, injuries, burns and bruises.

2. Internal origin wounds

Internal origin wound causes destruction of skin and nearby tissues with chances of bacterial infection.

Ex: skin ulcers and calluses.

MATERIAL & METHODS:

The pharmacognostic investigations were carried out on the polyherbal formulation of

Acacia nilotica

Family: *Fabaceae* ^[13]

Adrographis echiodides

Family: *Acanthaceae* ^[14]

Canna indica

Family: *Cannaceae* ^[15]

Plant collection and Authentication

The leaves of *Acacia nilotica*, *Adrographis echiodides* 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 ^[16]

Preparation of plant extract: The leaves of *Acacia nilotica*, *Adrographis echiodides*, 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.

• 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.

• 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.

• 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.

• 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:^[17]

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 ^[18]

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 echinoides	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 5% glucose solution orally (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.^[18,19]

Incision wound model

The rats were anesthetized prior to and during the creation of wound with ketamine hydrochloride (100mg/kg i.m). The dorsal fur of the animals was shaved with an electric clipper. A longitudinal paravertebral incision, six centimetres in length was made through the entire thickness of the skin and cutaneous muscle on either side of vertebral column with the help of a sharp scalpel. After complete haemostasis, the wound was closed by means of interrupted sutures placed at approximately 1cm apart with the help of black silk surgical thread (no. 000) and a curved needle (no. 11).

Figure: Incision Wound Model



Figure: Measurement of incision wound size



The animals were randomly divided into 6 groups and each group containing 6 animals and were treated once daily from 0th day to 9th post- wounding day.

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 echinoides* ointment (1% w/w formulation)- Formulation-II

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)

One day before performing the experiment (measurement of breaking strength) the sutures were removed from the stitched wounds of rats after recovery and the treatment was continued. The wound breaking strength was measured on the tenth post wounding day ^[20,21,22].

Determination of Wound Breaking Strength

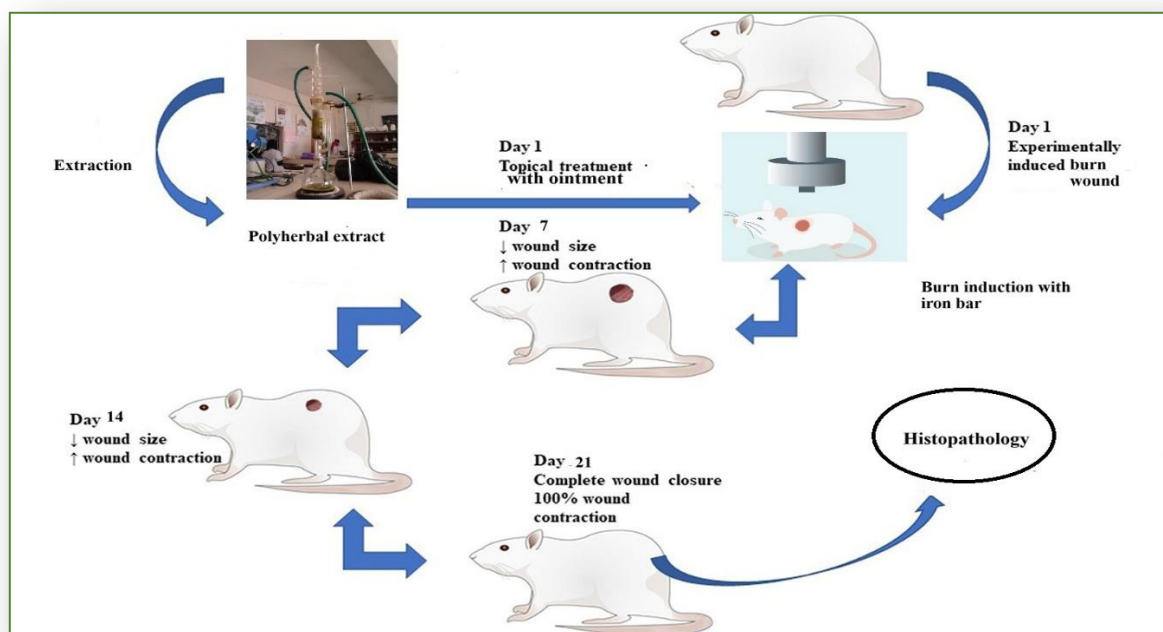
The anesthetized animals were secured to the table, and a line was drawn on either side of the wound 3mm away from the line. This line was gripped using

forceps one at each end opposed to each other. One of the forceps was supported firmly, while the other was connected to a freely suspended light weight metal plate. Weight was added slowly and the gradual increase in weight, pulling apart the wound edges. As the wound just opened up, addition of weight was stopped and the weights added was noted as a measure of breaking strength in grams. Three readings were recorded for a given incision wound. The mean reading for the group was taken as an individual value of breaking strength. The results were analysed by one-way Anova followed by Dunnett's t test²³.

Burn wound model

The rats were anesthetized prior to and during the creation of wound with ketamine hydrochloride (100mg/kg i.m). The dorsal fur of the animals was then shaved and sterilized with povidone-iodine solution. A standard iron bar of 1cm in diameter was maintained at a temperature of 100°C and applied for 15 seconds to create second degree burn wounds. Care was taken to ensure equal pressure and exposure time across all animals and they were resuscitated immediately with lactate ringers solution. The rats were then placed in an isolated cages to prevent transmission of infection.

Figure: Burn Wound Model



The animals were divided randomly into 6 groups and each group containing 6 animals and were treated once daily for a period of 21 days.

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 echinoides* ointment (1% w/w formulation)- Formulation-II

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)

The resulting wounds were treated with the respective formulations once daily. Wound healing progress was monitored by measuring the wound area on day 3, 7, 14, 21. The data obtained were used to calculate wound contraction percentage similar to the excision wound model^{24,25}.

Estimation of Hydroxyproline

The neutralized acid hydrolysate of tissue from all the groups was used for estimation of hydroxyproline. The sample of tissue was sealed in a screw capped glass tube. The tubes were autoclaved at 151.056 kg/cm² for 3 hours. The tubes were marked as sample, 1ml of sample was added to test tube marked as sample, 1ml of standard solution added to test tube marked as standard and blank containing 1ml of DM water.

1ml 0.01M copper sulphate solution was added to all the test tubes followed by addition of 1ml of 2.5N sodium hydroxide and 1ml of 6% hydrogen peroxide. The solutions were occasionally mixed for 5 minutes and then kept for 5 minutes in water bath at 80°C. Tubes were chilled in ice cooled water bath and 4ml of 3N sulphuric acid was added with agitation. 2ml of p-dimethyl amino benzaldehyde was then added and heated in water bath at temperature 70 for 15 minutes then the absorbance was measured at 540nm using colorimeter.

The results were analyzed by one-way Anova followed by Dunnett's t test²⁶.

RESULTS:

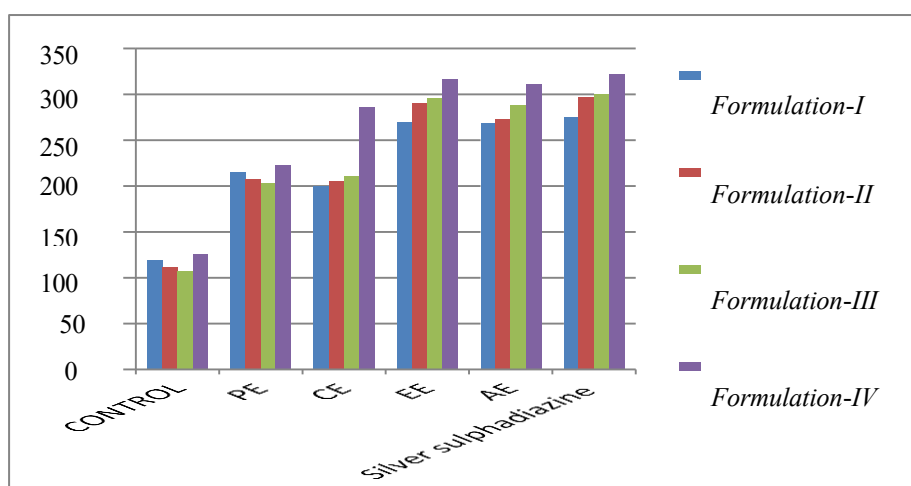
Incision Wound Model

Effect of Petroleum Ether Extract, Chloroform Extract, Ethanol Extract and Aqueous Extract on diabetic wounds using rat incision wound model

Table: Effect of Petroleum Ether Extract, Chloroform Extract, Ethanol Extract and Aqueous Extract on diabetic wounds using rat incision wound model

Group	WOUND BREAKING STRENGTH			
	Formulation-I	Formulation-II	Formulation-III	Formulation-IV
Control	119.33±6.67	111.82±5.21	107.64±2.51	125.55±2.01
Petroleum ether extract	215.25±2.36	207.52±3.60	203.76±6.50	222.65±6.45
Chloroform extract	200.30±1.72	205.44±6.90	210.88±8.97	286.58±3.50
Ethanol extract	270.23±1.37	290.38±3.75	295.73±6.65	316.19±8.05
Aqueous extract	268.41±2.31	272.71±6.11	287.87±9.15	311.45±3.91
Silver sulphadiazine	274.91±8.50	296.55±7.52	300.40±8.00	322.60±4.00

Figure: Effect of Petroleum Ether Extract, Chloroform Extract, Ethanol Extract and Aqueous Extract on diabetic wounds using rat incision wound model



PE = Petroleum ether extract, CE = Chloroform extract, EE = Ethanol extract and AE = Aqueous extract

Burn wound model

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

Group	% WOUND CONTRACTION			
	Day-3	Day-7	Day-14	Day-21
Control	16.70±0.8075	37.04±0.0420	54.54±0.8829	67.48±0.6681
Formulation-I	21.36±0.5824*	41.72±0.6418*	59.84±0.7215*	71.26±0.6943*
Formulation-II	18.94±0.6157*	39.26±0.7524*	56.72±0.6841*	69.18±0.6275*
Formulation-III	23.18±0.7342*	44.63±0.6825**	62.47±0.7952**	75.36±0.7154**
Formulation-IV	26.74±0.6825*	48.52±0.7241**	66.38±0.6947**	81.27±0.6382**
Silver sulphadiazine (Standard)	30.48±0.8282	54.04±0.7532	74.37±0.7418	89.43±0.6681

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

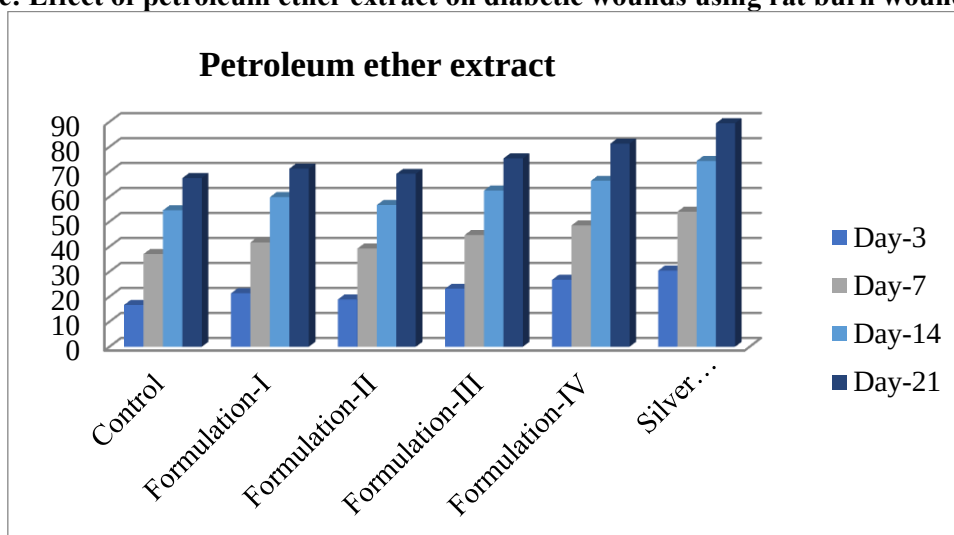


Table: Effect of chloroform extract on diabetic wounds using burn wound model

Group	% WOUND CONTRACTION			
	Day-3	Day-7	Day-14	Day-21
Control	16.70±0.8075	37.04±0.0420	54.54±0.8829	67.48±0.6681
Formulation-I	24.62±0.6345*	46.38±0.6927**	64.25±0.7248**	77.42±0.6835**
Formulation-II	20.74±0.5872*	42.16±0.7164*	59.68±0.6825*	71.35±0.6417*
Formulation-III	25.86±0.7451*	48.72±0.6735**	66.84±0.7582**	80.26±0.6954**
Formulation-IV	28.47±0.6928*	51.83±0.7416**	70.52±0.6837**	85.62±0.5748**
Silver sulphadiazine (Standard)	30.48±0.8282	54.04±0.7532	74.37±0.7418	89.43±0.6681

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

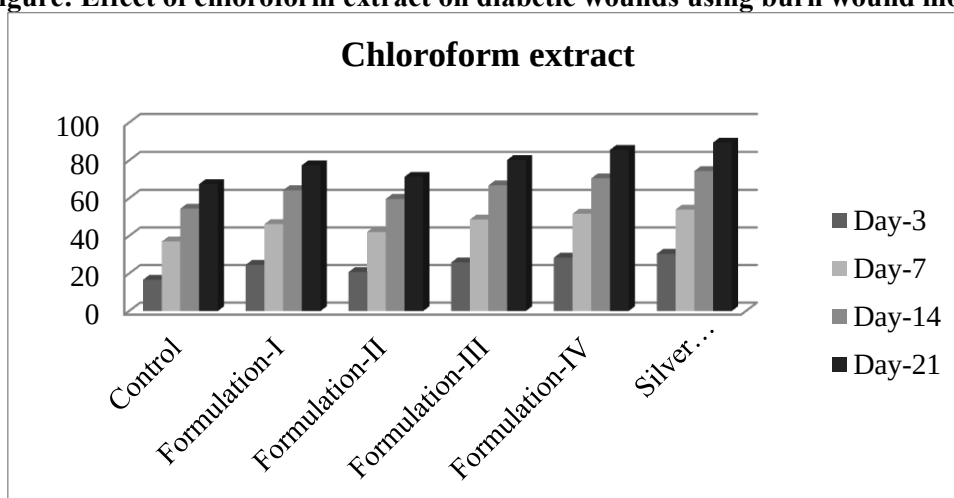


Table: Effect of ethanol extract on diabetic wounds using rat burn wound model

Group	% WOUND CONTRACTION			
	Day-3	Day-7	Day-14	Day-21
Control	16.70±0.8075	37.04±0.0420	54.54±0.8829	67.48±0.6681
Formulation-I	26.48±0.6053**	48.81±0.6819**	68.70±0.6904**	80.15±0.6812**

Formulation-II	19.26±0.6379*	38.20±0.8297*	55.37±0.6478*	68.04±0.6487*
Formulation-III	20.48±0.7973**	39.98±0.6577**	57.32±1.1070**	73.37±0.6812**
Formulation-IV	29.51±0.0876**	52.26±0.7932**	71.81±0.6276**	88.15±0.4045**
Silver sulphadiazine (Standard)	30.48±0.8282	54.04±0.7532	74.37±0.7418	89.43±0.6681

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

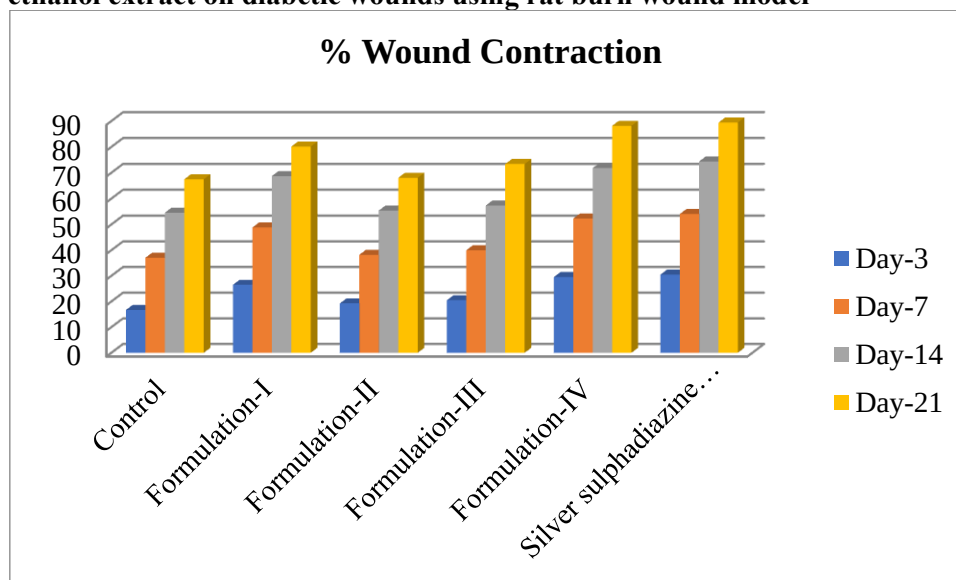
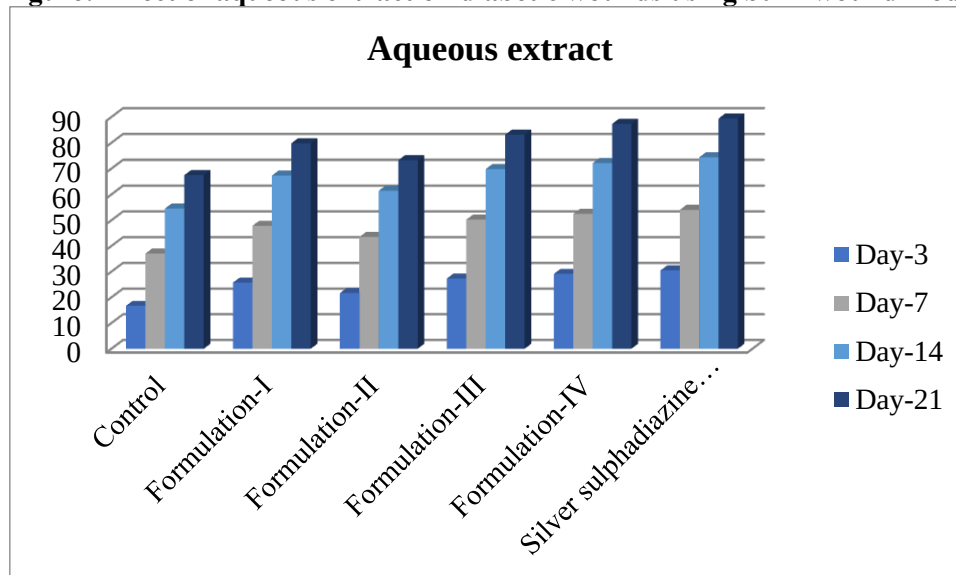


Table: Effect of aqueous extract on diabetic wounds using burn wound model

Group	% WOUND CONTRACTION			
	Day-3	Day-7	Day-14	Day-21
Control	16.70±0.8075	37.04±0.0420	54.54±0.8829	67.48±0.6681
Formulation-I	25.74±0.6158*	47.86±0.6842**	67.28±0.7315**	79.84±0.6725**
Formulation-II	21.68±0.6427*	43.52±0.7248*	61.47±0.6952*	73.26±0.6487*
Formulation-III	27.35±0.7184*	50.24±0.6753**	69.86±0.7841**	83.15±0.6832**
Formulation-IV	29.16±0.6875*	52.48±0.7326**	72.14±0.6728**	87.36±0.5947**
Silver sulphadiazine (Standard)	30.48±0.8282	54.04±0.7532	74.37±0.7418	89.43±0.6681

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



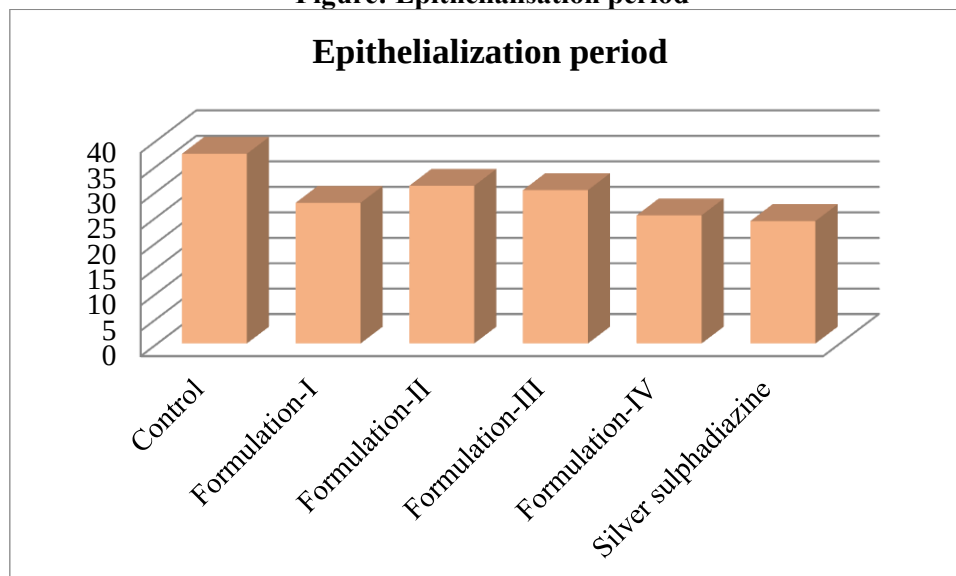
Epithelialisation period

Table: Epithelialization period

Group	Epithelialization period
Control	37.26±1.67
Formulation-I	27.66±1.12
Formulation-II	31.00±0.76
Formulation-III	30.15±0.22
Formulation-IV	25.22±2.12
Silver sulphadiazine (Standard)	24.10±2.00

Epithelialisation period

Figure: Epithelialisation period



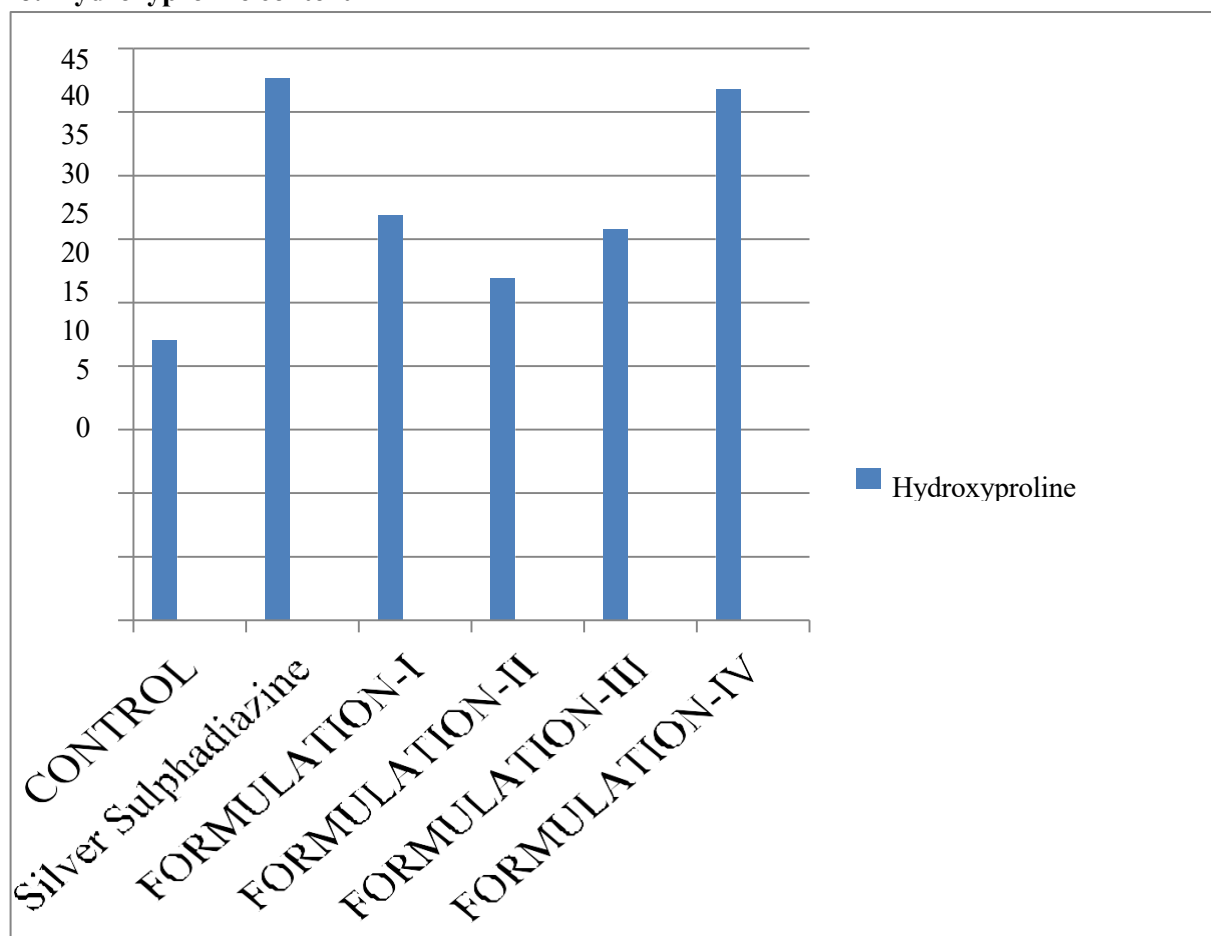
Hydroxyproline content

Table: Hydroxyproline content

S.No	Treatment Group	Hydroxyproline
		Mean $\pm$ SEM(mg/gm of tissue)
1	CONTROL	22.05 $\pm$ 0.41
2	STANDARD	42.67 $\pm$ 0.48
3	FORMULATION-I	31.85 $\pm$ 0.41
4	FORMULATION-II	26.95 $\pm$ 0.46
5	FORMULATION-III	30.75 $\pm$ 0.40
6	FORMULATION-IV	41.75 $\pm$ 0.42

Hydroxyproline content

Figure: Hydroxyproline content



DISCUSSION

The present study demonstrated that all four extracts petroleum ether, chloroform, ethanol and aqueous improved wound healing in diabetic rats, as indicated by increased wound breaking strength compared with the control group. The control group showed relatively low wound breaking strength, ranging from 107.64 $\pm$ 2.51 to 125.55 $\pm$ 2.01, whereas all extract-treated groups showed considerably higher values. Among the extracts, the ethanol extract produced the highest wound breaking strength, particularly with Formulation IV (316.19 $\pm$ 8.05), which was closely comparable to the Silver Sulphadiazine standard (322.60 $\pm$ 4.00). The aqueous extract showed the next

highest activity, with Formulation IV producing 311.45 $\pm$ 3.91, followed by the chloroform extract (286.58 $\pm$ 3.50) and petroleum ether extract (222.65 $\pm$ 6.45). In each extract group, Formulation IV showed the highest wound breaking strength compared with Formulations I–III, indicating that it was the most effective formulation. The increased wound breaking strength suggests improved collagen formation, maturation and strengthening of the newly formed tissue during wound repair. Overall, the findings indicate that the ethanol extract, particularly Formulation IV, showed the strongest wound-healing activity, followed by the aqueous, chloroform and petroleum ether extracts, with

the ethanol Formulation IV showing an effect close to that of the standard treatment.

With burn wounds, Formulation IV consistently showed the best wound-healing activity, irrespective of the extract used, and its effect was close to that of the Silver Sulphadiazine (standard). The higher wound contraction and hydroxyproline content, along with the shorter epithelialization period, suggest enhanced collagen formation, tissue regeneration, and faster wound closure. When compared with other extracts, the ethanol formulation showed a particularly strong response, with Formulation IV producing an effect closely comparable to the standard. Overall, these findings indicate that Formulation IV possesses promising wound-healing potential in diabetic burn wounds as well as in incision wound model.

Strengths of the Study

- The study evaluated the wound-healing potential of a polyherbal formulation in an experimentally induced diabetic rat model, which is relevant to the impaired healing conditions associated with diabetes.
- Both incision and burn wound models were employed, allowing assessment of different aspects of wound repair, including wound contraction, wound breaking strength and epithelialization.
- The study compared individual herbal formulations with the polyherbal formulation, thereby providing a comparative assessment of the contribution of individual plant extracts and their combined formulation.
- Multiple extraction solvents, including petroleum ether, chloroform, ethanol and water, were investigated, providing comparative information regarding the influence of extract type on wound-healing activity.
- Silver sulphadiazine was included as a reference standard, providing a conventional comparator for evaluating the observed wound-healing response.
- Hydroxyproline content was assessed as a biochemical indicator associated with collagen deposition and tissue repair, complementing the macroscopic wound-healing parameters.
- The study used predefined treatment groups and repeated wound-healing assessments during the burn wound experiment, allowing comparison of the progression of wound closure over time.

Limitations of the Study

The study has certain limitations. First, the experiments were conducted in an animal model, and therefore the findings cannot be directly extrapolated to humans without further validation. Second, the study primarily evaluated gross wound-healing parameters and hydroxyproline content; detailed histopathological, microbiological, inflammatory and molecular assessments were not performed. Third, the precise phytochemical constituents responsible for the observed activity were not identified or quantified.

Fourth, the study did not investigate the detailed molecular mechanisms underlying the wound-healing effects of the polyherbal formulation. Fifth, the duration of follow-up was limited to the experimental observation periods, and therefore the long-term safety, recurrence of wounds and sustained therapeutic effects could not be established. Finally, additional studies involving larger sample sizes, standardized extract composition and appropriate dose-response assessment are required to confirm the reproducibility and therapeutic relevance of the findings.

Future Scope

Future studies should focus on standardization and quantitative phytochemical characterization of the polyherbal formulation to identify the major bioactive constituents associated with wound-healing activity. Histopathological evaluation, together with assessment of inflammatory cytokines, oxidative stress markers, angiogenesis-related factors, collagen organization and relevant molecular signaling pathways, would help clarify the underlying mechanisms of action. Dose-response studies and formulation optimization should also be performed to determine the most effective and safe concentration. Long-term toxicity and safety studies are required before considering clinical translation. Further investigations using additional diabetic wound models and larger experimental groups may help establish reproducibility. Ultimately, well-designed preclinical studies followed by appropriately controlled clinical trials will be necessary to determine the efficacy, safety and clinical applicability of the polyherbal formulation for diabetic wound management.

CONCLUSION

In conclusion, the present research provides scientific and experimental support for the wound-healing potential of the investigated polyherbal formulation, particularly Formulation IV and its ethanol extract, establishes a foundation for its further pharmacological and pharmaceutical development as a potential herbal therapeutic approach for the management of diabetic wounds.

The 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.

DECLARATIONS

Ethics Approval and Animal Welfare

The experimental protocol involving laboratory animals was conducted in accordance with the applicable institutional and national guidelines for the care and use of laboratory animals. Ethical approval

was obtained from the Institutional Animal Ethics Committee (IAEC) of the concerned institution before commencement of the study. The animals were handled by trained personnel, and appropriate measures were taken to minimize pain, distress and unnecessary suffering throughout the experimental procedures.

Consent for Participation

Not applicable, as the study involved experimental laboratory animals and did not involve human participants.

Consent for Publication

Not applicable. The manuscript does not contain identifiable information relating to individual human participants.

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Conflict of Interest

The authors declare that they have no conflict of interest related to the study, formulation, data analysis or publication of this manuscript.

Data Availability Statement

The data generated and/or analyzed during the present study are included in the manuscript. Additional information or datasets may be made available by the corresponding author upon reasonable request, subject to applicable institutional and ethical restrictions.

Author Contributions

Amtul Mukheet: Conceptualization, experimental investigation, methodology, data collection, formulation preparation, analysis and drafting of the manuscript.

Dr. Lalitha Repudi: Supervision, study design, methodological guidance, interpretation of findings, critical review and final approval of the manuscript. Both authors read and approved the final version of the manuscript.

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