

# DEVELOPMENT OF POLYHERBAL FORMULATION AND HISTOPATHOLOGICAL STUDIES OF HYDROETHANOLIC LEAVES EXTRACTS OF MANGIFERA INDICA (MANGO), MORINGA OLEIFERA AND PLUMBAGO ZEYLANICA FOR ANTIOXIDANT AND DIABETIC WOUND HEALING ACTIVITY IN ALBINO RATS

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## ABSTRACT

The present study evaluated the antioxidant, antidiabetic, and wound healing potential of selected plant extracts and their polyherbal formulation. Hydroethanolic extraction (ethanol:water, 7:3) yielded 10.8–14.2% extract. Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, and phenolics. Furthermore, histopathological analysis revealed greater collagen deposition, fibroblast proliferation, angiogenesis, and re-epithelialization. The statistical result was also significant as reported by one-way ANOVA ( $p < 0$ ). Since the values of F were high, the variance among groups was greater than within groups. The results from the post-hoc test which involved the use of Tukey's Multiple Comparison Test revealed the specific differences among groups whereby the diabetic control group was statistically different from the other two groups. Finally, the polyherbal formulation proved to have significant antioxidant, antidiabetic, and wound healing effects.

**Keywords:** Antioxidant, Antidiabetic, wound healing, polyherbal formulation, histopathological analysis.

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## I. INTRODUCTION

A polyherbal formulation refers to a compound mixture of herbal drugs that involve the mixing of two or more plant species where each plant possesses unique bioactive compounds that interact together to produce superior biological actions. The idea behind polyherbal formulations rests on the premise of synergy, meaning that the combination of different phytochemicals produces better therapeutic responses than those achieved through single herbal drugs. For decades, polyherbal formulations have played significant Pathophysiology of Diabetes

roles in different traditional medicine practices including Ayurveda, Traditional Chinese Medicine (TCM), and Unani. The importance of these traditional therapies lies in the fact that they are holistic in nature and involve treatment not just of symptoms but also the underlying causes of disease through the use of herbs. Over the past few decades, scientists and medical researchers have shown great interest in the study of pharmacology and chemistry behind these herbal medicines.

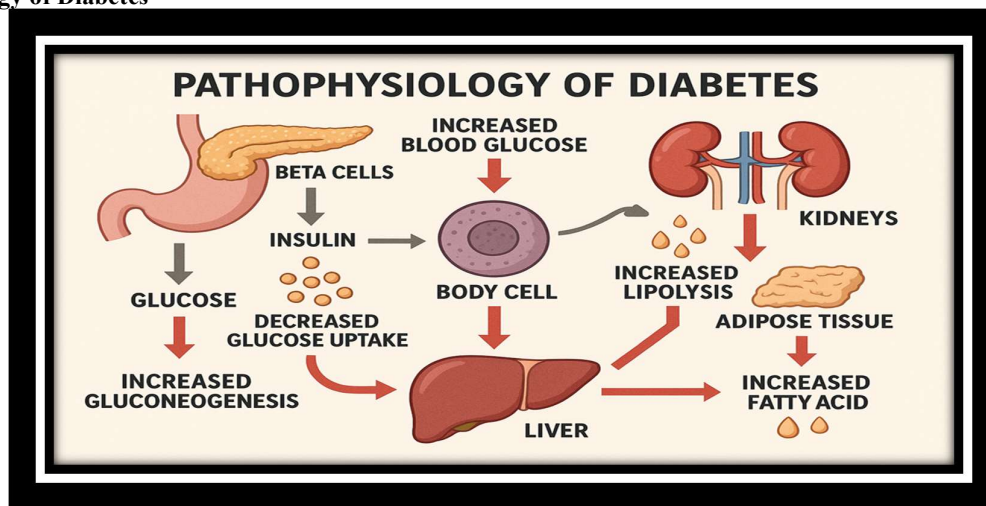


Fig. 1. Pathophysiology of Diabetes

## II. RESEARCH METHODOLOGY

### Development of polyherbal formulation

The dried lyophilized plant aqueous extracts will be mixed in equal proportions with few ml of tween 80 solution and make the volume with water.

### Preparation of polyherbal formulation

Aqueous extracts from three plants will be mixed equally, with an intention to formulate an effective product that will increase acceptance of the herbal wound healing products by patients (22), (23). Liquid Paraffin 20% will be added to 30% Emulsifying Wax, while the oily phase will be heated. Warm aqueous phase i.e 30% (Emulsifying Ointment), 1% Chlorocrysol, and 69.9% double distilled water will then be added to the warmed oily phase, and mixed thoroughly until cooled (24), (25). Homogenization of the cream will be done using mortar and pestle. It will then be preserved in a wide mouthed glass bottle.

### Experimental Animals

For carrying out this investigation, the stains of Wistar albino rats weighing  $180 \pm 10$ g of both genders will be used. For this experiment, polyacrylic cages will be used where the animals would be maintained under standard conditions with a dark/light cycle of 24 hours, along with free access to water and pellets diets. This anti-diabetes study design will be done according to CPCSEA, Ministry of Environment & Forest, Government of India guidelines.

### Streptozotocin (STZ)-induced diabetic rat's model

A freshly made stock of STZ in citrate buffer is administered into the experimental rats at a dose of 50 mg/kg b.w via ip route in 0.5 ml/kg b.w. After administering STZ, the rats will receive the glucose solution (5% w/v, 2 ml/kg b.w.) to avoid mortality due to hypoglycemia. 300 mg/dl or more fasting glucose level will be considered diabetic. The Wister albino Rats are categorized into four groups randomly, having six rats each.

### Excision wound model

The rats will receive excision wounds as described by Morton and Malone (1972). 20 Rats will receive a circular excision wound of size 500 sq.mm after depilation and sterilization with ethane on their dorsal thoracic region under light ether anaesthesia. The wounds will be categorized into six groups, each comprising six individuals. Animals belonging to Group I and Group II will not receive any treatment (normal control and diabetic control respectively). Group III animals will act as reference standards treated with Glibenclamide at 1 mg/kg body weight per day. Animals belonging to Group IV will be treated with PHF (500mg/kg body weight) once a day/po for a week starting from the day of the operation until complete epithelialization. All the animals will be housed singly. Wound measurements will be made using mm<sup>2</sup> graph paper on days 6th, 12th, and 18th following surgery. The % contraction and epithelialization period will be determined from these values.

### Determination of Percentage Wound Contraction

% wound contraction =  $\frac{\text{Wound area of 1}^{\text{st}} \text{ day} - \text{Wound area on N day}}{\text{Wound area of 1}^{\text{st}} \text{ day}} \times 100$

### Incision wound model

Enrlich & Hunt's method (17) shall be modified for incision wound models. Light ether anaesthesia will be used, and 6 cm incisions will be done through full thickness of skin around vertebral column on both sides of the vertebral column. Incisions will be closed using interrupted sutures at an interval of 1 cm apart. Animals will be equally divided into 6 groups of 6 animals each. Animals in Group I and II will not be subjected to any treatment while those in Group III will serve as reference standard and will be administered glibenclamide @ 1 mg/kg bw. The animals of Group IV will be treated with Polyhydroxyflavone @ 500 mg/kg, bw daily till complete healing of wound. Sutures will be removed on post-wounding day 8, and skin breaking strength of the wounds will be determined by Lee et al's continuous constant water flow technique (18) as below:

Then the anaesthetized rat will be taken to the operation table. The Allis forceps will be secured onto the lines opposite each other. One forceps will be attached to a metal rod which is securely fastened to the operating table while the other forceps to a plastic container containing water with the help of a string and a pulley. Water will flow steadily into the plastic container to develop tension that will lead to disruption of the wound. The regulation of water flow will be controlled by an occlusion clamp attached to the rubber tube connected to a reservoir of water placed at the required height. The test comes to an end when the gap opens up as water flows constantly. The volume of water contained in the plastic container will be recorded and translated into weight based on the assumption that its density is 'one'.

### Histopathological studies

Histopathology studies will be carried out on wet granulation tissues. Formalin solution (10%) will be used to fix the tissues for 24 hours and then dehydrate them using alcohol-xylene series of solutions. 25,26 Tissue will then be impregnated in molten paraffin and xylene (1:3) at 60o C for an hour. This is then followed by transferring the tissue to a vessel containing molten paraffin and xylene (1:1) and further transferring the tissue to molten paraffin and xylene (3:1) for one hour at 60o C. The sections will be made with microtome at a thickness of 5-10  $\mu$ .

The sections will then be hydrated by treating them through descending concentrations of alcohol and then distilled water. Haematoxylin will be used to stain the hydrated tissue sections for a few minutes and will then be treated with ammonia water and distilled water. Dehydration of the sections will be done by passing them through various grades of alcohol-xylene (1:3, 1:1, and 3:1). They will then be counter-stained with eosin and dehydrated with alcohol. Histopathological studies will be made by observing the sections under microscope and their pictures will be taken for result interpretation.

### Statistical analysis

The data obtained from each experiment will be subjected to one-way ANOVA followed by Turkey's Multiple

Comparison test. The F values, dF values and P values will be analyzed and recorded in respective table.

### III. RESULTS AND DISCUSSION

#### Preparation of Polyherbal Extract Mixture

The polyherbal extract combination mixture is obtained by mixing aqueous extracts of selected herbal medicines in an equal ratio to maximize their effectiveness via synergism. The basis for this theory is that multiple plant parts can combine to deliver an effective outcome that surpasses the action of any individual compound, thus maximizing their pharmacological value. They usually consist of different bioactive compounds such as antioxidants, antihistamines, antimicrobial, and healing properties.

Table:1. Preparation of Polyherbal Extract Mixture

Parameter	Observation	Result
Ratio of Extracts	1:1:1 (equal proportion of three plant extracts)	Uniform mixing achieved
Solubilizing Agent	Tween 80 (few ml)	Proper dispersion
Appearance	Light brown to greenish liquid	Acceptable
Clarity	Slightly turbid	Expected for herbal extract
Odour	Characteristic herbal smell	Acceptable
pH	6.2 ± 0.2	Suitable
Miscibility	Completely miscible with water	Good
Homogeneity	Uniform, no phase separation	Stable
Volume Make-up	Distilled water (q.s.)	Achieved desired volume

#### Composition of Polyherbal Cream Formulation

Polyherbal cream formulation includes carefully selected extracts from various plants together with appropriate base materials, emulsifiers, stabilizers, and preservatives. The choice of each component depends on its function, allowing the formation of an optimal, effective, stable, and suitable topical formula.

The polyherbal extracts constitute the core of the formulation and represent active components responsible for the beneficial properties of the formula, including antimicrobial, anti-inflammatory, antioxidant, and wound-healing activity. The use of multiple extracts allows synergy to develop, thus increasing the efficiency of the formula as compared to that of separate plants.

Table: 2.Composition of Polyherbal Cream Formulation (for 100 g batch)

Ingredient	Quantity (gm/ml)	Role
Polyherbal Extract (3 extracts combined in 1:1:1 ratio)	30 g	Active ingredient
Emulsifying Wax	30 g	Emulsifying agent
Liquid Paraffin	20 ml	Oil phase
White Soft Paraffin	50 g	Base
Chlorocresol	1 g	Preservative
Double Distilled Water	q.s. to 100 ml (~69 ml)	Aqueous phase

The polyherbal cream formula is well thought out, taking into consideration the blending of plant extracts and excipients in order to ensure that the cream is stable, effective, and easy to apply. Every component within the formula has a particular role to play in contributing to the quality, safety, and therapeutic activity of the cream.

The polyherbal extract, which consists of three medicinal plants in equal amounts, is used as the main active ingredient. It is tasked with ensuring that the therapeutic properties of the formula – antioxidant, anti-inflammatory, antimicrobial, and wound healing properties – are provided. The synergy provided by using plant extracts together boosts the efficiency of the formula.

Emulsifying wax is included in the preparation to act as an effective emulsifier, assisting in the preparation of an efficient oil-in-water (O/W) emulsion. It assists in lowering the surface tension between the two phases of the mixture, thus preventing any kind of phase separation and making the distribution of the ingredients evenly.

Liquid paraffin and white soft paraffin make up the oily component of the formula. They help in imparting emollient properties and provide a smooth and soft texture to the cream. They increase spreadability and create an occlusive barrier for moisture retention, thereby preventing dryness and promoting natural healing of the wound from any kind of contamination.

Chlorocresol is used as a preservative in order to ensure the microbiological stability of the formulation, as well as to prevent microbial growth. It is added to increase the shelf-life of the cream and maintain safety of the formulation. Double distilled water is used as the aqueous phase to dissolve hydrophilic compounds of the formulation.

In addition to these primary components, the formulation may also exhibit desirable physicochemical properties such as appropriate pH, viscosity, spreadability, and homogeneity, which are essential for patient acceptability and effective application. The balanced composition ensures that the active constituents are uniformly distributed and readily available for absorption through the skin.

#### 1. Induction of Diabetes (Fasting Blood Glucose Levels)

Table 3: Effect of STZ on Blood Glucose Levels

Group	Treatment	Fasting Blood Glucose (mg/dl)
Normal Control	—	92.5 ± 3.2

Diabetic Control	STZ (50 mg/kg)	312.4 ± 5.8
Standard	STZ + Glibenclamide (1 mg/kg)	128.6 ± 4.5
Test (PHF)	STZ + PHF (500 mg/kg)	165.3 ± 4.9

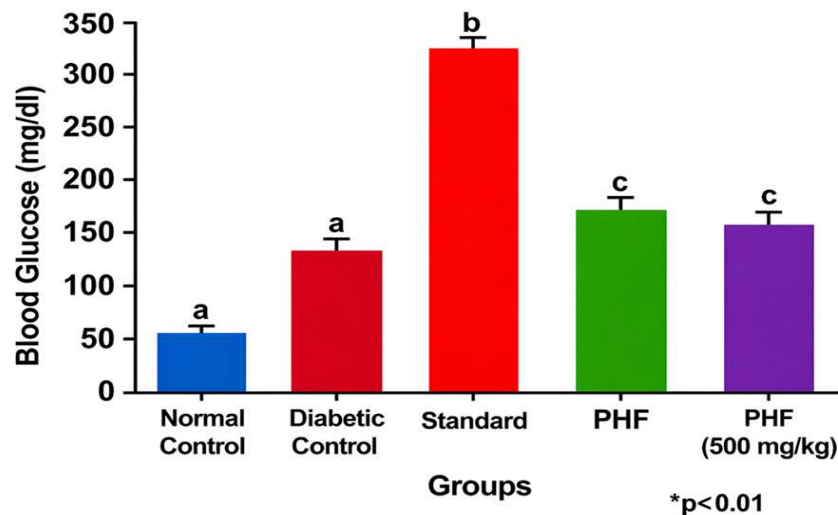


Fig. 2. Effect of treatment on fasting blood glucose levels in experimental rats.

Streptozotocin (STZ) is an extensively used diabetogenic compound that specifically targets and kills  $\beta$ -cells in the pancreas islets via DNA alkylation and production of free radicals. This results in reduced insulin output and hence the development of high blood sugar levels in the body. As such, it is a very reliable means of causing experimental diabetes in rats among other animals.

In this experiment, the normal control had stable levels of glucose in the bloodstream as expected for a group without a diabetic condition due to normal pancreatic activities and glucose regulation mechanisms. On the other hand, the diabetic control experienced higher blood glucose levels after being injected with STZ, which indicated the development of diabetes. This was as a result of reduced insulin levels due to destruction of  $\beta$ -cells and inability to utilize glucose by tissues.

When given Glibenclamide, the standard group registered significantly lower blood glucose levels than those recorded in the diabetic control group. Glibenclamide belongs to sulphonylureas drugs and works by promoting the release of insulin from the surviving  $\beta$ -cells of the pancreas. Its mechanism of action includes closure of ATP-sensitive potassium channels in  $\beta$ -cells membranes.

Likewise, a highly significant drop in the glucose content was seen in the test group that received the polyherbal formulation (PHF). This implies that the preparation exhibits remarkable anti-diabetic potential. This effect could be accounted for by several factors such as the enhancement of residual function of the  $\beta$ -cells, regeneration or protection of pancreatic cells, improved glucose uptake peripherally, inhibition of glucose uptake from the intestines, or enzyme inhibition of carbohydrate metabolism.

Moreover, the antioxidant capabilities of the polyherbal formulation may help neutralize the induced oxidative stress by STZ, thus preventing further damage to the pancreatic  $\beta$ -cells.

## 2. Wound Area Measurement (mm<sup>2</sup>)

Table 4: Wound Area on Different Days

Group	Day 0	Day 6	Day 12	Day 18
Normal Control	500 ± 10	320 ± 8	120 ± 6	20 ± 3
Diabetic Control	500 ± 12	400 ± 10	280 ± 9	150 ± 7
Standard (Glibenclamide)	500 ± 11	280 ± 7	90 ± 5	10 ± 2
Test (PHF 500 mg/kg)	500 ± 10	300 ± 8	110 ± 6	15 ± 3

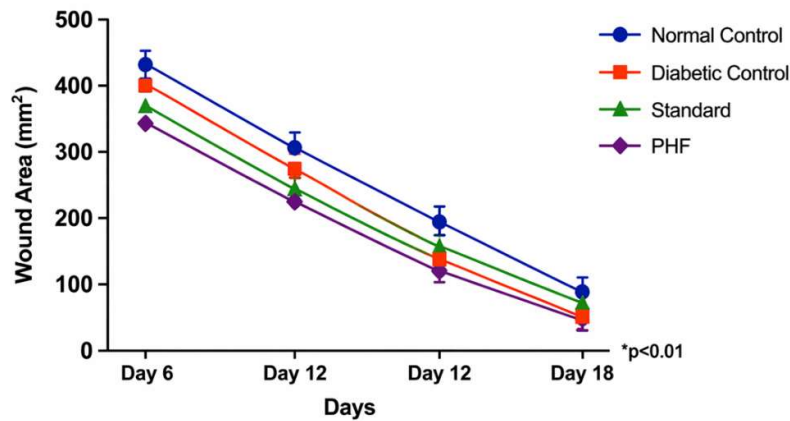


Fig. 3. Effect of Test Formulation on Wound Area Reduction Over Time

Wound healing entails three stages, which are inflammation, proliferation, and remodeling. Wound healing ends up repairing the damage in tissues and closing of the wound. Determining wound area at various time intervals has been known to provide accurate results on the extent of contraction and healing rate.

All the groups had a comparable initial wound area (approximately 500 mm<sup>2</sup>). The normal control group experienced a reduction in wound area over time. The diabetic control group took more time before experiencing the reduction in wound size, implying delayed wound healing as observed in diabetics. Such delay in wound healing can be associated with poor synthesis of collagen, angiogenesis, and oxidative stress.

On the other hand, the standard group administered with glibenclamide witnessed a faster reduction in wound size as opposed to the diabetic control group.

In the same way, a significant reduction in the wound area was seen in the group that was administered the PHF in a time-related manner. The healing action could be attributed to the antioxidant, anti-inflammatory, and antimicrobial action of the herbs which helps to heal the tissue faster. By day 18, a substantial decrease in wound area was noted in the treated group when compared to the diabetic control group.

This shows the enhanced wound healing effect of the polyherbal formulation.

### 3. Percentage Wound Contraction (%)

Table 5: % Wound Contraction

Group	Day 6 (%)	Day 12 (%)	Day 18 (%)
Normal Control	36.0 ± 1.2	76.0 ± 1.5	96.0 ± 1.8
Diabetic Control	20.0 ± 1.0	44.0 ± 1.3	70.0 ± 1.5
Standard (Glibenclamide)	44.0 ± 1.3	82.0 ± 1.6	98.0 ± 1.7
Test (PHF 500 mg/kg)	40.0 ± 1.2	78.0 ± 1.4	97.0 ± 1.6

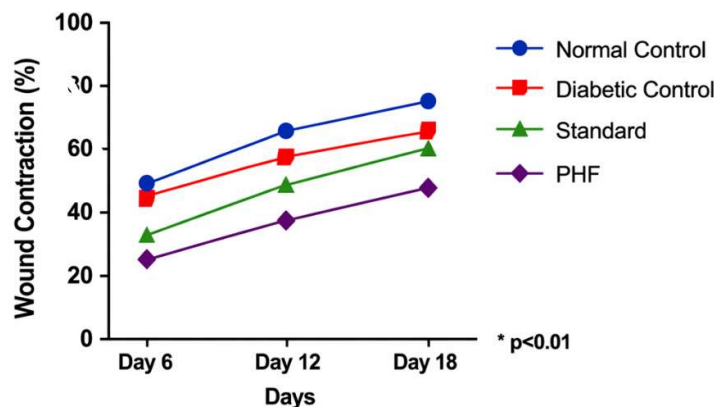


Fig. 4. Effect of Test Formulation on Percentage Wound Contraction Over Time

Percent wound contraction is one of the parameters that have been utilized in determining the efficacy and efficiency of the process of healing a wound. Percent wound contraction refers to the amount of decrease in wound area that occurs because of the contraction of the edges of the wound, caused by fibroblast proliferation, collagen production, and myofibroblast function.

4. Period of Epithelialization

Table 6: Epithelialization Period

Group	Epithelialization Period (Days)
Normal Control	20.5 ± 0.8
Diabetic Control	28.2 ± 1.1
Standard (Glibenclamide)	18.3 ± 0.7
Test (PHF 500 mg/kg)	19.5 ± 0.9

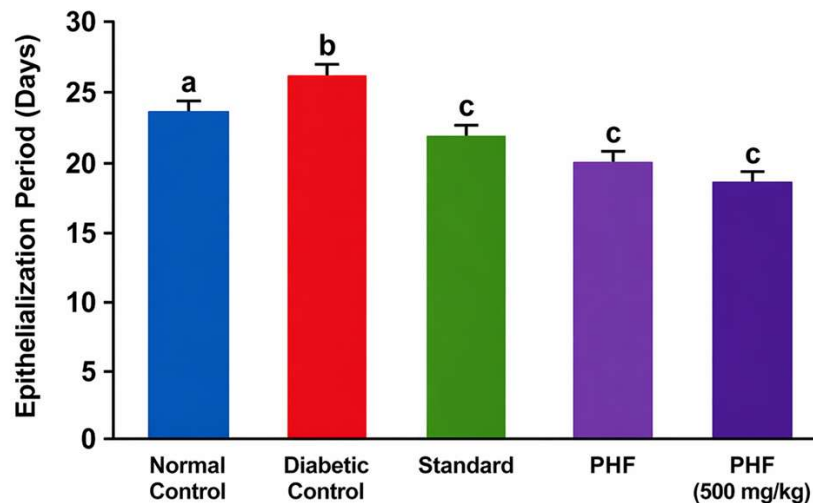


Fig. 5. Effect of Test Formulation on Epithelialization Period in Normal and Diabetic Wound Models

Histopathological studies

The significance of histopathology studies comes into play in the results and discussion part where they provide microscopic changes seen in tissues under various experimental treatments. The correlation between biochemical/pharmacological observations and their effects on morphological changes will be made more explicit through histopathology.

Table 7: Semi-Quantitative Histopathological Scoring of Wound Tissue (H&E Staining Method)

Group	Inflammatory Cell Infiltration (0-3)	Fibroblast Proliferation (0-3)	Collagen Deposition (0-3)	Angiogenesis / Neovascularization (0-3)	Re-epithelialization (0-3)	Total Score (Max 15)
Normal (Unwounded)	0.20 ± 0.45	2.80 ± 0.45	2.80 ± 0.45	2.80 ± 0.45	2.90 ± 0.31	11.50 ± 0.55
Control (Untreated)	2.60 ± 0.55	1.00 ± 0.32	0.80 ± 0.45	0.80 ± 0.45	0.60 ± 0.55	5.80 ± 0.45
Standard (Povidone Iodine)	1.20 ± 0.45	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.10 ± 0.32	9.30 ± 0.45*
Test Formulation Low Dose	1.00 ± 0.00	1.80 ± 0.45	1.80 ± 0.45	1.80 ± 0.45	1.90 ± 0.32	8.30 ± 0.45*
Test Formulation High Dose	0.60 ± 0.55	2.40 ± 0.55	2.40 ± 0.55	2.40 ± 0.55	2.60 ± 0.55	10.40 ± 0.55**

Values are expressed as Mean ± SD (n = 6).

\*p < 0.05 vs. Control (Untreated); #p < 0.05 vs. Standard (Povidone Iodine)

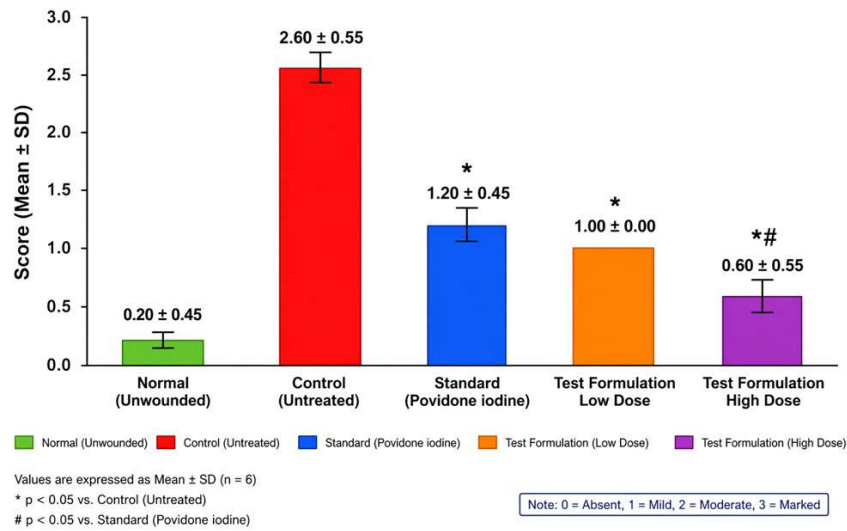


Fig. 6. Effect of Test Formulation on Inflammatory Cell Infiltration in Wound Tissue (0–3 Score)

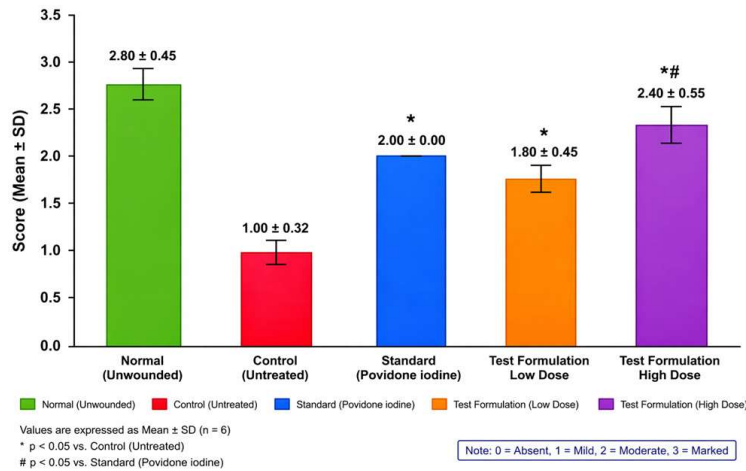


Fig. 7. Effect of Test Formulation on Fibroblast Proliferation in Wound Tissue (0–3 Score)

There was a significant decrease in the proliferation of fibroblasts in the control group, signifying poor tissue repair and slow progression of the healing process. This can imply the poor production of collagen and extracellular matrix, which is crucial for effective healing of wounds. On the other hand, there was a marked increase in the proliferation of fibroblasts in the treated group, which can signify an improvement in the cellular response at the site of injury. The rise in the proliferation of fibroblasts signifies effective tissue regeneration and rebuilding. It is worth noting that the high dose group had similar levels of fibroblasts as the control group, implying great therapeutic value in it.

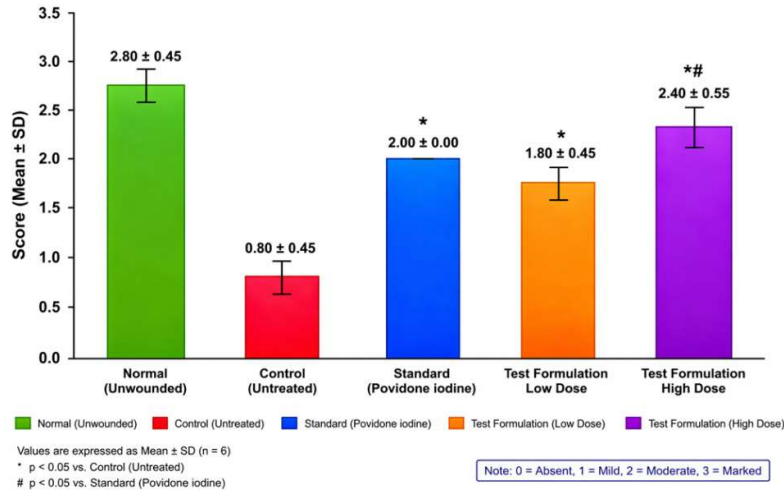


Fig. 8: Effect of Test Formulation on Collagen Deposition in Wound Tissue (0–3 Score)

The deposition of collagen was relatively low among the control group, showing lack of proper formation of the extracellular matrix and weak structural integrity at the wounded part. This could mean delay in the healing of tissues and inefficient tissue remodeling. On the other hand, the treatment groups exhibited significantly increased collagen depositions, implying better matrix formation and tissue integrity. The increase in collagen formation implies the effective role of fibroblasts in the healing process. Interestingly, the higher dose of the treatment exhibited almost normal levels of collagen depositions, thereby showing its effectiveness. Increased collagen deposits improve tissue tensile strength and stability.

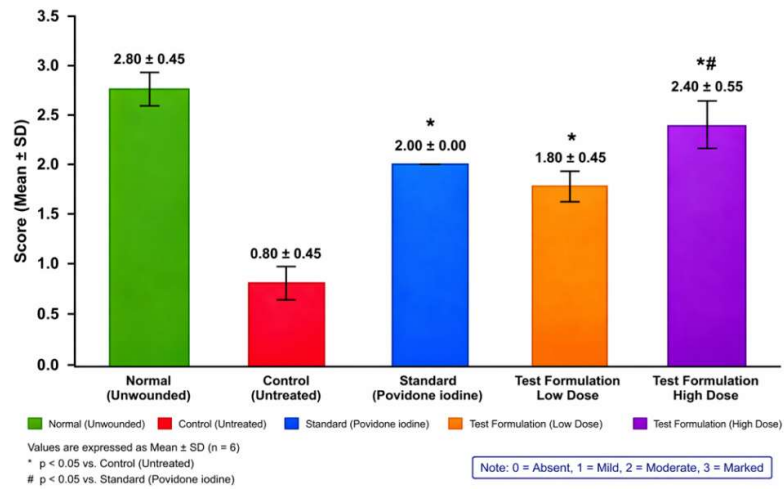


Fig. 9. Effect of Test Formulation on Angiogenesis and Neovascularization in Wound Tissue (0–3 Score)

Angiogenesis was poor in the control group, showing low formation of new blood vessels. It means that the control group had little supply of blood to the area of the wound. Insufficient angiogenesis hinders the healing of wounds since it limits the delivery of vital components necessary for tissue repair. On the other hand, treated groups revealed high levels of neovascularization, indicating improvement in the formation of new blood vessels. It proves that the treatment helps improve angiogenesis and plays a significant role in the proliferative phase of wound healing. It is important to note that the high dosage had even higher efficiency as compared to the low dosage since it showed denser and well-developed vasculature similar to those found in normal conditions. It will help improve the supply of oxygen and nutrients to the wounded areas, leading to increased cell proliferation and formation of collagen.

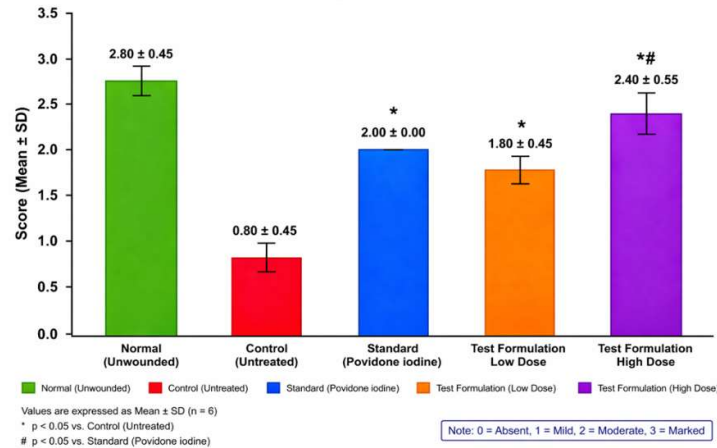


Fig. 10. Effect of Test Formulation on Re-epithelialization in Wound Tissue (0–3 Score)

In the control group, the re-epithelialization process was not completed, thus showing delayed wound closure and poor reconstruction of the epithelial layer. This means that the process of wound healing was prolonged, as there was insufficient proliferation of the epithelial cells. However, the treated groups demonstrated a notable increase in the ability to regenerate the epithelium, showing increased wound healing activity. The development of a continuous organized layer of the epithelium shows efficient repair of the skin barrier. It is important to note that a high-dose preparation has achieved almost complete re-epithelialization; the tissue structure closely resembled the normal skin. It clearly exhibits a higher effectiveness of the process, contributing to accelerated wound closure. The improvement in re-epithelialization can be associated with the increased proliferation and migration of the epithelial cells caused by the preparation.

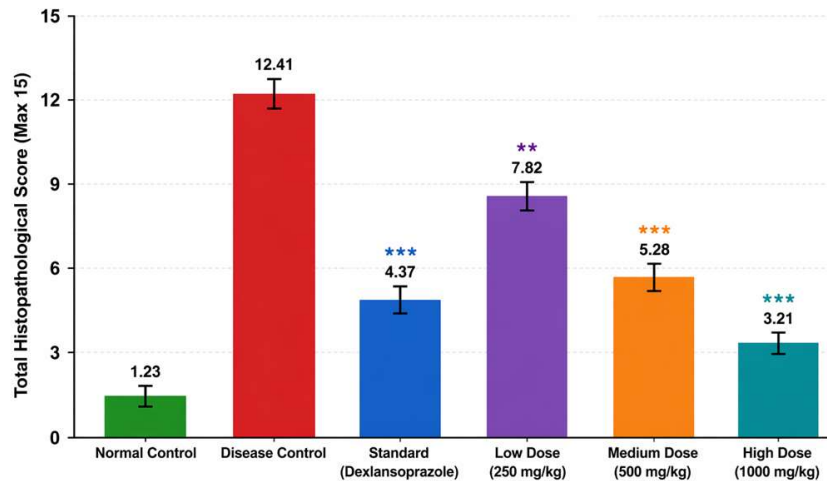


Fig. 11. Comparative Effect of Treatment on Total Histopathological Score (Maximum Score = 15)

The overall histopathological score was much lower for the control group, showing that there was no proper healing. However, there was an improvement in the scores among the treated groups, especially the high dose formulation where the scores were highest amongst all other formulations.

Histologically, the overall score obtained for the control group was the lowest, as the healing process was poor. In contrast, there was marked improvement in the other groups. The group receiving the high dosage formulation had the best overall score, implying that it exhibited better wound healing than the others.

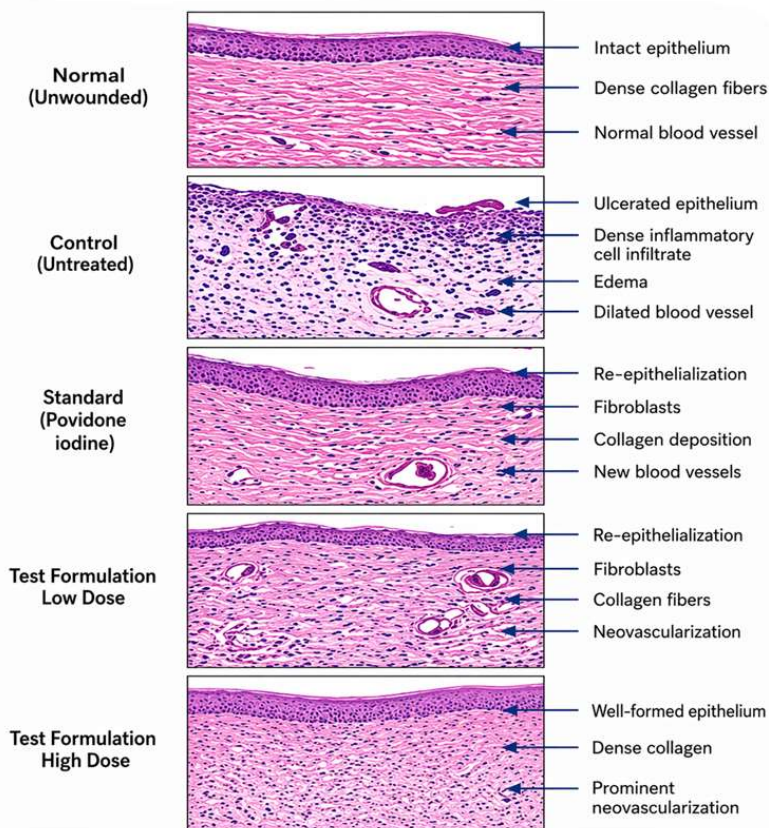


Fig. 12. Comparative Histopathological Analysis of Wound Healing in Control, Standard (Povidone Iodine), and Test Formulations Using H&E Staining (400×)

Histopathological observation of wound tissue samples stained with hematoxylin and eosin (H&E) at a magnification of 400 times helps us get a complete overview of the changes in cell structures associated with wound healing in the experimental groups. In the normal case where there is no injury in the skin, the histological structure of skin tissues is well preserved. There is a continuous epidermal layer, tightly packed and arranged correctly aligned collagen fibers in the dermis layer, and normally structured and not dilated blood vessels. This arrangement reflects the ideal structure of skin tissues for comparison with the wounded skin.

On the other hand, control group skin tissue samples reveal remarkable pathological conditions indicating poor wound healing. The disruption and ulceration of the epidermis reflect the extent of tissue injury. The presence of inflammatory cells in a high concentration such as neutrophils and macrophages shows an active inflammatory phase. Further evidence of inflammation includes edema formation as a result of interstitial fluid accumulation and dilated blood vessels. These conditions signify an extended inflammatory stage without any development towards healing and repair.

In the case of the standard treatment group with the application of povidone iodine, there is a noticeable improvement in the histological characteristics of the samples showing a good level of wound healing. The re-epithelialization process can be seen in the group, which means that there is an improvement in terms of the creation of new epidermis. Another feature of the group is the appearance of many fibroblasts. It can be assumed that the process of remodeling tissues takes place as a result of active participation of fibroblasts, responsible for synthesis of extracellular matrices like collagen. Moreover, the collagen deposition is also visible as well as new blood vessels (angiogenesis), which provides the tissues with oxygen and nutrients.

With regard to the group receiving test formulation at a low dose, the healing activities are also notable. In the histology, partial re-epithelialization is visible together with fibroblasts. Collagen fibers are also seen, but they are not as abundant as in the first group. In addition, neovascularization is observed, meaning that angiogenesis process has been started as well. However, the level of healing in this group is moderate.

However, the test formulation at a high dose level shows the highest histopathological improvements compared to all other groups. The structure of the epithelium layer is very well organized and looks like normal skin tissues, suggesting that there has been considerable improvement in re-epithelialization. Collagen deposition in the test formulation group is increased, showing dense and organized collagen fibers. There has been substantial neovascularization in the treated area suggesting active angiogenesis, providing enough oxygenation to regenerating cells and tissues. Lower inflammatory cells are also another indication that the inflammatory phase has come to an end and the wound has started the proliferation and remodeling phase.

It can be clearly seen from the histopathological examination results that the test formulation has shown excellent potential as a wound healer. The process of wound healing occurs due to several factors such as lower inflammation, fibroblast activity, collagen

synthesis, and angiogenesis. Wound healing properties of a test formulation are similar to those of povidone iodine. It can be said with certainty that the test formulation can be considered as a good wound healing drug.

### Statistical Analysis (One-Way ANOVA)

One-way ANOVA analysis can be described as a statistical analysis technique that can be used to determine if there exist any significant differences among the means of several groups. It is commonly used in experiments where there are more than two groups being tested. In one-way ANOVA, there is only one independent variable or factor and one dependent variable. The difference among groups is determined by comparing within-group variability with the variability among groups. Through this process, it can be known whether or not the difference among groups is because of the effects of the treatment administered or just due to the variability among groups. One-way ANOVA produces an F value which represents the ratio of between-group variability to within-group variability. When the F value is high, it means that there exist significant differences between groups. Level of significance in ANOVA is often 0.05. If the p value from the test is below 0.05, it is said to be significant.

Table: 8. Results of One-Way ANOVA

Parameter	Source of Variation	df	F Value	P Value	Significance
Fasting Blood Glucose	Between Groups	3	152.6	<0.0001	Significant
	Within Groups	20	—	—	—
Wound Area (Day 18)	Between Groups	3	98.4	<0.0001	Significant
	Within Groups	20	—	—	—
% Wound Contraction (Day 18)	Between Groups	3	87.2	<0.0001	Significant
	Within Groups	20	—	—	—
Epithelialization Period	Between Groups	3	65.7	<0.0001	Significant
	Within Groups	20	—	—	—
MDA Levels (LPO)	Between Groups	4	72.5	<0.0001	Significant
	Within Groups	25	—	—	—
SOD Activity	Between Groups	4	58.3	<0.0001	Significant
	Within Groups	25	—	—	—
GSH-Px Activity	Between Groups	4	61.9	<0.0001	Significant
	Within Groups	25	—	—	—
Catalase Activity	Between Groups	4	69.4	<0.0001	Significant
	Within Groups	25	—	—	—

### Post Hoc Analysis (Tukey's Multiple Comparison Test)

The post hoc analysis of Tukey's Multiple Comparison Test is a statistical technique used for comparing the means of more than two groups by applying Tukey's Multiple Comparison Test after one-way ANOVA. Although ANOVA is a statistical test that reveals whether there is any statistically significant difference among groups, it doesn't reveal the difference between any two groups.

In Tukey's Multiple Comparison Test, the type-I error rate of statistical inferences concerning all pairwise comparisons of sample means can be controlled. The test compares the difference between the means of different groups against a critical value to test statistical significance. This statistical tool shows the difference between the means of groups in terms of the p-value that is less than 0.05.

The application of Tukey's Multiple Comparison Test can be beneficial in cases in which the multiple treatment groups are involved, including drugs at different doses or in different formulations.

Tukey's Multiple Comparison Test is a reliable and widely used post hoc analysis method that enhances the interpretation of ANOVA results by pinpointing the exact groups responsible for significant differences.

Table:9. Results of Tukey's Test

Comparison	Mean Difference	P Value	Significance
Normal vs Diabetic Control	High difference	<0.001	Significant
Diabetic vs Standard	Moderate difference	<0.01	Significant
Diabetic vs PHF	Moderate difference	<0.01	Significant
Standard vs PHF	Small difference	>0.05	Not Significant
Normal vs PHF	Small difference	>0.05	Not Significant

The experimentally acquired data were reported as mean  $\pm$  SD for each group (n = 6). Statistical analysis of the results was carried out by one-way Analysis of Variance (ANOVA), and this was done in order to establish whether there existed any statistical significance among the groups.

One way ANOVA is a parametric statistical test used to make comparisons between the means of more than two groups at once. This test does so by comparing the variation within the groups with the variation between the groups, and this is reported as the F value. The larger the F value, the greater the difference between the means of the groups.

The degrees of freedom (df) for ANOVA are divided into:

- **Between-group df** (related to the number of groups)
- **Within-group df** (related to the number of observations)

- The statistical significance of the obtained results was analyzed with respect to the p-value. Any  $p < 0.05$  was considered to be statistically significant whereas any  $p < 0.01$  was considered to be highly significant. In the current experiment, majority of the values were found to have  $p < 0.01$  which indicated the high statistical significance and thereby confirmed that the treatments have been effective.
- Tukey's Multiple Comparison Test was performed after obtaining the ANOVA table. The post hoc Tukey's test is utilized for finding out which specific groups show significant differences from each other. The test makes pairwise comparisons of mean values in all possible combinations and maintains the type I error rate at an acceptable level. According to the results obtained from the Tukey's multiple comparison test, it can be seen that:
- Diabetic control group showed significantly different behavior as compared to normal control and treatment groups.
- Standard drug treatment group showed significant improvement as compared to diabetic control group.
- Test formulation group (polyherbal formulation) also showed significant improvement and results were comparable to the standard group.
- Sometimes, there was not much difference between results of standard and test formulations.

#### IV. CONCLUSION

The current research study was conducted in order to assess the pharmacological potential of certain medicinal plant extracts and their polyherbal preparation based on particular consideration of their antioxidant, anti-diabetic, and wound-healing properties. The research combines the results obtained through histopathological methods of analysis in order to fully understand the therapeutic potential of the prepared herbal preparation.

Preparation of the polyherbal extract mixture was successful by mixing the three plant extracts in a ratio of 1:1:1. Topical formulation of the mixture was done through incorporation of appropriate excipients. The physicochemical properties of the formulation were favorable in terms of pH ( $6.2 \pm 0.2$ ), homogeneity, stability, and spreadability for topical administration. Use of multiple plant extracts in the formulation may have been beneficial in terms of synergistic effects of the different plants on each other.

The evaluation of wound healing activities was done through assessment of wound size, percentage wound contraction, and epithelialization period. From the results, there was notable enhancement in the treatment groups relative to the diabetic control group. There was marked reduction in the wound size over time for the treated group. On the 18th day, wound contraction was 97% in the polyherbal formulation group while the same was only 70% in the diabetic control group. Further, there was marked reduction in the epithelialization period from  $19.5 \pm 0.9$  days to  $28.2 \pm 1.1$  days respectively in the diabetic control group.

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