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Research Article

EVALUATION OF WOUND HEALING POTENTIAL OF POLYHERBAL FORMULATION IN EXPERIMENTALLY INDUCED DIABETIC RATS

Amena Shafia*¹, Dr. Sumer Singh², and Dr. Mohd Rafiq³¹Ph. D Scholar, Singhania University, Rajasthan.²Professor, Singhania University, Rajasthan.³MAM College of Pharmacy, Gulbarga, Karnataka.

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Abstract:

For a wound to successfully heal, four extremely involuntary automated overlapping stages must occur. These stages include hemostasis, the inflammatory phase, proliferation, and remodelling. The objective of the study was to determine efficacy of polyherbal formulation (i.e, Echinops echinatus, Tabernaemontana divaricata, and Portulaca quadrifida) extract against wound healing activity upon topical application. The collected polyherbal plants was extracted with ethanol. Results showed that The wound healing activity of ethanolic extract of selected polyherbal formulation was studied by using excision wound model and the extract showed significant wound healing activity when compared to control and similar to standard FSC (Framycetin sulphate cream). It can be concluded based on findings that polyherbal formulation extract may be used as an alternative medicine in the healing of wound.

Keywords: Polyherbal formulation, Excision model, Wound healing, Diabetic Rats

Corresponding author:**Amena Shafia,**

Ph. D Scholar, Singhania University, Rajasthan.

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INTRODUCTION:

Considering various adverse effects associated with synthetic medicines, researchers are shifting their thinking towards herbal based medicines which are safe to use. Throughout the world tribal's as well as the folklore traditions used a huge number of plant extracts for various elements including curing cuts, wounds, bruises and burns.

Wounds are physical injuries that result in an opening or breaking of the skin. Whereas healing is a natural phenomenon by which body itself overcome the damaged to the tissue but the rate of healing is very slow and chance of microbial infection is high. Therefore, proper healing of wounds is essential for the restoration of disrupted anatomical stability and disturbed functional status of the skin¹.

The present study is designed to assess the wound healing potential of polyherbal formulation (*Echinops echinatus*, *Tabernaemontana divaricata*, and *Portulaca quadrifida*) in experimentally induced diabetic rats.

Echinops echinatus which belongs to the family *Asteraceae* (*Compositae*) commonly known as "Usnakantaka" is a xerophytic herbaceous plant found in India, Pakistan and Sri Lanka. It is an erect, rigid, pubescent, annual herb about 1 m in height. It has short, stout stems with branches widely spreading from the base. The leaves are alternately arranged, sessile, oblong, deeply pinnatifid, Flowering occurs between December and January². the plant shows a wide range of pharmacological activities such as antifungal, analgesic, diuretic, reproductive, hepatoprotective, antioxidant, anti-inflammatory.

Tabernaemontana divaricata belongs to the Apocynaceae family. The generic synonym of *Tabernaemontana divaricata* is *Ervatamia coronaria* and widely distributed in tropical countries as a garden plant. *Tabernaemontana divaricata* is a shrub or small tree, usually glabrous, found in the Konkan, North Kanara, Western Ghats in Malabar, throughout North India^{3,4,5}. In traditional medicine, *Tabernaemontana divaricata* is used to treat various diseases such as diarrhea, abdominal tumors, arthralgia, asthma, epilepsy, eye infections, fever, fractures, headache, inflammation, leprosy, mania, edema, paralysis, piles, rabies, rheumatic pain, skin diseases, urinary disorders, strangury, toothache⁶.

Portulaca quadrifida which belongs to the family *Portulacaceae* is the small diffused herb and commonly grown at river banks. It is available in both wild and cultivated plants. It is also used as Vegetable. The plant is sour, bitter, hot, alterative, laxative; causes biliousness and "Kapha;" cures low grade fever, asthma, cough, urinary discharges, inflammation; good for eye diseases, skin diseases and ulcers(Ayurveda) and also cures jaundice, cardiovascular diseases and gonorrhea. *Portulaca quadrifida* has been reported to possess Centrifugal activity against *Aspergillus Fumigates* and *Candida albicans*⁷

MATERIAL AND METHODS:

Solvent Extraction⁸:

The drug was subjected to systematic phytochemical screening by ethanolic extract by qualitative chemical analysis.

Preparation of Plant Extracts:

Plants were collected and shade dried, powdered in a mechanical grinder and passed through a sieve no.40 to obtain powder of desired particle size.

The powder material was subjected to solvent extraction in soxhlet assembly with ethanol for 48 hours using soxhlet apparatus. The filtrate is collected and evaporated to dryness under reduced pressure using a Rotary flash evaporator. The extract obtained with each solvent was weighed; its percentage was calculated in terms of air-dried weight of poly herbal plant material. The dried extract is stored in dry sterilized small containers at 4°C until further use.

Preparation of alcohol extraction:

The dried marc from the above process was extracted successively with methanol to get alcoholic extract. The marc was collected, dried and used for further investigation.

PREPARATION OF SIMPLE OINTMENT BASE:

Wool fat, hard paraffin, cetostearyl alcohol, white soft paraffin were obtained from our pharmaceutical technology laboratory.

Simple ointment base:

1. Wool fat	5%
2. Hard paraffin	5%
3. Cetostearyl alcohol	5%
4. White soft paraffin	85%

Procedure:

In preparing simple ointment base melt hard paraffin and cetostearyl alcohol on water bath. To this incorporate wool fat and white soft paraffin. Stir until all ingredients are melted. Examine the content for the foreign particle. Decant or strain if required. Stir the mixture thoroughly until cold.

Preparation of ointment for *POLY HERBAL FORMULATION* of methanolic extract:

The Poly herbal formulation ointments for two different concentrations were prepared by using simple ointment base.

Poly herbal formulation (10%) ointment for alcoholic extract:

Simple ointment base	-	
90% (90gm)		
Alcoholic extract	-	10%
(10gm)		

Procedure:

In order to obtain the best results the extract (S) is triturated with small amount of simple ointment on an ointment slab with the help of a stainless steel spatula with long broad blade. Gradually add the remaining quantity of simple ointment with thorough trituration until a homogeneous mass is obtained.

The wound healing property of different extract of Poly herbal formulation were investigated by excision wound model, using albino rats. The methods and animals used in wound healing study are as follows.

ANIMAL SELECTION & ACCLIMATIZATION OF ANIMALS

Albino Wistar rats used in wound healing activity and Swiss albino mice used in Acute Oral Toxicity Study will be housed in polypropylene cages and feed with standard rodent pellet diet and water ad libitum. Room temperature - 21-25 °C. Relative humidity-50-60%. The animals will be exposed to alternate cycle of 12 hours of darkness and 12 hours' light. Albino mice weighing 20-30 g are used in acute toxicity study. Ethical clearance for performing the experiments on animals was obtained from the Institutional Animal Ethics Committee (IAEC).

INDUCTION OF DIABETES:

ALLOXAN AS DIABETOGENIC AGENT MECHANISM

Alloxan hydrate is a urea analog which was reported to produce permanent diabetes in animals. Alloxan is a highly reactive molecule that readily reduces to diuleric acid, which is then auto-oxidized back to alloxan resulting in the production of free radicals. These free radicals damage the DNA of β -cells and cause cell death. The second mechanism of alloxan is

its ability to react with protein SH group, especially the membrane proteins like glucokinase on the β -cells, finally resulting in cell necrosis⁹.

PROCEDURE: The animals are fasted overnight and were made diabetic with intraperitoneal injection of alloxan monohydrate (150mg/kg body weight) dissolved in normal saline. The blood glucose levels were checked before and after administration of alloxan to confirm the diabetes. Since Alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 30 percent glucose solution orally at different time intervals after six hours of alloxan induction, and 5 percent glucose solution was kept in bottles in their cages for the next 24 hr to prevent hypoglycemia. After 10 days, rats with blood glucose range of 250 to 375mg/dl were used for this experiment.

EVALUATION OF WOUND HEALING ACTIVITY.

EXCISION WOUND MODEL

The animals were anaesthetized prior to creation of the wounds, The rats were inflicted with the excision wounds as described by Morton and Malon. The dorsal fur of the animal was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area of about 300 mm and 2 mm depth was created along the markings using toothed forceps, a surgical blade and pointed scissors. The entire wound was left open.

The animals were divided into three groups with six animals each. Group I (Normal control- receives plain drinking water), Group II (Diabetic control- receives plain drinking water), Group III (Diabetic group- receives 1000mg/kg body weight of extract), orally until complete healing takes place. The wound closure rate was assessed by tracing the wound on alternate days of post wounding using transparent paper and a permanent marker. The wound areas recorded were measured using graph paper. The day of eschar falling off, after wounding, without any residual raw wound was considered as the time until complete epithelialization.¹⁰⁻¹⁶

Percentage of wound contraction= Initial wound size – Specific day wound size/Initial wound size x 100
The results were analyzed by one – way ANOVA followed by Dunnet's test.

RESULTS AND DISCUSSION:**Table -01: Percentage yield extract of selected polyherbal formulation.**

Sl. No	Extract	Nature of extract	Colour	Weight (gm)	% Yield (w/w)
01	Ethanolic extract	Semi solid	Dark Brown	17.43	1.7

Table - 02: Effect of ethanol extract of polyherbal and nanoparticle on excision wound

Treatment Groups in diabetic affected Rats	Percentage wound contraction on				Epithelization Period (Days)
	4th day	8th day	12th day	16th day	
Simple ointment base (B.P) (Control)	20.70±0.36	32.34±3.35	46.10±3.32	51.50±2.79	33.12±2.72
Polyherbal formulation	40.08±1.63	38.08±1.72	89.40±0.87	99.86±0.30	21.00±0.91
Framycetin sulfate	36.00±2.26	58.34±3.13	86.23±2.81	93.45±2.30	21.00±0.56

Values are mean ± S.E.M of 6 animals in each group. Numbers in parenthesis indicates percentage of wound contraction. ** p < 0.001 vs respective control by students 't' test.

In excision wound model study, the tropical application of ethanolic extract of polyherbal showed significantly greater wound healing activity when compared to control animals.

CONCLUSION:

The wound healing activity of ethanolic extract of selected polyherbal formulation was studied by using excision wound model and the extract showed significant wound healing activity when compared to control and similar to standard FSC (Framycetin sulphate cream) Moreover the extract did not produce any adverse effect and because of this it can be strongly recommend in different wound healing models like burn wound, dead space wound, injury by X-ray radiation and ultraviolet light etc.

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