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SCREENING OF WOUND HEALING ACTIVITY USING MATURATION OF SILVER NANOPARTICLES EXTRACTED FROM POLYHERBAL FORMULATION IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Nanoparticles are materials with overall dimensions in the nanoscale, ie, under 100 nm. In recent years, these materials have emerged as important players in modern medicine, with applications ranging from contrast agents in medical imaging to carriers for gene delivery into individual cells. Nanoparticles have a number of properties that distinguish them from bulk materials simply by virtue of their size, such as chemical reactivity, energy absorption, and biological mobility. The present work was aimed to detect the wound healing and maturation of silver nanoparticles (SNP) extracted from polyherbal plants (*Semecarpus Anacardium*, *Vachellia Nilotica*, and *Portulaca Quadrifida*). 20g of selected polyherbal contains 20mg of SNP. SEM and XRD analysis showed the size and characterization of the silver particle was less than 100nm. Thermal wound induced in diabetes animals model was used for wound healing effects of SNP. The wounds decreased in size gradually with time, closed at 2 weeks in normal mice and at 3 weeks in diabetic mice. In diabetic mice at 15 days, a significant difference in ($P<0.01$) wound closure was noted on the skin. Histopathological observation of skin showed improvement in the surface epithelium. The results of the present study revealed that the SNP from selected polyherbal plants was considered as a potential drug target for tissue injury.

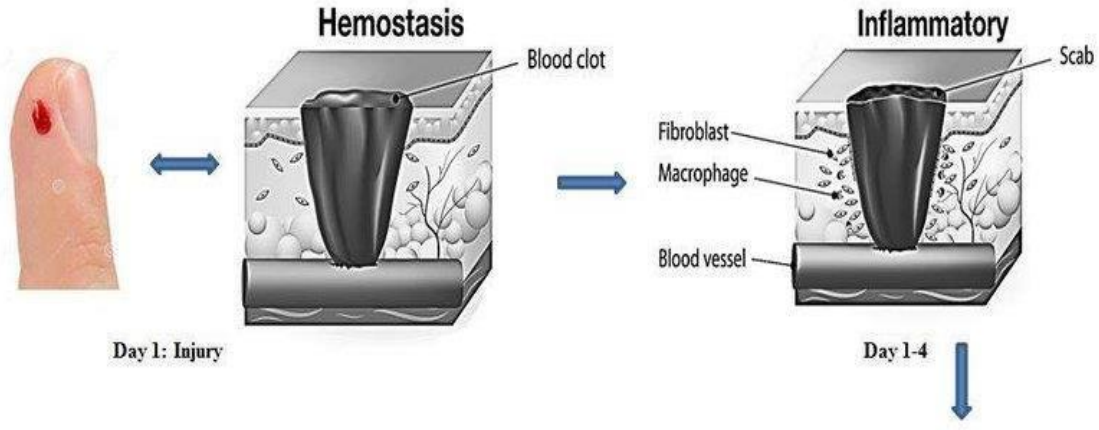
KEYWORDS: Wound healing, Polyherbal, Diabetes mellitus, Silver nano particles.

INTRODUCTION

Plants are served as major natural resources for traditional as well as modern medicinal system all over the world. The therapeutic potential of plants and plant products can be traced back to thousands of years ago. The information with respect to medicinal benefits of plants with other therapies has been preserved in several documentations.[1] 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.[2] Medicinal plants play vital roles in disease prevention and their promotion and use fit into all existing prevention strategies.

The exploitation of metals as antibacterial agents is traced back to early civilizations. During the past ten years, the use of metals and metal nanoparticles to fight infection has increased. Numerous metal nanoparticles are now easily accessible on the market and utilized more regularly in hospitals for dressings.[3] Metal nanoparticles like silver, gold, and zinc are the main options for wound dressing's development because of their antibacterial capabilities and low toxicity.[4] Noble metal nano particals is a white shiny metallic element found widely in the human external environment. Low concentrations of noble metal nano particals are present in the human body through inhalation of particles in the air while in inspiration and contamination of the diet and drinking water, but traces amount of noble metal nano particals serves no effect in the human body.[5,6]

Wounds result from disruption of the normal anatomical epithelial lined tissue barriers and may be caused by trauma, tissue resection, or burns.[7]



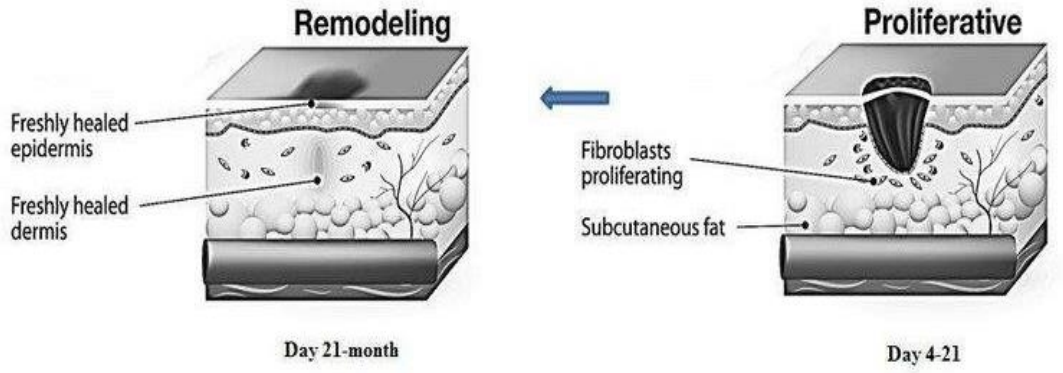


Figure 01: Stages of wound healing.

Some wounds fail to heal in a timely fashion and become chronic as a result of co-existing conditions such as diabetes or peripheral vascular disease. Failure to heal might also result from post-operative wound infections which are estimated to affect up to 4% of patients who undergo surgery. Chronic non-healing wounds represent a growing health and economic burden and are associated with a high morbidity that adds significantly to the cost of medical care.[8] Non-healing wounds represent a growing problem due to their high morbidity and cost. Despite recent advances in wound healing, several systemic and local factors can disrupt the weighed physiologic healing process.[9] This paper critically reviews and discusses the role of nanotechnology in promoting the wound healing process using polyherbal formulation (*Semecarpus Anacardium*, *Vachellia Nilotica*, and *Portulaca Quadrifida*) in experimental animals.

Semecarpus Anacardium (Family: *Anacardiaceae*) is distributed in tropical and central parts of India, Western peninsula and North Australia. The fruit is kidney shaped, drupaceous nut with a fleshy pear-shaped receptacle. The nut is commonly called as ‘marking nut’ and in the vernacular as *Ballataka* or *Bhilawa*.[10]

Acacia Nilotica (*Mimosaceae*) indigenously known as '*Babul*' or '*Kikar'* is a proverbial, medium sized tree and is broadly scattered in tropical and subtropical countries. It has an inspiring range of medicinal uses with potential anti-oxidant activity. This plant contributes a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes.[11]

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 *Asphergillus Fumigates* and *Candida albicans.*[12]

MATERIALS AND METHODS

Experimental animals Male Wister albino rats of four (4) weeks, weighing between 150 and 200 g were obtained from well-maintained central animal house at MRMC Campus, Gulbarga, Karnataka state, India and it was further used for wound healing investigation. The animals were housed in standard environmental conditions of temperature (31 ±1oC), humidity (60± 0.2%) and 12 hours light and 12 hours dark cycle. Rats were fed with standard rodent diet and tap water ad libitum. The study was carried out according to the guidelines of laboratory animal care.[13]

Extraction of plant materials[14]

2000 gm of various plants mentioned were shade dried, powdered and made to pass through sieve no.40. Then the powdered of *Semecarpus Anacardium*, *Vachellia Nilotica*, and *Portulaca Quadrifida* made to undergo soxhlation with 90% ethanol individually. The extraction procedures were carried out till a sufficient quantity of extract was obtained. After extraction the filters of different plants were pooled and concentrated under reduced pressure to obtained dried solid mass. The percentage yield was calculated and tabulated (Table 1).

Preparation of ointment by fusion method for topical application[15]

(a) Preparation of simple ointment Wool fat - 2 gm: Hard Paraffin-2 gm; Cetostearyl alcohol - 2 gm; White Soft Paraffin-34 gm. Each ingredient was mixed and heated gently with stirring then cooled. The base was then packed in a wide mouth container.

(b) Formulation of different ointments:[16] Four different formulations were prepared. All formulations were having simple ointment base. Formulations I, II, III were 1% w/w of each concentration of extracts of *Semecarpus Anacardium*, *Vachellia Nilotica*, and *Portulaca Quadrifida* and Formulation IV was 3% w/w concentration (i.e. 1% w/w of each extract) which is used for activity as polyherbal formulation with nano materials.

Synthesis of silver nanoparticle[17]: 1mM AgNO3 aqueous solution of silver nitrate was freshly prepared for synthesis of silver nanoparticles. 20ml of Silver nitrate solution was

added to 20gm of polyherbal extract added in a conical flask. The extract with the substrate was incubated in dark (to minimize the photo activation of silver nitrate) at 37 °C under static condition. A control set up was also maintained without polyherbal formulation extract. The quantity of the synthesized silver nanoparticle was assessed.

Procedure

Honey

Honey is a popular folk treatment for burns and other skin injuries. Preliminary evidence suggests that it aids in the healing of partial thickness burns 4–5 days faster than other dressings, and moderate evidence suggests that post-operative infections treated with honey heal faster and with fewer adverse events than with antiseptic and gauze.

First step: Solution A

- Take 25 g of honey was dissolved in 100 ml of distilled water.
- Then the solution is kept on the hot plate with continuous magnetic stirring at 25°c.

Second step: Solution B

Silver nitrate is an inorganic compound with chemical formula AgNO3. This salt is a versatile precursor to many other silver compounds, such as those used in photography. It is far less sensitive to light than the halides.

- Take 0.169 g of silver nitrate is mixed with 100 ml of distilled water and it is kept aside.
- And Take 20 ml of honey Prepared solution and added to 25 ml aqueous solution of AgNO3 (10−3 M) and stirred well for 15 min.
- To initiate the reduction of Ag ions.
- And the pH was adjusted to 6.5 using NaOH.

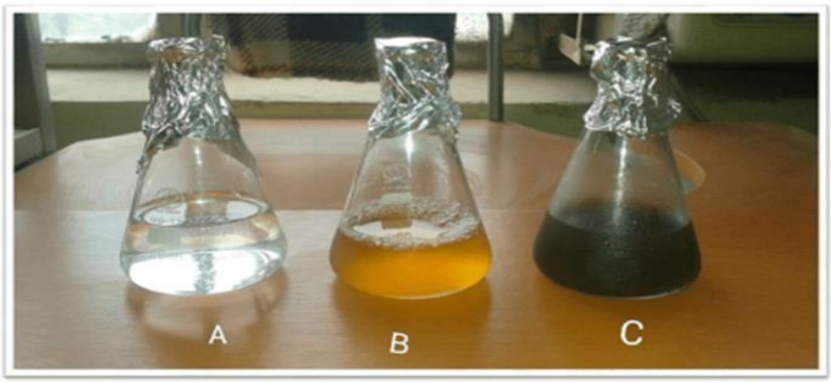


Figure 02: A-C: Polyherbal extract showing the color change in the synthesis silver nanoparticles on incubation time.

Characterization of silver nano particles

UV-VIS spectra analysis: The reduction of metallic silver ions was monitored by measuring the UV-VIS spectrum after about incubation. A small aliquot was drawn from the reaction mixture and a spectrum was taken on a wave length from 300 nm to 800nm on UV-VIS spectrophotometer.

XRD analysis: For the XRD and SEM analysis, the suspension of nano particles was dried into powder and about 1mg fine powder was used for the analysis. XRD analysis was recorded by using philips PW 1830 X-ray generator operator at a voltage of 40 Kev.

SEM analysis: SEM analysis was carried out on JEOL JSM 6360 A (SEM) and using JEOL JSM 1600 A fine coated for uniform.

Induction of alloxan as diabetogenic agent

Mechanism

Alloxan monohydrate is a urea analog which is reported to produce permanent diabetes in animals. Alloxan is a highly reactive molecule that is readily reduces to dialuric 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 necrosis.[18]

Procedure

Diabetes is induced in Wistar rats by single intra peritoneal injection of alloxan monohydrate in sterile normal saline to overnight fasted animals at a dose of 120 mg/kg b.w. The fasting blood glucose level will be determined after 72 hours of alloxan injection. The rats having blood glucose levels above 200 mg/dl is used for the study. The diabetic animals is allowed free access to tap water, pellet diet, and maintained at room temperature in plastic cages.[19]

Oral administration of Extract /Drug

As ethanol extract of selected plants and Pioglitazone is insoluble in water, they are suspended in 5% Tween80 (w/v), administered orally according to the dosage in the respective groups using an intra-gastric feeding tube. Method of collection of blood samples: Tail cut method from tip of the rat tail. Estimation of blood glucose Blood glucose estimation is done by using Accu-chek Active glucometer. It uses glucose oxidase specific strips and

works on principle called as Reflectance Photometry. It is easy to use, quick to perform and reliable. There is a reasonable co-relation between laboratory results and those obtained with glucometer.[20,21]

Wound healing activity

Excision and incision wound models were used to evaluate the wound-healing activity of ethanolic extract of polyherbal formulation. The study was approved by the Institutional Animal Ethical Committee of luqman College of Pharmacy, P & T colony, Gulbarga – 585102 – Karnataka - India, registered under CPCSEA, India. (346/CPCSEA)

Excision wound: The rats were inflicted with excision wounds under light ether anesthesia. A circular wound of about 2.5 cm diameter was made on depilated dorsal thoracic region of rats.[22] The animals were divided into three groups, each group containing six animals. Group-I was considered as control and received simple ointment base (B.P), Group-II served as reference standard and received framycetin sulfate cream 1% w/w. Group-III received the test formulation. The ointments were applied topically once in a day, till the epithelization was complete starting from the day of experiment. The parameters studied were wound closure and epithelization time. The formulation was applied until complete wound healing. The percentage of wound closure and the period of epithelization were calculated. The period of epithelization was calculated as the number of days required for wound healing. (Results were tabulated in Table 02).

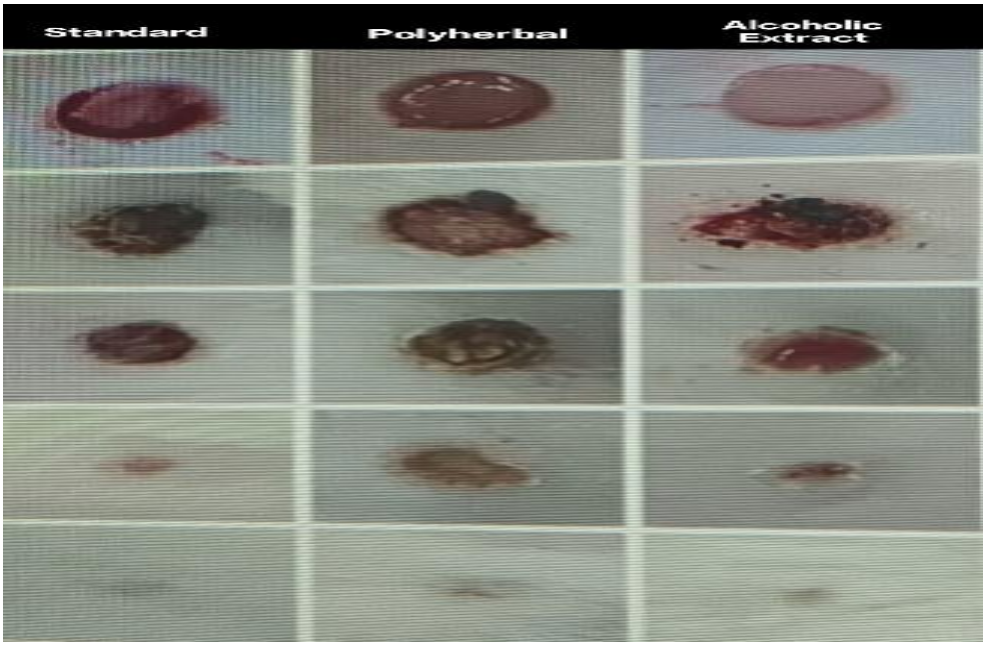


Figure 03: Excision wound model day wise.

Incision wound: In incision wound model, 6 cm long paravertebral incisions were made through full thickness of the skin on either side of the vertebral column of the rat.[23] The wounds were closed with interrupted sutures of 1 cm apart. The animals were divided into three groups, each group containing six animals. The categorization and treatment of experimental animals was similar to that of excision wound model. The ointment containing the test formulation was applied topically once in a day. The sutures were removed on 8th post wound day and the tensile strength of the healed wound was measured on 10th day following continuous water flow technique.[24] (Results were tabulated in Table 03). Statistical analysis: The data is expressed as mean ± SEM and subjected to students-t'test and the level of significance was set at $p < 0.001$.

RESULTS AND DISCUSSION

Table 01: Percentage yield extract of polyherbal formulation.

Sl. No	Extract	Nature extract	Colour	Weight (gm)	% Yield (w/w)
01	Ethanolic extract	Semi solid	Dark Brown	27.56	2.7

Table 02: Effect of ethanol extract of Polyherbal and Nanoparticle on excision wound.

Treatment Groups	Percentage wound contraction on				Epithelization Period (Days)
	4th day	8th day	12th day	16th day	
Simple ointment base (B.P) (Control)	18.50±0.34	30.34±1.33	44.10±1.30	49.50±0.77	31.12±0.70
Polyherbal with nano particle	36.08±1.63	36.08±1.72	87.40±0.87	98.86±0.30	19.00±0.89
Framycetin sulfate	34.00±0.24	56.34±1.11	84.23±0.79	91.45±0.28	19.00±0.34
Values are mean ± S.E.M of 6 animals in each group. Numbers in 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 and almost similar result with silver nitrate 1%.

Table 03: Effect of ethanolic extract of polyherbal formulation with nanoparticles on wound healing in incision wound.

Group	Treatment	Tensile strength in g ± SEM
1	Simple ointment base (BP) control	121.33±6.69
2	Polyherbal with nanoparticle	329.41±8.79**
3	Framycetin sulfate	309.23±1.35**
Values are mean ± S.E.M of 6 animals in each group. ** $p < 0.001$ vs respective control by student —t‖ test.		

Figure 04: Incision wound model.

In incision wound model study, significant increase was observed in the skin tensile strength of ethanolic extract of Polyherbal treated group on 10th post wounding day when compared to control.

The results of ethanolic extracts of Polyherbal on both excision and incision wound model showed significant acceleration in the process of wound healing by decreasing the surface area of the wound and increasing the tensile strength.

Our present study emphasized the present need of medicinal plants against synthetic drugs on wound healing potentials.

CONCLUSION

The wound healing activity of ethanolic extract of polyherbal formulation was studied by using excision and incision wound model and the extract showed significant wound healing activity when compared to control and almost similar to standard silver nitrate 1 %. 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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