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Exploration of Wound Healing Activity of Polyherbal Formulation

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ABSTRACT

The present study was prepared wound healing activity of polyherbal formulation (*lablab purpureous, Nerium indicum, Tebernamontana derivitacate)* in Wister rat in excision and insision wound model. The appropriate gel formulation applied on tropically. Wound healing was monitored on Days 4, 8,12 and 16 days. and histological evaluation was carried out on the samples. The wound area of each animal was measured at the intervals of 24-48 hrs using transparent polythene graph paper and then tracing the area of wound on it. Contraction was calculated from the days of measurements of wound area.

Keywords: Poly herbal formulation, Wound healing, Wound contraction.

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INTRODUCTION

Wound may be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue[1]. Wound healing is a complex phenomenon involving a number of processes, including induction of an acute inflammatory process, regeneration of parenchymal inflammatory process[2], migration and proliferation of both parenchymal and connective tissue cells, synthesis of extracellular matrix (ECM) proteins, remodelling of connective tissue and parenchymal components, and acquisition of wound strength[3]. All these steps are orchestrated in a controlled manner by a variety of cytokines including growth factors[4]. Some of these growth factors like platelet-derived growth factor. (PDGF), transforming growth factor B (TGF-B), fibroblast growth factor (FGF) and epidermal growth factor (EGF) have been identified in self-healing wounds[2]. In chronic wounds, the normal healing process is disrupted due to some unknown reasons, and in such cases, exogenous application of certain growth-promoting agents or compounds which can enhance the in situ generation of these growth factors is required to augment the healing process. Several factors delay or reduce wound healing, including bacterial infection, necrotic tissue, interference with blood supply, lymphatic blockage and diabetes mellitus. Generally, if the above factors could be inhibited/controlled by any agent, increasing healing rate could be achieved[5].Optimum healing of a cutaneous wound requires a well-orchestrated integration of the complex biological and molecular events of cell migration and proliferation and of extracellular matrix deposition and remodelling. Wound care and maintenance involves a number of measures including dressing and administration of painkillers, use of anti-inflammatory agents, topical systemic antimicrobial agents, and healing promoting drugs. The aim of wound care is to promote wound healing in the shortest time possible with minimal pain, discomfort, and scarring to the patient and must occur in a physiological environment, conducive to tissue repair and regeneration. [6] Plants have the immense potential for the management and treatment of wounds. A large number of plants are used by tribal and folklore in many countries for the treatment of wounds and burns. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. These phytomedicine are not only cheap and affordable but are also safe. The presence of various life-sustaining constituents in plants has urged scientist to examine these plants with a view to determine potential wound healing properties. Alkaloids and tannins are already reported to promote the wound healing through several cellular mechanisms, chelating of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts.[7] Flavonoids are strong scavengers of reactive oxygen species provide enabling support to the healing process initially by the moderation of superoxide anions and later by enhancing the expression of vascular endothelial growth factor (VEGF), thereby enhancing angiogenesis and flow of blood as the wound repair process advances.[8,9]

Alkaloids, flavonoids and triterpenoids also exhibit the potential anti-microbial activity which also serves as a key factor in the prevention of wounds from microbes for quick and noninfectious healing.[10] The content of this study the ingradients of plants like ethanolic extract of *lablab purpureous, Nerium indicum, Tebernamontana derivitacate*. The extraction having hydroalcoholic. The formulations are developed based on the principle of ayurvedic. Where plants are wound healing activity indiced by excision wound model. In the present study the excess the effect of polyherbal formulation on wound healing, wound contraction.

MATERIALS AND METHODS

Plants collection and preparation of plant extract

Fresh leaves of *lablab purpureous, Nerium indicum* and *Tebernamontana derivitacate* were collected locally in the month of February from Govindpura, Bhopal, Madhya Pradesh, India. All the medicinal plants were dried under the shade in the laboratory. It was pulverized to moderately coarse powder. The coarse powder of leaves was passed through sieve No. 16 to maintain uniformity and stored in cool and dry place. All the medicinal plant powdered samples (100 g) were defatted by treating with pet-ether and then extracted with ethanol by using soxhlet apparatus. The solvent was removed under through distillation and dried to get the solid mass. The residue was weighed and stored in water and air proof container at room temperature.

Screening of powder

Loss on drying

About 10 gm. of the powdered drug was weighed in a tarred Petridish. It was dried at 105°C for 1 hour in hot air oven and then reweighed. Loss on drying was determined from calculating the initial and final weight.

Total ash value

About 5 gm. accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon in muffle furnace. It was then cooled and weighed. The % *w/w* of ash with reference to the air-dried drug was calculated.

Acid insoluble ash value

Accurately weighed 1 gm. ash was boiled for 5 minute with 25ml hydrochloric acid by covering the crucible with a watch-glass on water bath then cooled. The watch-glass wasrinsed with 5 ml of hydrochloric acid and this liquid was added in to the crucible. Then the content was filtered on a previously weighed Whattman filter paper and filtrate was dried and weighed. Acid insoluble ash value was determined by calculating the % content remaining after deducting the weight of filter paper.

Water soluble ash value

Accurately weighed 1 gm. ash was boiled for 5 minute with 25ml distilled water by covering the crucible with a watch-glass on water bath then cooled. The watch-glass was rinsed with 5 ml of distilled water and this liquid was added in to the crucible. The % of remaining content was deducted from initial % of ash taken (i.e. 100%) to determine the water soluble ash value.

Foaming index

About 1 gm. coarse powder was weighted and transferred to a 500 ml conical flask containing 100 ml of water. It was maintained at moderate boiling for 30 minute on water bath. It was cool and filtered in to a 100 ml volumetric flask. Volume was diluted by adding sufficient amount of water. The decoction was poured in test tube, and then shaken in a lengthwise motion for 15 seconds. They were allowed stand for 15 minutes and the height of foam was measured to determine the foaming index[11].

Phytochemical analysis of crude extract

The crude extract obtained by solvent extraction was subjected to various qualitative tests to detect the presence of common chemical constituents as: alkaloid, glycoside, carbohydrate, phytosterol, saponin, tannin, flavonoid and protein etc[12,13].

Animal care and handling

The experiment was carried out on Wistar albino rats of 4 months, of both sexes, weighing between 170 to 200 g. They were provided from Pinacle Bio-medical Research Institute, Bhopal, (M. P.). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44-56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water *ad libitum* during experiment. The experiment was approved by the Institutional Animal Ethics Committee and as per CPCSEA guidelines (Approval no. PBRI/IAEC/PN-17100).

Acute toxicity studies

Acute toxicity study was performed according to Organisation for Economic Co-operative and development guidelines No. 423.[14] Healthy adult albino rats of either sex, fasted overnight, were divided into 6 groups (n = 6 per cage) and were fed with increasing doses (1, 2, 4, and 5 g/kg body wt.) of the ethanolic extract. The total ethanol extract, administered orally in doses of up to 2000mg/kg body wt., Extract did not show any signs of toxicity up to the dose of 2000 mg/kg po.

Formulation of polyherbal formulation

The formulation of gel was prepared for the proposed study i.e. wound healing activity of polyherbal formulation. Three dose levels 1% w/w, 2% w/w and 5% w/w was selected for the preparation of gel of plyherbal formulation. A defined quantity of carbapol and the measured quantity of extracts were dispersed in 80 ml of distilled water with continuous mixing using a magnetic stirrer at 800 rpm for 1 h. Glycerol 5 ml was added to the mixture under continuous stirring. The mixture was then neutralized by drop-wise addition of tri ethanolamine. Mixing was continued until a transparent gel was formed. Fresh gel formulation was prepared for each treatment[15].

Table 1 Composition of polyherbal gel formulation

Polyherbal Formulation	Extract (%)	Carbopol (%)	Methyl Paraben (%)	Propyl Paraben (%)	Propylene Glycol (%)	Ethanol (%)	Water	Triethalamine
Gel (1%)	1	1	0.2	0.02	4	3	Upto 100	Q.S
Gel (2%)	2	1	0.2	0.02	4	3	Upto 100	Q.S
Gel (5%)	5	1	0.2	0.02	4	3	Upto 100	Q.S

Wound healing models

Wound healing activity was studied using two models viz. i) excision wound model ii) incision wound model.

Excision wound model

In the experiment, a total of 24 rats were used. The rats were divided into 4 groups comprising of 6 animals in each group as follows:

Group I:Left untreated and considered as control.

Group II: Served as standard and treated with Healgel.

Group III: Treated with 1% (w/w) polyherbal gel formulation daily, O.D.

Group IV:Treated with 2% (w/w) polyherbal gel formulation daily, O.D.

Group V:Treated with 5% (w/w) polyherbal gel formulationdaily, O.D.

The wound was created using excision method. Hair was removed from the posterior sides of rats using hair remover cream. An area about of 10 mm diameter was measured with sterile scale and this area was marked with a marker pen. The rats were anesthetized with ketamine (50mg/kg ip). After 15 minutes of anesthesia, the marked area of skin was excised with the help of surgical blade No. 18 and forceps. The skin was removed after creating the wound.Formulated gel and marketed gel (Healgel) were applied, starting from the day of the operation, till complete epithelialization time. The parameters studied were percent wound closure and epithelialization time. The wound was measured using transparency paper, a marker, scale and area was calculated. The period of epithelialization was calculated as the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound (Manjunatha, B.K. *et al,* 2005 with slight modification).[16]

The percentage wound contraction was determined using the following formula:

Percentage of wound contraction =$\frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$

Incision wound model

The animals were grouped and treated as mentioned in excision model. The rats were anesthetized with ketamine (50mg/kg i.p.). Paravertebral incision of 6 cm length was made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete haemostasis, the wound were closed by means of interrupted sutures placed at approximately 1 cm apart. For stitching, stitched with black silk surgical thread (No. 000) and a curved needle (No. 11) were used. Animal were treated daily, as mentioned above under excision wound model from 0th day to 9th post-wounding day. One day before performing the experiment (measurement of breaking strength) the sutures were removed from the stitched wounds of rats after recovery. The wound breaking strength was measured on 10th post wounding day.[17]

Statistical analysis

All the values are expressed as Mean± Standard error of mean (S.E.M.) and analyzed by One-way ANOVA and Posthoc Tukey multiple comparison testby employing statistical software, Graph Pad InStat 3. Differences between groups were considered significant at $P < 0.05$ levels.

RESULTS AND DISCUSSION

Extract was subjected to various chemical tests for preliminary identification of various phytoconstituents.Flavonoids and triterpenoids are also known to promote the wound-healing process mainly due to their astringent and antimicrobial property, which seems to be responsible for wound contraction and increased rate of epithelialisation (Table 2&3).

Table 2 Phytochemical screening of ethanolic extract of herbs

Identification Test	Test name	*lablab purpureous*		*Neriumindicum*		*Tebernamontanaderi vitacate*	
		Present	**Absent**	**Present**	**Absent**	**Present**	**Absent**
Alkaloids	Mayer's test	+		+		Present	Absent
	Dragendorff's test	+		+		+	
	Wagner's test	+		+		+	
Glycosides	Killer-killani test	+			-	+	
	Baljet test		-		-	+	
	Foam test	+		+			-
Carbohydrates	Molisch's test		-_	+			_-
	Fehling test	+		+		+	
	Iodine test	+		+		+	
Tannins	Vanillin-HCL test	+		+		+	
	Gelatin test		-	+		+	
	Ferric chloride test	+		+		+	
Flavonoids	Lead acetated test	+			-	+	
	Shinoda test	+			-	+	
	Alkaline reagent test	+			-	+	

(+) = Present, (–) = Absent

Table 3 Physiochemical analysis of powder of herbs

S. No.	Parameters	*lablab purpureous*	*Neriumindicum*	*Tebernamontanaderivitacate*
1	Loss on drying	0.92	0.72	0.6
2	Total ash value	4.11	5	7
3	Acid insoluble ash value	1.8	1.7	2.6
4	Water soluble ash value	0.9	1.12	1.25
5	Foaming index	8 (ml)	12 (ml)	22 (ml)

The studies on excision wound healing model reveal that all the groups showed day to day decrease in wound area. However, on 16th post wounding day, control animals group-I showed 68.0% of wound contraction whereas group-II standard group animals showed that of 85.58% and extract treated group-III & IV exhibited that of 76.3& 80.21 % wound contraction respectively. When compared with the standard, the activity of both formulations of extract was found to be lesser. It was also observed that reducing the epithelization period of 2% extract (20.73) and 5% extract (19.85) group in comparison to control group (Table-4). The time required for complete epithelization of the excision wound is an important parameter to assess the wound healing process.

Table 4 Effect of polyherbal formulation on excision wound model.

Groups	% Wound contraction				Epithelisation period (Days)
	4th day	8 th day	12th day	16th day	
Control	9.46 ±1.22	28.2±1.19	52.2±1.53	68.0±1.51	24.33±0.73
Standard	19.63 ±0.44 a***	40.62±0.66 a***	69.67±1.88 a***	85.58±2.76 a***	19.0±0.67 a***
Formulation 1%	18.46±1.5 a***	33.46 ±0.48 a***,b**	56.14 ±1.83 a***,b*	74.51±2.36 a***	22.14±1.29 a**
Formulation 2%	19.0±1.07 a***	35.9±0.86 a***,b**	59.38±2.21 a***,b*	76.3±1.43 a***	20.73±1.45a**
Formulation 5%	19.33±1.1 a***	38.5±0.43 a***, b**	66.6±1.77 a***	80.21±2.27 a***, b**	19.85±0.72 a***

Values are mean ± SEM from a group of four animals. *p<0.05, **p<0.01 and***p<0.001,
a- Significance difference in compare to untreated group, b-Significance difference in compare to standard treated group

The promotion of wound healing activity is also well gazed by its tensile strength of the incision wound. Generally, wound-healing agents have the properties to enhance the deposition of collagen content, which provides strength to the tissues and forms cross-linkages between collagen fibres. The tensile strength of the extract treated (5%w/w) groups was found to be (248.33gm) which was higher than that of extract treated (2%) group (228.66gm). Standard group showed maximum tensile strength (267.66gm) of on 10th post wounding day, which indicate wound healing strength of the extract (Table 5).

Table 5 Effect of polyherbal formulation on incision wound model.

Groups	Treatment	Wound breaking strength (g)
I	Control	218.5±3.33
II	Standard	267.66±4.33a***
III	Formulation 1%	216.45 ±3.39 a**, b*
III	Formulation 2%	228.66±3.0a*, b**
IV	Formulation 5%	248.33±4.08a***,b*

Values are mean ± SEM from a group of four animals*p<0.05, **p<0.01, ***p<0.001
a- Significance difference in compare to untreated group
b- Significance difference in compare to standard treated group

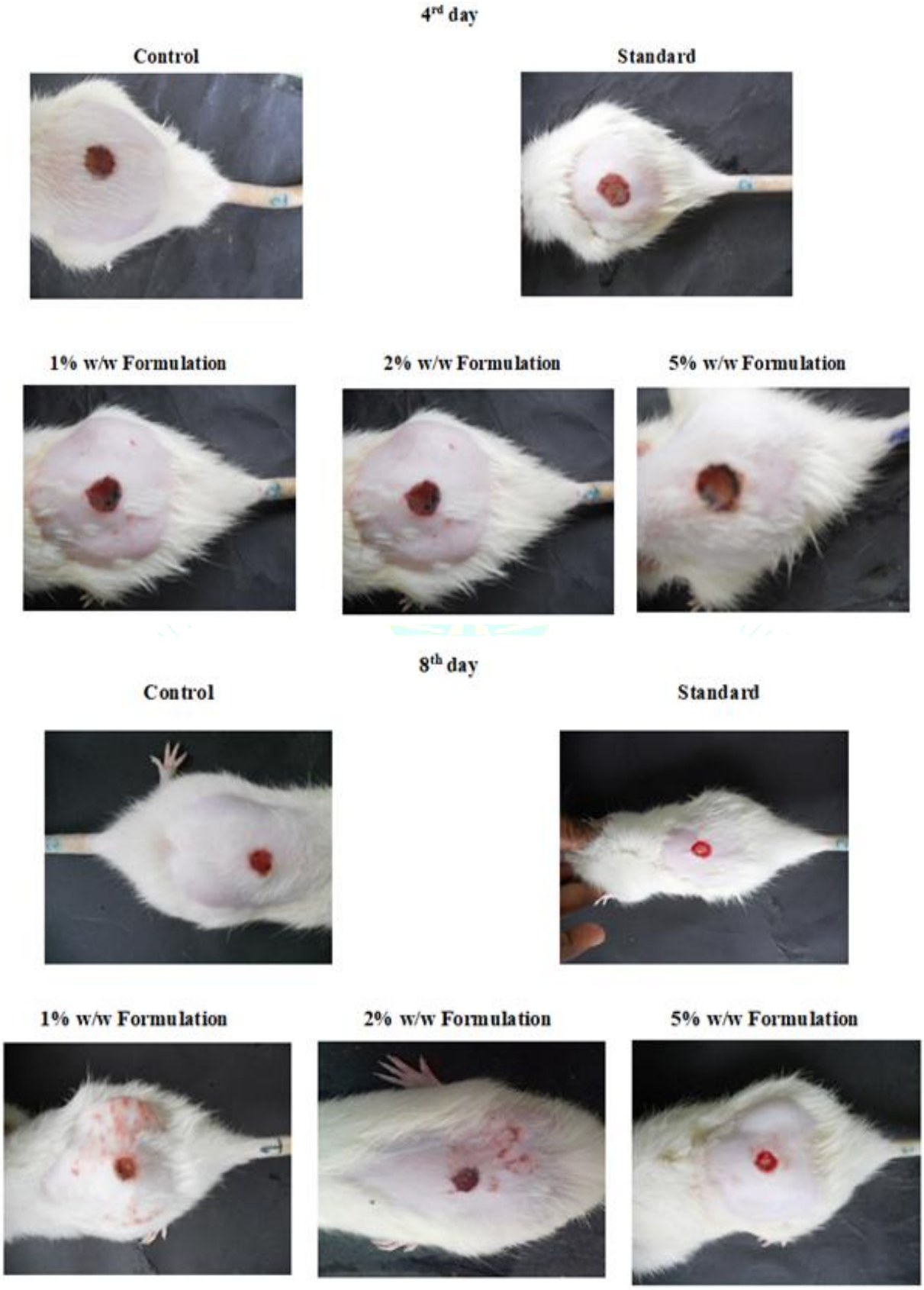

Figure 1(A): Effect of polyherbal formulation on incision wound model.

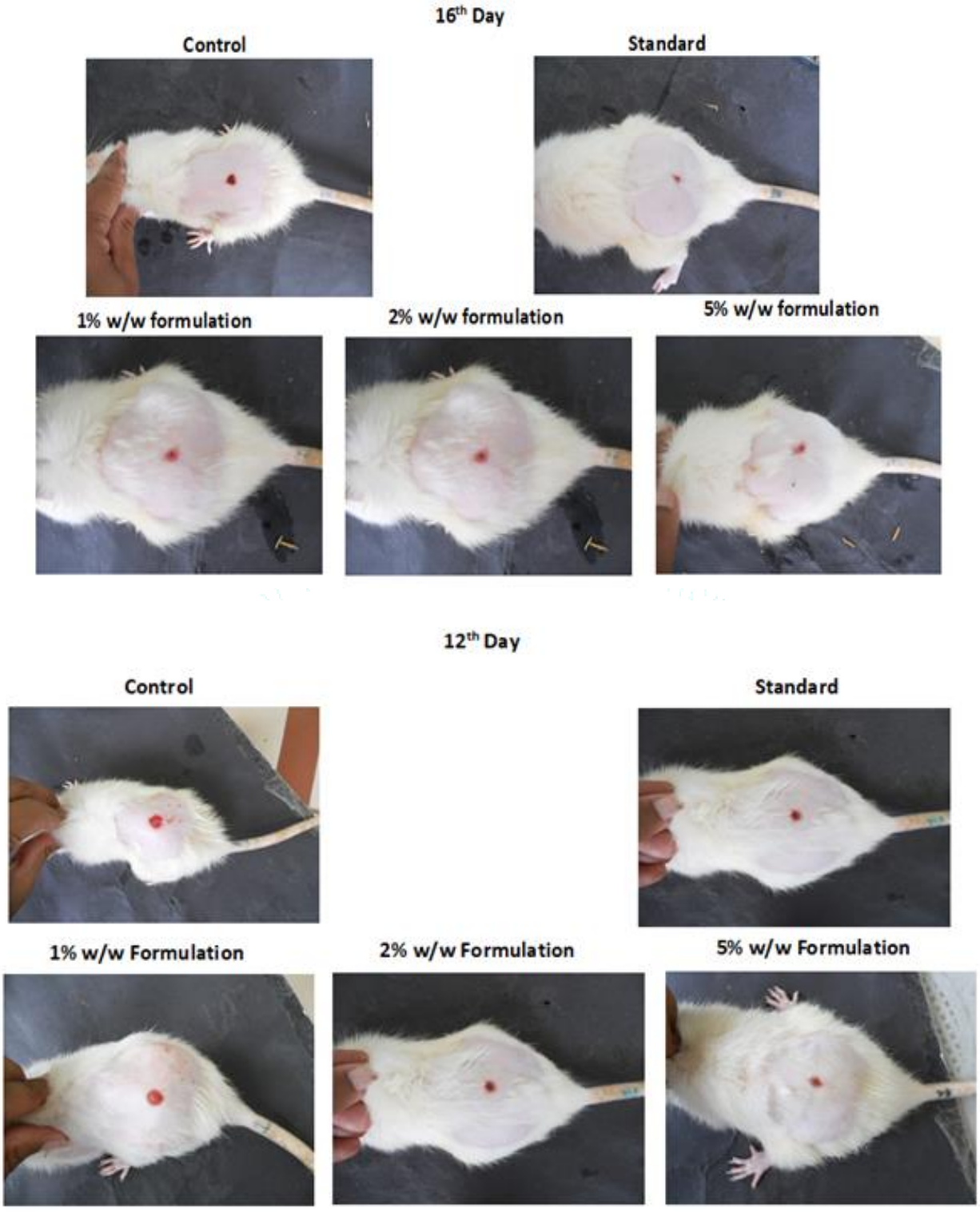

Figure 1(B): Effect of polyherbal formulation on incision wound model.

Thus, wound-healing property of the polyherbal formulation may be attributed to the phytoconstituents they contain, which may be either due to their individual or additive effect that fastens the process of wound healing. At this stage, it is difficult to say which component(s) of the polyherbal formulation are responsible for the wound healing activity. However, further phytochemical studies are needed to isolate the active compound(s) responsible for these pharmacological activities.

CONCLUSION

The polyherbal formulation of exhibits significant wound healing activity in excision and incision wound model which is compared with Silverex Heal Gel (Sun Pharmaceutical Industries Ltd). It was observed that the poly herbal formulation in traditional treatment of wounds or burns associated with bacterial infections.

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