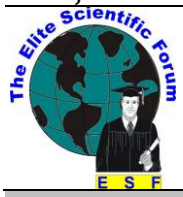
Pak. j. life soc. Sci. (2016), 14(3): 158-166 E-ISSN: 2221-7630;P-ISSN: 1727-4915



Pakistan Journal of Life and Social Sciences
www.pjlss.edu.pk

RESEARCH ARTICLE

Efficacy of *Azadirachta indica* and *Solanum nigrum* for Skin Regeneration in Mice

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ARTICLE INFO
Received: Aug 25, 2016
Accepted: Dec 15, 2016
Online: Dec 16, 2016

Keywords
Angiogenesis
Granulation
Re-Epithelialization
Regeneration
Reinnervation

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ABSTRACT

Skin is a protective shield of body against external hazards of environment which can interfere with its structural and metabolic mechanism. Consequences of any chemical or physical injury integrity are significant in medical sciences. Rate of skin recovery and regeneration indirectly ensures the revitalization of internal normal functioning of body. In the present animal model, ethanolic extracts of two local herbs, *Azadirecta indica* and *Solanum nigrum* were applied for skin wounds healing up to 4 weeks. Male mature mice were grouped as Intact control, Experimental control, A (*A. indica*+ glycerin treated), B (*S. nigrum* + glycerin treated), C (*A. indica* + *S. nigrum* treated) and D (glycerin treated). Histopathological results revealed the controlled microbial attacks in groups A and B, as compared to the wounded sites of groups C and D. Re-epithelialization and granulation rates were noted higher in group B after 1st week of wound induction. Significant regeneration of hair follicles and reinnervation in group B were recorded with mean diameters 61.63µm and 57.13µm, respectively, at first week of following wound induction. Similarly, angiogenesis rate was found better in group B with capillaries' average diameter of 14.25µm at 1st week and this re-growth delayed till 2nd week for other experimental groups. Moreover, in morphological examination, it was noticed that body weight steadily increased during the study period for the group A; a trend which was not observed for the other experimental groups. Wound surface area also reduced quickly down to 187.5µm^2 in group A during at 1st week of post trauma. Hematological analysis showed that ratios of direct leukocyte count and platelets were less in blood smears of groups A, B and C than in experimental control and group D. Results of the present study recommend that low cost local and natural flora should be preferred over harmful synthetic produce to make stimulatory drugs for enhanced skin wound healing and with better regeneration rate accompanied with minimum side effects.

INTRODUCTION

Skin is the largest organ of our body and is in utmost direct contact with external environment. So it is more prone to injuries, harmful effects of particulate matter, soluble chemicals and solar radiations. Besides sensing external changes such as recognizing sensation and temperature control, it also has a barrier function of preventing the loss of body fluid and protecting the body from toxic materials, microbial attack, physical stimulation, and ultraviolet irradiation (Mecheleidt et al., 2002; Choi et al., 2005; Cork et al., 2006). Meanwhile with the increase in pollution dermal disorders are increasing day by day. No doubt, synthetic medicines are working well but they frequently induce the side effects. Therefore, dermatologists are looking forward for natural remedies other than synthetic ones in the current era.

Dermal wounds are the result of injuries to the skin that disrupt other soft tissues. Healing of a wound is a multifaceted and protracted process of tissue regeneration and remodeling. Various plant products have been used in treatment of wounds over the years. Wound healing herbal extracts promote blood clotting, fight infection, and accelerate the process of skin regeneration (Tabassum and Hamdani, 2014). That's

why, the study of wound healing mechanisms and factors affecting it are of great importance. Injury care can be traced back to early civilizations and many of the treatments were based on the use of herbal remedies (Villegas et al., 1997). Skin regeneration and wound healing pass through different phases such as homeostasis, granulation (inflammation), proliferation, collagenation, collagen maturation, scar maturation and migration of different cell types which are concurrent but independent to one another (Charles et al., 1995; Lodhi et al., 2006). In other words, wound healing is a compound multifactorial process that results in the contraction and closure of the wound and regeneration of a functional barrier (Sidhu et al., 1999; Chattopadhyay et al., 2002).

There are various ways which are adopted for dermal wounds and injuries' recovery including application of anthropogenic chemicals, use of raw herbs, their extracts and other natural products like honey. Several of the remedies are in practice for centuries, and many of them are even today, considered effective (Lodhi et al., 2006). Thus herbal medicines have been enjoying revitalization among the clients all over the world. There are hundreds of medicinal plants that have a long history of curative properties against a range of diseases and ailments. However, screening of plants for their specific medicinal activities is essential and needs vital attention in order to know the importance of a particular plant. The assessment of plants for their therapeutic action is done on the basis of either their chemotaxonomic examination or ethnobotanical information for a particular disease (Juneja et al., 2007). Fortunately, Pakistan is rich in production of local herbs concerned with dermal wound recovery. Among them *Adhatoda vasica, Allium sativum, Embeliaribes, Mallotus phillippensis, Picrorrhiza kurrooa, Ricinus communis, Elaeagnus hortensis, Swertia chirata, Solanum nigrum, Centella asiatica, Solanum xanthocarpum, Oxtostagia limbata, Cassia angustifolia* and *Polygonum viviparum* have been frequently examined for their therapeutic potential by different researchers (Malik et al., 2011).

Role of herbs in wounds and skin disorders' healing is established and significant (Shivhare et al., 2010). For instance, *Solanum nigrum* and *Azadirachta indica* have been reported as potent dermal wounds healers (Shtayeh et al., 1998; Shale et al., 1999; Rajendran et al., 2003). *S. nigrum*, Black night shade locally called makkoh has been declared effective healing agent during experimental investigations involving both animal model based studies as well as from clinical data of wrinkles reduction and for recovery of rat bite, skin eruption, cuts, wounds, sprains and inflammation. For this purpose, its whole body parts like leaves, fruits, stems and roots have been tested (Chopra et al., 1956; Rajendran et al., 2003; Hwa, 2007).

A. indica, Indian lilac locally called as neem, has been checked in various surveys and research studies and obtained data have proved that it is a potent healer for skin damage after eczema and other issues (Rahman and Jairajpuri, 1996) and is being used as remedial constituent of soap and other cleaners (YashRoy and Gupta, 2000) as it is an effective antiseptic in nature (Thakur et al., 2011). Different animal based investigations have also been organized for verifying its application as suitable remedy for poisonous bites of animals (Ayyanar and Ignacimuthu, 2011). Leaf extract of the herb has been applied topically on boils and blisters for skin regeneration and healing (Joshi and Joshi, 2007). In another study, skin tumors were induced in mice and later on it was revealed that the chemopreventive *A. indica* is potent against skin cancer (Arora et al., 2011). It is important to note that reduction in biodiversity is being translated in many natural products. The present study was designed to study the effects of applications of ethanolic extracts of *A. indica* and *S. nigrum* on the processes of skin regeneration and wound healing following incision injuries to identify natural product(s) for rapid damaged skin regeneration. The animal based experimental study provided a model for predicting and controlling scar formation with the application of suitable herbal extracts to injured skin.

MATERIALS AND METHODS

Herbs' collection and extract formation: Plants of *A. indica* and *S. nigrum* were collected from different areas of Punjab. Stems and leaves of *A. indica* and *S. nigrum* were washed and separately dried in sun light, crushed and the obtained powder was stored in air tightened clean labeled jars. The ethanolic extracts of both species' were made (Purohit et al., 2013). A 5% w/w ointment formulation was prepared by incorporating the leaves and stems' ethanolic extract in 10% aqueous solution of glycerin for external application of the drug in the wound model.

Animals: Male albino mice (*Mus musculus*) of 6weeks age were placed at room temperature (25°C±2°C). After 10days' acclimatization, they were divided in to 6 groups; two groups served as intact and experimental controls, whereas remaining groups A, B, C and D were topically treated with ethanolic extract of *A. indica* and *S. nigrum* and, their blends (*A. indica* +*S. nigrum*) in glycerin, respectively. Effectiveness of the herbal extracts was checked for the injured skin regeneration and the process of wounds' healing following the skin full thickness para-vertebral linear incision wound model employed by different workers (Ehrlich and Hunt, 1968; Nakajima et al., 2013; Purohit et al., 2013; Patel et al., 2014).

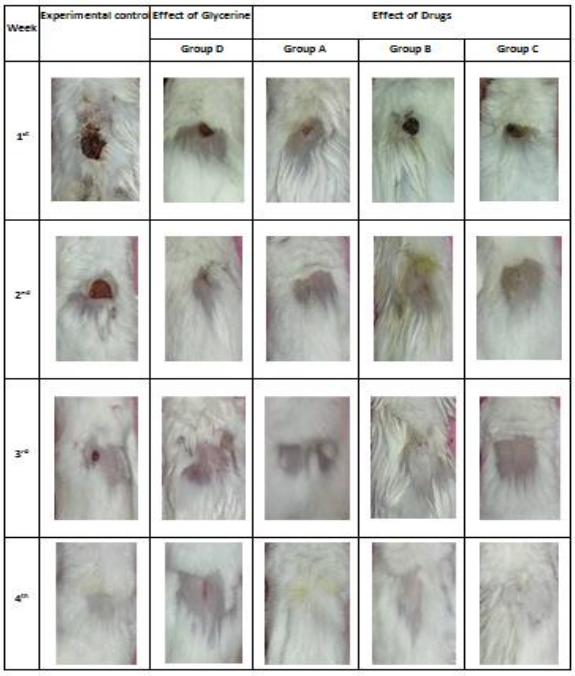

Fig. 1: Morphological comparison of wound healing in mice

Experimentation: The test preparations were topically applied once a day maximally for 28days excluding the members of intact control group. The experimental animals were sacrificed at every 7-days intervals post injury. Samples of wounded skin and blood were collected.

Histopathology: The skin samples were fixed in Bouin's fixative over night and then were processed further for microtomy and stained with haematoxyline and eosin to examine under light microscope at 400X (Rehman et al., 2012). Wound healing (granulation and epithelialization), angiogenesis, nerves reinnervation (Qazi and Mufti, 1998; Qazi et al., 2011) and hair follicular re-growth (Sundberg et al., 2005) were selected parameters for observation record and for photomicrographs.

Hematology: Hematological selected parameters were WBCs and platelets counts made by using wandering method of DLC of blood smears (Rehman et al., 2012; Patel et al., 2014).

Morphological observations: Immediately after single wound induction of 1cm long and about 2mm deep cut to all groups' members excluding members of intact control, with sterilized blade, the morphological features of wounded sites were recorded at regular intervals.

Body weight observation: Initial and final body weights of mice were recorded on day 1 and on day of dissection which were 7th, 14th, 21th or 28th days following the incision. This practice was conducted to observe the effect of employed drugs on over all body physiology.

Fig. 2: Effect of *A. indica* skin wound treatment on mice body size and weight

Wound surface area: Morphometric analysis of the 7th day healed wounds was performed by measuring surface area of outlines of the wound pictures by K-E polar planimeter (Qazi and Mufti, 1998).

Statistical analysis: All the collected data were subjected to ANOVA statistical analysis by SPSS version 22 at 0.1% probability level.

RESULTS

Morphological observations: Over all healing rate was much faster for the both experimental groups. Mice of experimental control group died more frequently but same pattern was also noticed in groups B and C during first two weeks of wound induction. The phenomenon of self eating was also observed among surviving group members during 1st week (Figure No. 1).

Effect on body weight: Group A showed over all maximum weight gain of 2.64g (Figure No. 2) than other groups after one week of wound induction whereas comparison with intact group values exhibited that groups B, C and D presented body weight loss for same duration. By the end of 2nd week, experimental control group presented significant body weight reduction of 3g where groups A and B mice gained weight than of groups C and D. Similarly the 3rd week readings also presented the same body weight gain/loss pattern among the all groups. At 4th week group A gained maximum weight of 7.9g than other groups and among them, significant weight loss 0.50g was showed by members of group B.

Comparison of length of Re-epithelialization: Better regeneration was found for group B with 231.7µm (Figure No.2) than other groups during 1st week. Whereas 2nd week data presented maximum re-epithelialization length for group D (Table No.2).

Comparison of width of re-epithelialization: It was noted that group D histological sections showed newly formed epithelium with thickness of 114.33µm and

127.7µm during 1st and 2nd weeks of post injury, respectively (Figure No.2) which were relatively higher than the other groups' observations (Table No.3).

Comparison of width of Granulation tissue layer: At 1st week, re-formed dermis thickness was 250µm in histological sections of both groups B and C (Figure No.2) and it was higher than groups A and D. Whereas at 2nd week readings of Group A showed sudden increase in width of granular area up to 237.7µm than intact dermis width (Table No.4).

Regeneration of hair follicles: Following one week of effective herbal application, outcome was observed in group B with mean diameter value of 61.63µm which was higher than of other groups. A shift of pattern was noticed at 2nd week in group D histological sections revealed more growth 63.75µm of hair follicles. During 3rd week group A data presented significant rise with diameter value of 62.83µm while in 4th week observations the combined herbal effect was found noteworthy 76.63µm in group C (Table No.5).

Innervation and growth of nerves: During first three weeks no re-growth was observed excluding the histological sections of group B but the results were found significant regarding reinnervation of nerves in wounded area during healing phase 4th week and are group A showed maximum reinnervating nerves' mean diameter up to 60.53µm than other experimental groups (Table No.6).

Effect on angiogenesis: At wounded site regeneration of blood vessels was observed and their diameters were recorded to estimate healing rate. In this regard, statistical comparison for arteries was found significant. During 1-3 weeks no proper regeneration was visible in histological sections but during 4th week, group A showed remarkable growth of arteries with mean diameter of 56.1µm than other groups (Table No.7).

Table 2: Comparison of length of re-epithelialization (µm)

Week	Intact control	Experimental control	Group A	Group B	Group C	Group D
1st	238.33±12.25	175±43.30 (3)	105±29.3 (3)	231.7±10.68 (3)	162.7±21.65 (3)	221.7±14.84 (3)
2nd	(3)	144.3±6.7 (3)	175±38.2 (3)	125±7.22 (4)	157.7±4 (3)	225±14.43 (3)

Values of Mean ±S.E.M (n). Data of respective columns were compared by employing single factor analysis of variance and significant difference was not found.

Table 3: Comparison of width of re-epithelialization (µm)

Week	Intact control	Experimental control	Group A	Group B	Group C	Group D
1st	17.7±4.33	37.7±18.76 (3)	85±51.33 (3)	125±28.87 (3)	107.7±37.5 (3)21.65	114.33±4.05 (3)
2nd	(3)	63.3±2.6 (3)	138.7±53.84 (3)	106.25±23.1 (4)	85.33±18.7 (3)	127.7±21.65 (3)

Values of Mean ±S.E.M. (n). Data of respective columns were compared by employing single factor analysis of variance and significant difference was not found.

Table 4: Comparison of width of granulation tissue layer (µm)

Week	Intact control	Experimental control	Group A	Group B	Group C	Group D
1st	236±33.2	87.7±14.72 (3)	144.33±35.88 (3)	250±00 (3)	250±00 (3)	138.33±6.01 (3)
2nd	(3)	226±4.73 (3)	237.7±12.4 (3)	211.25±33.8 (4)	180±47.26 (3)	191.7±33.8 (3)

Values of Mean ±S.E.M. (n). Data of respective columns were compared by employing single factor analysis of variance and significant difference was not found.

Table 5: Effect of herbal skin wound treatment on regeneration hair follicles and on their diameter (µm)

Week	Intact control	Experimental control	Group A	Group B	Group C	Group D
1st	67.83±14.53	37.5±1.88 (3)	41.7±20.58 (3)	61.63±5.7 (3)	27.28±0.88 (3)	35.7±1.2 (3)
2nd	(3)8.389	38.33±2.02 (3)	49.42±13.3 (3)	57.88±11 (4)	37.13±0.73 (3)	63.75±4.04 (3)
3rd		48.25±1.28 (3)	62.83±20.16 (3)	49.1±13.41 (3)	60.58±19.05 (3)	54.5±2.17 (3)
4th		46.25±5 (3)	43.83±5.32 (3)	43.83±5.32 (3)	76.63±3.4 (3)	53.7±2.55 (3)

Values of Mean ±S.E.M. (n). Data of respective columns were compared by employing single factor analysis of variance and significant difference was not found.

Table 6: Effect of herbal skin wound treatment on reinnervated nerves' diameter (µm)

Week	Intact control	Experimental control	Group A	Group B	Group C	Group D
1st		-	-	57.13±7 (3)	-	-
2nd	60.58±9.86	-	-	23.75±1.45 (4)	-	-
3rd	(3)	82.33±1.04 (3)	-	45±22 (3)	52.75±2.24 (3)	55±3.9 (3)
4th		40.46±4.86* (3)	61.53±12.18* (3)	40.58±6.24 (3)	30.37±17.8 (3)	56.83±0.93 (3)

Values of Mean ±S.E.M. (n) and – sign indicates no re-growth was observed. Data of respective columns were compared by employing single factor analysis of variance and at 0.1% level significant difference (*) was found.

For veins' regeneration, same pattern was observed during week 1-3 and no proper growth was found whereas during 4th week groups A and D showed distinctive growth with mean diameter values of 62.75µm and 62.25µm respectively than other groups but the results were not found significant in comparison of intact veins' diameter which was 64.42 µm (Table No.8).

Moreover, it was noted that capillaries' regeneration started during 2nd week in majority experimental groups and on comparison with intact histological sections, the significant growth was observed in group B with mean diameter value of 15.63µm. Similarly it was also observed that in group D histological sections major increase in capillaries' diameter of 25.83µm was visible than other groups (Table No.9).

Wound surface area: Wound surface area was noted for 1-4 weeks and it was observed that excluding experimental group, the wounds were recovered by the end of 1st week whilst morphological wound healing completed in experimental control group at 3rd week. The fastest wound recovery and smallest wound size of 187.5µm^2 was seen in group A at 1st week stage (Table No.10).

Platelets: The number of platelets was found higher than others in blood smears of groups C and D at 1st week stage and their average values were 3.5 and 3.88 respectively. Later on, in remaining three weeks, decrease in platelets count was noticed (Table No.11).

WBCs count: Direct leukocyte count (DLC) was done by using wandering method and it was noted that during 1st week the ratios of lymphocytes and of basophils were significantly higher in groups A and C with values of 1.56 and 1.77, respectively. Similarly it was noted that the ratio of macrophages was lower in all groups in first two weeks but in 3rd week observation group A showed sudden rise in the parameter up to 1.38 (Table No.12).

Table 7: Effect of herbal skin wound treatment on diameter (µm) of regenerated arteries

Week	Intact control	Experimental control	Group A	Group B	Group C	Group D
1st	45.25±1.53	-	-	-	-	-
2nd	(3)	-	-	-	-	-
3rd		68.38±2.38 (3)	-	-	-	-
4th		44.63±8.45 (3)	56.1±13.5* (3)	33.58±1.81 (3)	33.75±6.5 (3)	41.35±1.7 (3)

Values of Mean ±S.E.M.(n)and – sign indicates no re-growth was observed. Data of respective columns were compared by employing single factor analysis of variance and at 0.1% level significant difference (*) was found.

Table 8: Effect of herbal skin wound treatment on diameter (µm) of regenerated veins

Week	Intact control	Experimental control	Group A	Group B	Group C	Group D
1st		No re-growth observed	No re-growth observed	No re-growth observed	No re-growth observed	No re-growth observed
2nd	64.42±5.5 (3)	No re-growth observed	No re-growth observed	No re-growth observed	No re-growth observed	No re-growth observed
3rd		79.13±17.83 (3)	No re-growth observed	No re-growth observed	No re-growth observed	No re-growth observed
4th		31.25±1.73 (3)	62.75±2.31 (3)	41.42±8.66 (3)	42.5±6.21 (3)	62.25±1.22 (3)

Values of Mean ±S.E.M. (n). Data of respective columns were compared by employing single factor analysis of variance and significant difference was not found.

Table 9: Effect of herbal skin wound treatment on diameter (µm) of regenerated capillaries

Week	Intact control	Experimental control	Group A	Group B	Group C	Group D
1st		No re-growth observed	No re-growth observed	14.25±1.4* (3)	No re-growth observed	No re-growth observed
2nd	15±2.89 (3)	4.83±1.55 (3)	No re-growth observed	15.63±5.28* (4)	No re-growth observed	8.75±1.16 (3)
3rd		12.12±3.1 (3)	No re-growth observed	13.1±3.5 (3)	11.17±0.98 (3)	11.25± 4.73 (3)
4th		14.13±3.4 (3)	11.42±3.5 (3)	14±4.2* (3)	8.38±0.94* (3)	25.83±1.23* (3)

Values of Mean ±S.E.M. (n). Data of respective columns were compared by employing single factor analysis of variance and at 0.1% level significant difference (*) was found.

Table 10: Comparison of wound surface area (µm^2) after one week of incision injury in different groups

Initial cut (IC)	Experimental control	Group A	Group B	Group C	Group D
4708.33±2403.7 (3)	1979.1±1548.5 (3)	187.5±62.53 (3)	527.1±198.6 (3)	812.5±355.42 (3)	396±116.05 (3)

Values of Mean ±S.E.M. (n). Data of respective columns were compared by employing single factor analysis of variance and significant difference was not found.

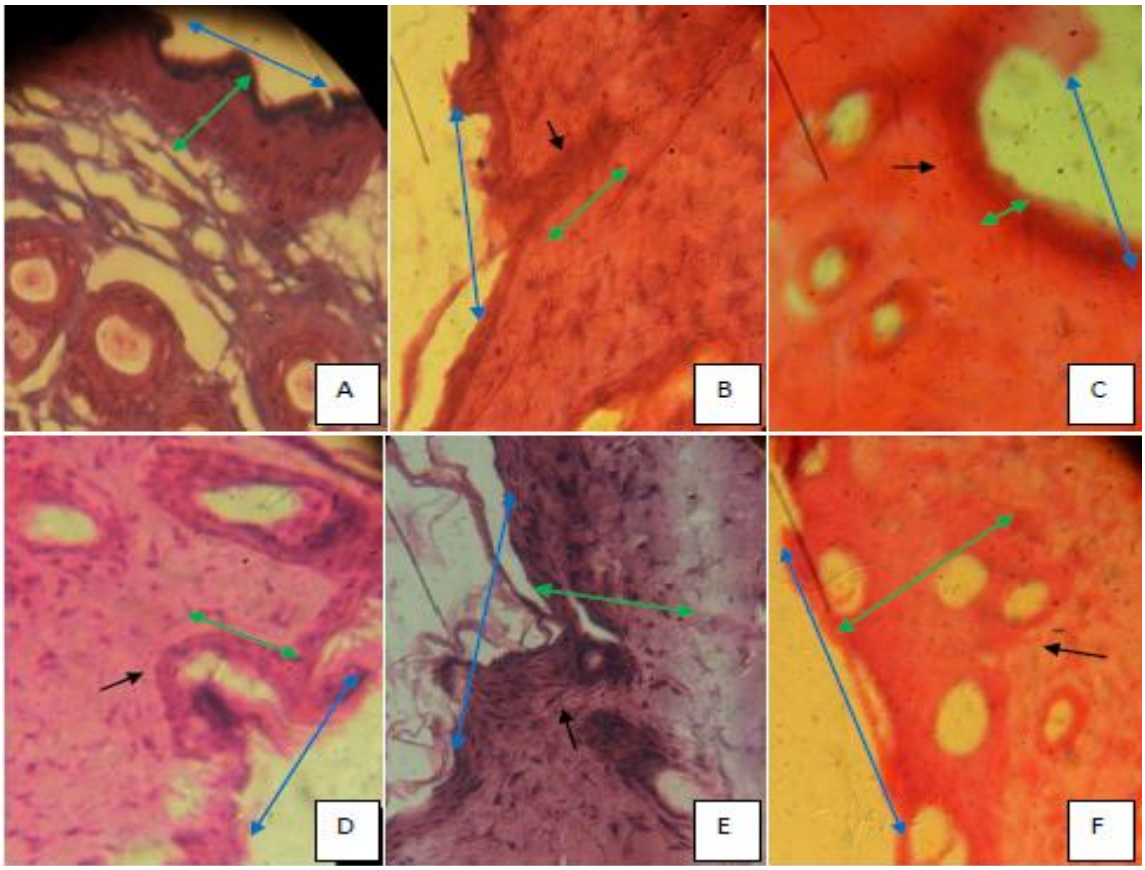

Fig. 2: 1st week T.S. Histological sections of A=intact, B=experimental control, C= group A, D= group B, E = group C, F= group D presenting the length (blue arrow heads) and width (green arrow heads) of re-epithelialization and granulation (µm) at wounded site (black arrows). All micrographs have 400X magnification, (H & E staining).

Table 11: Platelets average count

Week	Intact control	Experimental control	Group A	Group B	Group C	Group D
1st		1.47	1.43	1.92	3.5	3.88
2nd	2.17	0.84	0.6	0.33	3.2	2.23
3rd		0.503	0.17	0.68	0.92	0.833
4th		0.56	0.083	0.083	1.42	1.01

DISCUSSION

Wound repair is an essential physiological course of action that is vital for tissue homeostasis, but it can be impaired in disease and contributes to numerous pathologies. The wound healing process, predominantly in skin, has been well characterized in histological studies extending back more than 100 years (Shaw and Martin, 2009). This natural phenomenon of wound recovery and skin regeneration can be accelerated by the indulgence of natural or synthetic drugs. In this regard, synthetic ones are considered critical now a days due to their plenty of side effects. Thus in current era major focus of the pharmaceutical research is on establishment of economical and side effects free herbal compositions for dermal wounds faster recovery.

The present study also represents an effort in this regard in which wounded skin was treated topically with different compositions of *A. indica* and *S. nigrum* and selective morphological, histological and hematological parameters were examined to evaluate the efficacies of the local flora leading herbs. It was noticed that general morphology after wound induction of all experimental mice exhibited a positive response for the application of selective herbs. The mice mortality was observed in glycerin treated group D more frequently than others. Even inflammation was also visible at wounded sites in above mentioned group histological sections. Because glycerin is adhesive in nature (Vinkatraman and Gale, 1998) so it just covers the wounded site but lacks antimicrobial and anti-inflammatory activities. So in the presence of a powerful invading pathogen, the skin regeneration disrupts. Similarly eye infection was

observed in mice of groups C and D during 2nd and 3rd weeks of wound healing. Although both selective herbs are antimicrobial (Purohit et al., 2013; Li et al., 2007; Wang et al., 2007; Zakaria et al., 2006) in nature still their collective healing stimulatory response was not satisfactory following topical application only and this aspect should be focused in further investigations.

Wound healing initial sign is injured sites closure and contraction. Comparison of wounds' surface area reveled that group A mice cured earlier at one week whereas maximum delayed wound contraction was noticed in group C. The reason behind is that group A was treated with drug containing *A. indica* which kills a vast range of wounds invading pathogens and allows metabolic system to continue its regenerative process without interference (Purohit et al., 2013).

Weekly histological sections examination of wounds also revealed striking results. In this regard, at wounded site re-epithelialization and formation of granulation tissue which replaced the damaged epidermis and dermis, respectively were studied. In current effort, this specific recovery process was completed in approximately two weeks in drugs treated groups excluding the experimental control. More re-epithelialization thickness was noted in group D till 2nd week of post injury i.e. experimental drugs proved efficient than glycerin so their wounded sites recorded quickly with regressed wound area and completed the phase of dermal layers healing within 1st week.

For wound induction, the selected skin area was shaved so re-growth of hair was also included in complete recovery indicators of used experimental model. It is evident from the results that hair follicles development became visible in all post injury observations and their gradual maturation was noted in all the experimental groups' histological sections. During 1st week better hair follicles regeneration and reinnervation of nerves were recorded for group B than other groups which explained quick stimulatory action of *S. nigrum* for hair follicular growth and reinnervation. This pharmaceutically significant aspect has not been reported yet because still this herb is used for regeneration and recovery of internal organs.

Further, estimation of angiogenesis concluded that development of capillaries started earlier in group B mice than other groups, whereas regeneration of arteries and veins delayed till 3rd week of post injury in majority of the studied sections. It means that constituents of *S. nigrum* triggered angiogenesis better than *A. indica.*

Hematological data also revealed positive results about chosen herbs' treatment. Comparatively less number of platelets was recorded in blood smears, for groups A and B and higher for groups C and D. It indicated that both herbs have fair ability of blood coagulation.

Wound healing process cannot be completed without contribution of natural body defensive system of leukocytes. In current effort, DLC results provided indirect major confirmations of enhanced wound healing by following observations.

Phagocytosis was prominent in group D during 1st week because more macrophages were defending in the presence of adhesive chemical glycerin. Similar activity was also noted in sections of group C during 1st week of post recovery which might be result the of absence of a good anti-microbial action of herbal blend because mixture of selected herbs was biochemically found over

Table 12: Direct leukocyte count of different experimental groups

Stage	Group	Ratio of Leukocytes					
		Neutrophils	Eosinophils	Lymphocytes	Monocytes	Basophils	Macrophages
1st	Exp. control	0.29	0.21	0.341	0.19	0.831	0.11
	A	0.18	0.109	0.068	0.123	1.56	0.27
	B	0.024	0.00	0.214	0.12	0.23	0.42
	C	0.21	0.014	1.77	0.067	0.12	0.473
	D	0.024	0.00	0.131	0.141	0.16	0.52
2nd	Exp. control	0.30	0.15	0.18	0.0703	0.395	0.03
	A	0.26	0.015	0.215	0.063	0.131	0.50
	B	0.085	0.039	0.16	0.0813	0.37	0.31
	C	0.042	0.00	0.214	0.08	0.162	0.611
	D	0.031	0.031	0.211	0.223	0.10	0.41
3rd	Exp. control	0.21	0.023	0.19	0.032	0.36	0.223
	A	0.20	0.04	0.25	0.04	0.42	1.38
	B	0.073	0.00	0.279	0.20	0.24	0.214
	C	0.13	0.18	0.15	0.076	0.36	0.17
	D	0.164	0.051	0.074	0.141	0.244	0.29
4th	Exp. control	0.12	0.073	0.16	0.083	0.203	0.983
	A	0.011	0.00	0.42	0.091	0.25	0.24
	B	0.051	0.00	0.214	0.12	0.30	0.31
	C	0.072	0.23	0.20	0.111	0.158	0.22
	D	0.107	0.054	0.062	0.18	0.23	0.19
Intact		0.22	0.79	0.15	0.16	0.174	0.00085

all less effective rather than exhibiting any synergistic collective response and in this regard further investigation is required yet. Moreover, the ratio of basophils was higher for group A in 1st week which showed their enhanced immediate hypersensitivity response in the presence of *A. indica* (Kallos and Caruso, 1977).

Whereas in 1st week, group C elevated number of lymphocytes indicates increased chronic inflammatory response (Martin et al., 1990) as blend of both herbs was not found effective for anti-inflammatory action. So from obtained DLC data, it is evident that in all the experimental groups, there was a significant decrease in quantity of eosinophils which highlighted that mice were immuned and were not affected by allergens and related inflammatory reaction (Reilly et al.,2002) in the presence of applied herbs.

Conclusion(s)

This animal based experimental study was premeditated to evaluate the effects of applications of ethanolic extracts of *A. indica* and *S. nigrum* on the processes of skin regeneration and wound healing following incision injuries. It is concluded that the study provided a model for predicting and controlling scar formation with the application of suitable herbal extracts as an effective alternative of synthetic drugs to injured skin. To get improved recovery rate, drug dose optimization should be focused in future by the researchers.

Authors' contribution

Both authors have contributed equally towards the manuscript.

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