Treatment of External Wounds by Using Indigenous Medicinal Plants and Patent Drugs in Guinea Pigs

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Abstract: The present investigation was carried out in 40 guinea pig with the aim to investigate the efficacy of some indigenous medicinal plants in the treatment of external wounds. Two wounds of 2.00 cm length and 0.5 cm depth were made on the opposite thigh muscles and the effects of some antibacterial and some indigenous medicinal plants leaves were observed separately. Among the antibacterial used in this studies comparatively Nebanol powder showed the best result and was more effective for the treatment of fresh wounds in which healing occurred with in 11 days, Where as it was taken 13 days for sulfanilamide powder and 15 days for dusting powder treated groups. In the present experiment the leaves of the Neem, Ganda, Mehedi and raw Halid were applied as fresh aqueous pastes for the treatment of artificially produce wound in guinea pig. In comparison to their effect, Neem leaves paste showed the more effectiveness where healing was completed on 15 days, Halud paste and Ganda leaves pastes showed the same results, where healing was completed on 17 days. The Mehedi paste was the least effective in which healing occurred on 19 days. This investigation suggested that Neem leaves fresh paste are more effective for wound healing in guinea pig which may be suggested for the treatment of fresh wound healing of other livestock.

Key words: Medicinal plants, antibacterial, external wound healing, guinea pigs

Introduction

The use of laboratory animals have now been increased in various diagnostic laboratories and research institutes. Various types of tests and experiments are performed on laboratory animals. These include testing of a new drug for detection of its efficacy, safety and toxicity, vaccine and production and disease detection. So, laboratory animals play a vital role in the research program of veterinary and medical science.

The healthy and normal skin acts primarily as an organ of protection and maintain homeostasis; various skin diseases are prevalent in livestock which may cause a serious loss in the productive performance. Among the various skin disease external wounds are one of the important problems of the animals. The reported based on the data recorded at Bangladesh Agricultural University Veterinary clinic showed that external wounds possess the highest incidence among the surgical diseases in animals (Hossain *et al.*, 1986). External wounds reduce

the skin quality, decreased production and loss of draft power.

It has been demonstrated that "Himax" a product of Indian Herbs (Plants research) was effective against various infection in the wounds (Rai and Sastry, 1976). Recently, the world health organization (WHO) has recognized the necessity for investigation and mobilization of ancient medicinal practice to fulfil the primary health care of the animals and realizes that the traditional system of medicine may play an important role in the development of livestock of the world countries.

There are many unregistered veterinary practitioners in the Indian subcontinent who are using various herbal preparations for the treatment of animals (Sivdas, 1980). A variety of indigenous herbal preparations are manufactured by various pharmaceutical companies in India, Indonesia and Thailand. These drugs are commonly used by the veterinary practitioners in those countries. However, in Bangladesh, these resources are still now remain unexplored and as such our farmers and animal owners are depriving from the benefit of using to these cheaper and readily available herbal preparations.

There are many species of plants with medicinal importance are found in Bangladesh. A survey conducted by Botany Department of Dhaka University reveals that Babla, Pitraj, Sial kanta, Lazzabati, Ganda, Neem leaves and Thankuni have been reported to possess antibacterial property in the treatment of skin wound (Ali, 1957; Khan *et al.*, 1975; Amin, 1997).

Experimental investigations, therefore, is imperative to asses the therapeutic value of indigenous herbal plants and leaves and finally to develop an indigenous method of therapy for wound healing in livestock. Research work is this field is still now limited in our country. With these points of view the present study was therefore carried out with the following objectives: to investigate the comparative efficacy of some indigenous medicinal plants leaves pastes for the treatment of artificially induce fresh wounds in guinea pig and finally compared the results with that of locally available antibacterial.

Materials and Methods

The experiment was performed in the Department of Pharmacology in collaboration with the animal house of Microbiology Department, Bangladesh Agricultural University, Mymensingh, Bangladesh during the period from January to April, 1998. A total of 40 apparently healthy guinea pig (*Cavia cobaya*) of both sexes were used in this experiment. The animals were 6-8 months old and on an average 450-600 g body weight. During the entire experimental period the animals were kept in separated cages containing five guinea pig in each cage under same managemental conditions. All the guinea pig were maintained on good housing conditions and were provided with normal feed and water *ad libitum*. In addition to concentrate feed green grasses were also supplied to the animals

Grouping of experimental animals

After acclimatization, all the guinea pig were divided in to eight equal groups (A to H). Sulfanilamide powder, Dusting powder and Nebanol powder were applied twice daily after washing the wounds by distilled water with the help of sterile cotton in all animals of group A,

B and C. The experimental animals were handled carefully to avoid interference with the formation of granulation tissue. Paste of Ganda leaves, paste of raw Halud, past of Mehedi leaves and paste of Neem leaves were used for the treatment of wounds in all guinea pigs of group D,E,F and G. Only Vaseline was applied twice daily to the wounds in all animals of group H which remained as controlled group.

Rectal temperature, heart rates of all guinea pigs were recorded daily until wound healing. The changes of healing were recorded from various treatment groups after every three days until wound healing. A wound was assumed healed up when cicatrisation and pigmentation was found. A drop of blood was collected from the ear vein of each experimental animal before wounding and 3rd, 10th and 21st days after wounding. The blood was analyzed for the differential counts of Leukocytes (DCL) as described by Coffin (1953).

Preparation of artificial wounds

The guinea pigs were kept in eight separated cages containing five in each cage. Before making wounds on the animals all the animals were subjected to a through clinical examination to ensure that they were with in good physical condition and apparently free from infections. The site of the wounds were washed with soap and water then clipped, shared with separate sterile blades which was disinfected with losan (Novartis Bangladesh Ltd.). The sites were then desensitized by local infiltration with 2% Jasocaine (Lignocaine HCl, Jayson, Bangladesh Ltd). Two incised wounds of 2 cm length and 0.5 cm depth were made on the opposite thigh muscles following standard surgical procedure. The wounds produced in each animal of all groups were remained unsaturate.

Collection and preparation of medicinal plants and antibacterial

Sulfanilamide powder (Bangladesh Pharmaceuticals Ltd.), Dusting powder (Iodoform 1 parts, Boric acid 2 parts and Zinc oxide 2 parts), Nebanol powder (Neomycin sulphate, Square Pharmaceuticals Ltd, Bangladesh) were purchased from local market. The leaves of the Ganda (*Tagetes pasula*), Neem (*Azadirachta indica*) and Mehedi (*Duranta plumieri*) were collected from the Botanical Garden of Agricultural University, Mymensingh and the raw Halud (*Curcuma lonnga*) was purchased from the local market. The leaves and the raw Haluds were cleaned with water and air dried. Then the leaves and raw Halud were separately cursed thoroughly with the help of pestle and mortar. The crude extracts from the leaves of the separate plants and raw Halud were then mixed with distilled water to prepared pastes. Every day fresh pastes were prepared and preserved in separated plastic containers for the further use.

Statistical analysis

The data on length of wounds and differential counts of leukocytes percentage were presented as mean and standard deviation. The lymphocyte percentage of control group was compared with other treatment groups for significance test by " t" test in accordance with the Microsoft Excel Program on the PC Celeron Intel Inside. For statistically significant results, the data were analyzed with the values of $p < 0.01$.

Results and Discussion

Complete healing time of the artificially induced wounds in various treatment groups varies with the effectiveness of the treated materials. All antibacterial are seemed to be effective for wound healing. The comparative efficacy of the antibacterial against wounds are shown in Table 1. Among the all antibacterial comparatively Nebanol powder showed the best results, where healing was completed in 11 days. On the other hand sulfanilamide and dusting powder taken 13 and 15 days for complete wound healing. These present findings were compared and agreeable with the findings of Yasmin (1992). However, Furneaux (1975) reported that local application of topical antibiotics were effective in reducing the number of organisms in contaminated wounds.

The comparative efficacy of some indigenous medicinal plants against wound healing are shown in the Table 1. In comparison to their effect against wound healing, Neem leaves pastes were more effective than those of Halud, Mehedi leaves pastes and Ganda leaves, where complete healing occurred in 15 days. The Ganda leaves and raw Halud showed the same results where healing was completed in 17 days and was less effective than Neem leaves. Mehedi leaves was the least effective among all the plant leaves where healing was completed in 19 days. The control group took more time than all the plant leaves pastes and it was 21 days. The most desirable effect of the medicinal plants on wound healing was achieved in Neem leaves treated groups where complete healing occurs in 15 days time. These results are in agreement with the findings of Sivdas (1980) who stated that healing of wound with herbal plant preparations may occur in 15 days time.

In the present study experimental skin wounds were produced in the guinea pigs and the sequence of the events during wound healing were studied. The results showed that artificially induced wounds in guinea pigs have no significant effect on rectal temperature, pulse and respiration rate.

The characteristics clinical changes at different stages of wound healing with various antibacterial are shown in the Table 1. Moderate exudation occurred on the first day of wounding. The healing process was started from the second day characterized by scab formation due to drying of the exudate on the wound surface. Reddening was observed in all antibacterial treated groups for 3rd to 9th day. The reddening was prominent in Nebanol powder treated groups than other antibacterial. In Nebanol treated groups 90% cavity filling occurred within 9 days of wounding, at the same time it was 70, 60 and 45% in the sulfanilamide, dusting powder and Vaseline treated groups, respectively. Complete cavity filling was observed within 11 days in Nebanol powder treated groups, whereas it was 13 days for sulfanilamide groups, 15 days for dusting powder treated groups and 21 days for Vaseline treated groups. The wound cavity was filled up in between 9 to 21 days in various treatment groups and these results were similar with the findings of Ghani *et al.* (1986).

The appearance of pigmentation of cicatrisation observed from the 11th day in the sulfanilamide and dusting powder treated groups whereas it was appeared from the 15th day in the Vaseline treated groups. The present findings were in agreement with the earlier findings of Silver (1973).

Table 1: Characteristics clinical features at different stages of wound healing of guinea pigs with different antibacterial and medicinal plant leaves pastes

Days	Changes	Sulfanilamide powder	Dusting powder	Nebanol powder	Raw halud pastes	Mehedi leaves pastes	Ganda leaves pastes	Neem leaves pastes	Vaseline (control)
1st	Length of wound (cm) exudation	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
		+	+	+	+	+	+	+	+
3rd	Length of wound (cm) reddening	1.8±0.04	1.9±0.05	1.70±0.3	1.90±0.05	1.90±0.02	1.90±0.04	1.90±0.05	1.90±0.02
		++	++	++	++	+	++	+	+
5th	Length of wound (cm) reddening	1.50±0.02	1.70±0.03	1.30±0.05	1.80±0.04	1.80±0.01	1.75±0.03	1.70±0.03	1.80±0.01
		++	++	++	++	++	++	++	++
	Cavity filling(%)	30	25	40	20	20	20	25	15
7th	Length of wound (cm) reddening	1.20±0.03	1.50±0.03	0.90±0.04	1.60±0.03	1.70±0.03	1.60±0.02	1.5±0.03	1.70±0.02
		++	++	+++	++	++	++	++	++
	Cavity filling(%)	50	40	60	35	25	30	40`	25
9th	Length of wound (cm) reddening	0.90±0.04	1.20±0.02	0.40±0.03	1.40±0.01	1.35±0.04	1.45±0.03	1.20±0.02	1.40±0.04
		+++	+++	+	++	++	++	+++	++
	cavity filling(%)	70	60	90	50	40	45	60	35
11th	Length of wound (cm) cicatrisation	0.50±0.02	0.90±0.04	Complete healing	1.10±0.02	1.20±0.02	1.15±0.02	0.90±0.04	1.20±0.04
		++	++		++	+	++	++	_
	Pigmentation	++	++		++	+	++	++	_
	cavity filling(%)	90	80		70	60	75	80	45
13th	Length of wound (cm) cicatrisation	Complete healing	0.50±0.03		0.80±0.03	1.20±0.02	0.85±0.04	0.50±0.02	1.00±0.02
			+++		+++	++	+++	+++	_
	pigmentation		+++		+++	++	+++	+++	_
	cavity filling(%)		90		90	60	90	90	60
15th	Length of wound (cm) cicatrisation		Complete healing		0.40±0.02	0.50±0.01	0.40±0.01	Complete healing	0.70±0.03
					++	++	++		+
	Pigmentation					++	++	++	+
	cavity filling(%)					95	80	95	65
17th	Length of wound (cm) cicatrisation					Complete healing	0.20±0.02	Complete healing	0.40±0.02
							++		++
	Pigmentation							++	++
	Cavity filling(%)							90	80

Table 1: Continue

Days	Changes	Sulfanilamide powder	Dusting powder	Nebanol powder	Raw halud pastes	Mehedi leaves pastes	Ganda leaves pastes	Neem leaves pastes	Vaseline (control)
19th	Length of wound					Complete			0.20±0.01
	(cm) cicatrisation					healing			+++
	pigmentation								+++
	cavity filling(%)								95
21st	Length of wound								Complete
	(cm) Cicatrisation								healing
	Pigmentation								
	Cavity filling(%)								

In case of exudation - or + indicate absent or present of exudation; +, ++ indicate slightly, moderately reddening ; +, ++ and +++ indicates slight, moderate and complete cicatrisation; +, ++ and +++ indicate less, minimum and relatively normal pigmentation, respectively

Table 2: Effect of external wound on differential count of Leukocytes of guinea pigs in antibacterial treated and medicinal plant treated groups

Stage of study	Leukocytes (%)	Group-A (Mean±SD)	Group-B (Mean±SD)	Group-C (Mean±SD)	Group-D (Mean±SD)	Group-E (Mean±SD)	Group-F (Mean±SD)	Group-G (Mean±SD)	Group-H (Mean±SD)
Before Producing wound	Lymphocytes	55.77±0.29	55.62±0.39	55.95±0.37	55.82±0.03	55.57±0.66	55.63±0.42	55.15±0.51	55.98±0.28
	Lymphocytes	55.77±0.29	55.62±0.39	55.95±0.37	55.82±0.03	55.57±0.66	55.63±0.42	55.15±0.51	55.98±0.28
	Neutrophils	35.42±0.22	35.67±0.37	35.81±0.45	35.67±0.32	35.08±0.29	36.31±0.21	35.41±0.33	35.79±0.15
	Eosinophils	4.87±0.35	4.67±0.25	4.73±0.22	4.75±0.32	4.87±0.38	4.64±0.16	4.83±0.19	4.66±0.52
3 day after wound	Lymphocytes	56.02±0.35	61.78**±0.42	56.08±0.27	64.03**±0.15	64.43**±0.51	66.73**±0.53	58.95**±0.42	65.28**±0.65
	Neutrophils	35.73±0.32	29.92**±0.22	34.97±0.30	28.35**±0.62	27.03**±0.37	24.94**±0.48	25.62**±0.35	25.62**±0.35
	Eosinophils	4.64±0.22	4.90±0.24	4.77±0.10	4.64±0.22	4.90±0.25	4.77±0.10	5.09±0.24	5.08±0.24
10 day after wound	Lymphocytes	56.18±0.32	57.97*±0.322	56.6±0.33	56.18±0.32	57.97*±0.32	55.66±0.33	60.17*±0.10	60.17*±0.10
	Neutrophils	35.78±0.50	33.79*±0.12	35.63±0.26	35.78±0.50	33.79*±0.12	35.63±0.26	30.39*±0.31	30.39*±0.31
	Eosinophils	4.54±0.32	4.69±0.50	4.70±0.04	4.54±0.32	4.69±0.50	4.70±0.04	4.96 ± 0.29	4.96±0.29
After wound healing	Lymphocytes	55.97±0.22	56.80±0.27	55.66±0.33	55.97±0.22	56.80±0.27	55.66±0.33	55.63±0.37	55.63±0.37
	Neutrophils	35.63±0.26	34.97±0.30	35.63±0.26	35.63±0.26	34.97±0.30	35.63±0.26	35.78±0.30	35.78±0.30
	Eosinophils	4.70±0.04	4.77±0.10	4.70±0.04	4.70±0.04	4.77±0.10	4.70±0.04	4.48±0.18	4.48±0.18

*, ** Mean values of different superscripts in a column differ significantly (P<0.01)

Significant at 1% level of probability (P<0.01)

The characteristics changes at different stages of wound healing with various indigenous medicinal plants are shown in the Table 1. Moderate exudation occurred on the first day of wounding in all groups. Healing process started earlier in case of Neem leaves than other plants and Vaseline treated (control) groups. Prominent reddening was occurred in Neem leaves treated groups than all other groups. Complete cavity filling Neem leaves has taken less time than other groups. Complete cavity filling was observed within 15 days in Neem leaves treated groups, whereas at the same time it was 95, 80 and 65% in Halud, Mehedi leaves and Vaseline treated groups, respectively.

Cicatrisation and pigmentation was appeared earlier in the Neem leaves treated groups than in all other groups. In case of Halud and Ganda leaves treated groups cicatrisation and pigmentation appeared earlier but at thee same time tan in the Mehedi leaves and Vaseline treated groups.

In the study of hematological parameters, the effects of external wounds of Differential Count of Leukocytes are presented in the Table 2. The effect of external wounds on Differential Counts of Leukocytes on antibacterial treated groups showed that the noticeable changes were observed in lymphocytes. The percentage of lymphocytes before producing wounds in groups A, B, C and H were 55.77 ± 0.29, 55.62 ± 0.39, 55.95 ± 0.97 and $55.98 \pm 0.28\%$, respectively. These values increased significantly ($P<0.01$) to 61.78 ± 0.42, 65.28 ± 0.65 and 57.97 ± 0.32, 60.19 ± 0.01 in groups B and H, respectively. Vasin (1967) found leukocytosis during experimental wound healing in sheep and cattle. In group A and C after producing wounds lymphocytes also increased to 56.02 ± 0.35, $56.08 \pm 0.27\%$, respectively. However, these changes were not statistically significant. The mean values of neutrophils before producing wounds in group A, B, C and H were 35.42 ± 0.22, 35.67 ± 0.37, 35.82 ± 0.45 and $35.73 \pm 0.15\%$, respectively. After producing wounds these values on 3rd and 10th days started in group H on 3rd day. Significant changes was observed in group B and H. The percentage of neutrophils were started to increase in every groups after healing. The changes of eosinopils in various groups at different days were statistically insignificant.

The effect of external wounds of differential counts of Leukocytes in indigenous medicinal plant treated groups showed that, the percentage of lymphocytes in the groups D, E, F, G and H before producing wounds were 55.82 ± 0.03, 55.57 ± 0.66, 55.63 ± 0.42, 55.15 ± 0.51 and $55.98 \pm 0.28\%$, respectively. The highest values were observed on 3rd days. After healing these values were again started to decrease up to normal levels.

The neutrophils percentage were started to decrease significantly in every groups after 3 days of wounding and continued up to 10 days. After healing these values were again started to increase up to normal levels. The changes in eosinophils in various groups at different days were statistically insignificant. The present experiment showed that antibacterial therapy reduces excessive leukocytosis and decreases the length of healing time. These was agreeable with the findings of Vasin (1967) and Loktionova and Troistskaya (1974).

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