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Azadirachta Indica and Aloe Vera Leaves Extracts Enhances Excision Wound Healing Activity

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ABSTRACT

Wounds are major responsible for physical disabilities. Wound disease spread by the invasion of bacteria inside the body through skin. Most of pathogenic microorganisms responsible for wound include Staphylococcus aureus, Streptococcus pyogenes, Enterococci, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa. Bacterial infections on skin are the common ailment for generation of other diseases in the body. Wound infections represent the invasion of tissues by number of species of microorganisms. These infections disturb the immune system of body and cause delayed wound healing. Aloe vera leaves pulp is used for medicinal purposes, including treatment of constipation, asthma, diabetes, peptic ulcer, inflammation, wound healing, heart burn, heat stroke etc. While Azadirachta indica is useful in tiredness, cough, fever, loss of appetite, worm infestation. It heals wound, heat-rash, boils, jaundice, leprosy, skin disorders, eye disorders, stomach ulcers, chicken pox, excessive thirst, diabetes and insect poisons. It act as anti-leprotic, antimalarial, anti-hemorrhoidal and anthelmintic agent. The present study was undertaken on evaluation of combinational effect of ethanolic extract of Aloe vera leaves pulp and Azadirachta indica leaves for wound healing activity through topical route on excision wound model. The activity was compared with standard drug Povidone Iodine ointment(5% w/w). Combinational effect of both extract was showed faster wound healing effect than standard drug Povidone Iodine ointment on excision wound model.

Keywords: Aloe vera, Azadirachta indica, povidone iodine ointment, wound healing activity, excision wound model.

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INTRODUCTION

A wound arises due to invasion of microbial agents. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and junction of body[1]. Management of wound healing is a complicated and expensive step[2]. Restoration of damaged tissue (wound) is an important process which plays vital role in survival of life. It is imminent for the basis of all surgical manipulations. Many plants have proved to possess significant healing properties in different types of wounds. Using certain plants, possessing antiseptic, astringent, anti-inflammatory, antimicrobial property the rate of wound healing can be enhanced[3-4]. Such plant can increase the rate of tissue healing by providing different essential substances, required at various steps of wound healing. Plants being safer than allopathic drugs, so treatment by natural ways may be useful in veterinary practice, especially in India where these are found in plenty[5]. Indian Aloe is a plant commonly known as Aloe vera, belongs to the family Aloeceae[6-7]. Its leaves pulp is used for treatment of constipation, asthma, irritable bowel syndrome, diabetes, peptic ulcer, inflammation, wound, heart burn, stress etc[8-10]. Azadirachta indica is a plant commonly known as Neem, belongs to the family Meliaceae[6-7]. Its leaves are useful in tiredness, cough, fever, loss of appetite, worm infestation. It acts as anti-leprotic, antimalarial, anti-hemorrhoidal and anthelmintic agent etc[8-10]. Wound healing involves a highly dynamic integrated series of cellular physiological and biochemical processes that occurs in living organisms[11-12]. The majority of world population relies on traditional medicine for their health care[13]. This is also the case in the treatment of wounds. Many research proposed that wound healing can be improved by herbal drugs having antiseptic, antibacterial, antioxidant and anti-inflammatory properties[14-15]. Based on the above source of information, the present study was aimed to evaluate combinational effect of extract of leaves of Aloe vera and Azadirachta indica for wound healing activity through topical route on excision wound model against standard drug Povidone Iodine ointment (5% w/w).

MATERIALS AND METHODS

Collection and Extraction of plant materials

The fresh leaves of Azadirachta indica and Aloe vera were purchased from local nursery garden during the month of July 2006. The plant material were identified and authenticated at Botanical Survey of India, Jodhpur by Dr. P. J. Parmar. The fresh plant material of Azadirachta indica was dried under shade and then powdered using mechanical grinder and passed through sieve no.60 to get the powder of desired coarseness. Powdered material was preserved in an air tight

container for extraction purpose. 750 g of the coarsely powdered, dried leaves of Azadirachta indica were mixed with 4500 ml of ethanol, allowed to stand for 7 days. It was filtered and distilled under vacuum to get concentrated ethanolic extract. The ethanolic extract of Azadirachta indica leaves was stored under desiccators for further phytochemical and pharmacological screening.

The fresh mature leaves of Aloe vera were cleaned from outside with distill water to remove dirt and foreign impurity if any present on the leaves. The rind was removed with the help of sterilized knife. The colourless pulp was grounded in the blender and the pulp so obtained was placed in an air tight container and kept at a cool and dry place for further phytochemical and pharmacological screening.

Preliminary phytochemical analysis

A preliminary phytochemical screening was carried out for both the extract employing the standard procedure to reveal the presence of alkaloids, steroids, terpenoids, flavonoids, saponins, tannins, glycosides, carbohydrates, phytosterols and proteins[16].

Drug formulation

Two 5% w/w ointment formulations were made by incorporating individual extract of plant material with simple ointment base I.P.10 while third 5% w/w ointment formulation was made by mixing both the extract together in a ratio of 1:1 with simple ointment base I.P.10 for external application of the drug in the excision wound model.

Animals

Adult albino rats (wistar strain) of either sex weighing 180-200 g were taken. They were placed in polypropylene cages in a controlled room environment (25°C±2°C) at a natural day night cycle and they were provided with standard laboratory food and water *ad libitum*. Approval for the study was obtained from the institutional animal by the Institutional Animal Ethical Committee (IAEC), Reg No. 990, U.D.P.S.

Wound healing activity

Animals were assigned into 5 groups containing 6 animals in each group. All the wounds (control, standard and test groups A, B, C) were cleaned with the sterilized normal saline solution and then dried gently with sterilized gauze before every dressing. Group I was called as control group, was kept untreated, group II was called as standard group, was treated with market formulation (Povidone Iodine ointment), group III was called as test group in which it was divided into three subgroups comprising of test group III A, which was treated with leaves pulp extract ointment of Aloe vera, test group III B treated with ethanolic extract ointment of

Azadirachta indica leaves and test group III C, was treated with leaves extract ointment of both Aloe vera and Azadirachta indica in a ratio of 1:1. Excision wound model was selected for assessing wound healing activity of both the extracts on topical route of administration for 15 days. Animals were anaesthetized with ether and shaved on part to be exposed. A circular piece (500 mm^2 area) was impressed on the dorsal thoracic region 5cm away from ears and 1 cm away from the vertebral column. The animals were individually housed in separate cages. The test and standard preparation were topically applied once a day till epithelialization was complete, starting from the day of the operation. The wounds were traced on 1 mm^2 graph paper on the day of wounding and then subsequently on the 4th, 8th, 12th and 16th post wound days and thereafter daily until healing was complete. The parameters studied were percentage of wound contraction or wound closure and period of epithelialisation[17].

RESULTS AND DISCUSSION

The yield of leaves extract of Aloe vera and Azadirachta indica were found to be as 6.96%w/w and 8.56%w/w respectively. The Aloe vera leaves extract showed the presence of phytoconstituents like amino acids, anthraquinones, vitamins, lignins, monosaccharide, polysaccharides, salicylic acid, saponins and sterols. The Azadirachta indica leaves extract showed the presence of phytoconstituents like azadirachtin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, quercetin and salannin etc. During the initiation of study from the day 0 there was not much difference in the healing of wounds in all the groups. But after day 9, the healing process was observed faster in the groups treated with 5% w/w ointment formulation made by mixing both the plant material leaves extract together in a ratio of 1:1 as compared to the standard group which was treated with Povidone Iodine ointment as shown in table 1.

Table 1: Effect of topical administration of Aloe vera and Azadirachta indica leaves extract ointment on excision wound model

Comparative mean wound area of different groups (in mm^2)					
Post wounding days	**Group I (control)**	**Group II (standard)**	**Group III (test group)**		
			III A	**III B**	**III C**
0	242.45 ± 1.28	241.26 ± 1.22	240.06 ± 0.64	240.53 ± 0.22	241.92 ± 1.03
3	196.92 ± 1.06	173.86 ± 1.86	151.06 ± 1.42	113.76 ± 1.27	102.06 ± 1.45
6	152.04 ± 1.10	133.01 ± 1.74	127.01 ± 1.36	98.88 ± 1.08	58.81 ± 1.32
9	120.78 ± 1.07	101.58 ± 1.29	98.46 ± 1.34	31.89 ± 1.26	06.05 ± 0.13
12	92.58 ± 1.26	59.08 ± 1.56	48.05 ± 1.25	17.19 ± 1.25	0.00 ± 0.00
15	27.46± 1.12	10.05 ± 0.22	02.01 ± 0.31	01.00 ± 0.10	0.00 ± 0.00
18	12.06± 0.53	00.05 ± 0.03	00.00 ± 0.00	00.00 ± 0.00	0.00 ± 0.00

Statistical analysis was done by ANOVA and Dunnet's multiple comparison tests. Results are expressed as mean ± SE, n=6 in each group. Significant difference was compared to control

group at $p<0.01$. From the results, it was observed that in the topical route, test group III C shows faster wound closure and wound contraction and the results are significant ($P<0.01$) when compared with standard drug Povidone Iodine ointment.

CONCLUSION

Thus, it can be concluded that when combination of the leaves extracts of Aloe vera and Azadirachta indica (ratio of 1:1) were applied together externally on male albino rats by topical route through excision wound model then it showed faster as well as better wound closure and wound contraction as compared to other treatment groups. Therefore, we may interpret that the observation of such response may be due to the synergistic effect of the two plant materials possessing the presence of various phytoconstituents in them. However further studies can be performed to isolate the particular component responsible for generating the wound healing activity.

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