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EVALUATION OF WOUND HEALING ACTIVITY OF AEGELE MARMELOS LEAVES IN MALE ALBINO RATS

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ABSTRACT

Wounds are major cause of physical disabilities. Wound healing consists of orderly progression of series of events that establish the integrity of the damaged tissues. Aegele marmelos (Bael) is used for medicinal purposes, including treatment of dyspepsia, inflammation, asthma, hypoglycemia, cancer, hepatitis, diabetes, sinusitis, tuberculosis, diarrhea, dysentery, as astringent, digestive, stomachic etc. The present study was undertaken on ethanolic extract of leaves of Aegele marmelos on wound healing activity through topical route on excision wound model. The activity was compared with standard drug Povidone Iodine ointment (5% w/w). The leaves of Aegele marmelos ethanolic extract was found to have better and faster rate of wound healing effect than Povidone iodine ointment on excision wound model.

Key Words: Aegele marmelos, povidone iodine ointment, wound healing activity, excision wound model.

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INTRODUCTION:

Plants possess a variety of pharmacological activities like Turmeric *(Curcuma longa)* has anti-tumor, anti-diabetic and anti-microbial acitivities, Aak *(Calotropis gigantean)* has wound healing activity, Utaran *(Pergularia daemia)* has anti-inflammatory activity and Hapusha *(Spheranthus indicus)* possess antimutagenic activity[1-4]. Polyherbal Ayurvedic formulations like Avipattikar churna is useful for its gastro protective effects[5].

Wounds are major cause of physical disabilities. They arise due to physical, chemical or microbial agents. Healing is a survival mechanism and represents an attempt to maintain normal anatomical structure and junction[6]. Many immunosuppressant, cytotoxins and non-steroidal anti-inflammatory drugs suppress the wound healing. Management of wound healing is a complicated and expensive step[7]. 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[8]. 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[9]. Such plant can increase the rate of tissue healing by providing different essential substances, required at various steps of wound healing. Plants being cheaper and safer than allopathic drugs, so treatment by natural ways may be useful in veterinary practice, especially in India where these are found in plenty[10]. Aegele marmelos is a plant commonly known as Bael, belongs to the family Rutacae[11-12]. Aegele marmelos (Bael) is used for medicinal purposes, including treatment of dyspepsia, inflammation, asthma, hypoglycemia, cancer, hepatitis, diabetes, sinusitis, tuberculosis, diarrhea, dysentery, as astringent, digestive etc[13-15]. Based on the above source of information, the present study aimed to evaluate the wound healing activity of leaves of Aegele marmelos.

MATERIALS AND METHODS

Collection of plant materials

The fresh leaves of Aegele marmelos were purchased from local nursery garden during the month of November 2007. The plant material was identified and authenticated as RAU/AM/27 at Botanical Survey of India, Jodhpur by Dr. P. J. Parmar. The fresh plant material was dried under shade. Dried plant material was 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.

Extraction and drug formulation

750g of the coarsely powdered, dried leaves of Aegele marmelos was mixed with 4500 ml of ethanol, allowed standing for 7 days. It was filtered and distilled under vacuum to get concentrated ethanolic extract. The ethanolic extract of Aegele marmelos (EtAgEx) was stored under desiccators for further phytochemical and pharmacological screening. A 5% w/w ointment formulation was made by incorporating the extract EtAgEx with simple ointment base I.P10 for external application of the drug in the excision wound model.

Preliminary phytochemical analysis

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

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. All the experimental procedures and protocol used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC), Reg No. 990, U.D.P.S., Utkal University.

Wound healing activity

Animals were assigned into 3 groups containing 6 animals in each group. 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, was treated with ethanolic leaves extract ointment of Aegele marmelos (EtAgEx). Excision wound model was selected for assessing wound healing activity of EtAgEx on topical route of administration in a form of a 5%w/w extract in simple ointment base I.P.10 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 epithelialisation 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

Preliminary studies

The results of extractive value and preliminary phytochemical screenings studies for EtAgEx are shown in the table 1. The yield of Aegele marmelos leaves was found to be as 9.36%w/w. The Aegele marmelos extract showed the presence of phytoconstituents like skimmianine, steroids, aegeline, lupeol, cineol, citral, citronella, cuminaldehyde, eugenol, marmesinine etc.

Table 1: The percent yield and presence of phytoconstituents in ethanolic extract of Aegele marmelos.

Extract	Percentage yield (w/w)	Presence of phytoconstituents
Aegele marmelos leaves	9.36	Skimmianine, steroids, lupeol, cineol, citral, citronella, cuminaldehyde, marmesinine, eugenol

Table 2: Effect of topical administration of ethanolic extract of Aegele marmelos 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)**
0	226.85 ± 1.78	226.86 ± 1.98	219.96 ± 2.03
3	166.62 ± 1.96	153.86 ± 1.86	102.06 ±2.45
6	143.04 ± 1.20	113.04 ± 1.47	32.51 ± 1.26
9	94.88 ± 1.07	63.58 ± 1.56	07.43 ± 0.47
12	62.58 ± 1.46	7.08 ± 1.76	0.00 ± 0.00
15	37.46± 1.22	0.00 ± 0.00	0.00 ± 0.00

Pharmacological screening

Wound healing involves a highly dynamic integrated series of cellular physiological and biochemical processes that occurs in living organisms[18-19]. The majority of world population relies on traditional medicine for their health care[20]. 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[21-22]. In the present study, the excision wound model is considered for evaluation of wound healing activity of ethanolic extract of Aegele marmelos (EtAgEx) as topical application. The rate of wound contraction in mm^2 was taken as a measure of wound healing.

During the initiation of the study from the day 0 there was not much difference in the healing of wounds in all 3 groups. But after day 9, the healing process was faster in the group treated with test formulation of ethanolic extract of Aegele marmelos as compared to the standard group which was treated with Povidone Iodine ointment as shown in table 2. Statistical analysis was

done by ANOVA and Dunnet's multiple comparision 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 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 the when ethanolic extract of leaves of *Aegele marmelos* was applied 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 standard marketed formulation called as Povidone Iodine ointment. Therefore, we may interpret that the presence of such response may be due to the presence of various phytoconstituents which were found to be present in the leaves of Aegele marmelos. However further studies can be performed to isolate the particular component responsible for generating wound healing activity.

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