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THE STUDY OF THE EFFECTS OF MEDICINAL HERB INDIAN BARBERRY (*DARHALD, Berberis aristata*) ON ANALGESIC,WOUND HEALING AND ANTIINFLAMMATORY IN EXPERIMENTAL ANIMALS

Dabhade SA[1*], Dabhade SS[2], Rane BT[3] and Tiwari S.A.[4]

India.

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***Correspondence for Author**
Dr. Dabhade SA
India.

INTRODUCTION

Throughout the world about 6 million people are encumbered with chronic wounds. According to Mazumdar & Mukhopadhay prevalence of acute and chronic wound is 10.5 per 1000 population & 4.5 per 1000 population respectively.[1]

Wound healing is an important biological process involving tissue repair and regeneration. A wound is described as a break in the continuity of tissue from violence or trauma and is regarded as healed if there is a restoration of the wounded or inflamed tissue to normal condition. The process of wound healing can be broadly categorized into three stages; inflammatory phase (consisting of homeostasis and inflammation); proliferative phase (consisting of granulation, contraction and epithelialisation) and finally the maturation phase which determines the strength and appearance of the healed tissue.[2]

The acute phase of wound healing is partially triggered by activation of platelets through the release of platelet-derived growth factor (PDGF) and eicosanoids (prostaglandins and leukotrienes), which are known to facilitate hemostasis and the inflammatory response. Platelet-derived growth factor is responsible for chemotactic and mitogenic effects on fibroblasts, smooth muscle cells, macrophages, monocytes, and neutrophils. Neutrophils, in particular migrate rapidly to the site of injury and reach a maximal level at 2 days after wounding. In addition, neutrophils help secrete growth factors that stimulate the migration of fibroblasts, epithelial cells, and vascular endothelial cells into damaged tissue. After approximately 48 hours, macrophages become the predominant inflammatory cells in the

wound bed and serve to scavenge bacteria and tissue debris and to destroy neutrophils. As the process of wound healing progresses, the number of macrophages in the wound tapers off while fibroblasts become the major constituent of the wound bed.[3]

Biosynthesis of new collagens by fibroblasts has a key role in the healing process. Collagen deposition helps the wounds to gain tensile strength during repair. Another important factor in the wound repair process is transforming growth factor-β (TGF-β). These factors release from platelets at the site of injury and activate and infiltrate fibroblasts, macrophages and neutrophils and initiate wound tissue repair phase. In turn, these cells migrate into the wound site and release cytokines/chemokines that initiate granulation tissue formation.[4]

Indian Barberry (Berberis aristata) is an erect, glabrous, spinescent shrub with obovate to elliptic, subacute to obtuse, entire or toothed leaves. The flowers are yellow and in corymbose racemes. The fruits are oblong-ovoid or ovoid, bright red berries. *Berberis aristata* DC. (Berberidaceae) is commonly known as Daruharidra in Bengali, Daruhald & Rasaut in Hindi. It attain 3-6 m of height. It is distributed in temperate and subtropical parts of Asia, Europe and America. In India drug is largely collected in Chamba district of U.P. and sold in the markets of Chamba, Dehradun and Haridwar. The chief constituent is berberine, and other reported phytoconstituents are berbamine, armoline, palmatine and oxycanthine oxyberberine. Berberone hydrochloride, an alkaloid isolated from Berberis aristata, was found to have significant anti-inflammatory activity on acute, subacute and chronic types of inflammations produced by immunological and nonimmunological methods. The roots form a reputed drug in ayurvedic medicine and possess antibacterial and anti-inflammatory activities.[5,6]

AIMS AND OBJECTIVES

The aim of the study is to test the analgesic, anti-inflammatory and wound healing properties of Indian Barberry (Berberis aristata) In experimental animals.

MATERIALS AND METHODS

Pharma grade barbaris aristata powder was procured by Shaanxi Kingsci Biotechnology Co. Ltd. China (Mainland). The model of analgesic action[7] we used acetic acid induced writhing model. 24 Wister mice (weighing between 25-30g) eight in each group, of either sex were used. The animals were starved overnight. To ensure uniform hydration, the rats were given 0.5 ml of water by stomach tube (controls) or the test drug suspended in the same volume.

After 30 minutes of drug administration 0.1ml of 1% acetic acid solution was injected intra-peritoneally mice were placed under jar they were observed for a period of ten minutes and number of writhes was recorded in every animal.

Model for acute inflammation,[7] the Carrageenan Induced Paw Edema method was used. 24 Wistar rats (8 in each group) of either sex with a body weight between 150 and 250 g were used. The animals were starved overnight. To ensure uniform hydration, the rats were given 5 ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. Volume of the same paw was measured at Baseline (0h) and after 3h and at 6h after challenge by using mercury plethysmograph.

Amount of edema was obtained by subtracting volume of left hind paw at baseline before injecting carrgeenan from volume of the same paw at 3h and 6h each. The percent inhibition of edema due to control and treatment was calculated using the following formula.

Percent inhibition of edema = 100 x (1-Vt/Vc)

where, Vc = mean paw edema volume in control group.

Vt = mean paw edema volume in treated group.

Aspirin was used as standard control in Analgesic and antiinflammatory groups

The model for testing the effects on wound healing[8] was that of incised wounds on the back of the rats. The animals were housed in individual cages without saw dust, so that it would not stick to the wounds and the animals were fasted overnight. Animals were given water ad-libitum on the previous day of the study. The back of these animals was shaved using a sterile blade. The area shaved was cleaned with betadine solution. On the day of the study mild general anaesthesia with ether was used and an excision wounds was inflicted by cutting away approximately 2 cm x2cm full thickness of shaved skin of a predetermined area on the anterior-dorsal side of each rat under aseptic condition. Cotton pressure was maintained till bleeding stopped. The wound was cleaned with normal saline and sterile cotton bandage and the wound was left undressed. A total of 18 rats were used for this study out of which 9 received Indian Barberry extract and the 9 received normal saline 2 hours before the wounds. The animals were fed the respective extracts once a day for 7 days. Every alternate day the wounds were observed and healing was noted by the presence of appearance and nature of

granulation tissue. The percentage wound closure of original wound area in rat were recorded at the intervals of 4th, 8th, 12th and 16th day of post wounding. Slides for histopathology were collected on day 0,4,12 and 16th day.

The rate of contraction of wounds was measured by tracing the wound surface on to a transparent paper and then measuring the surface area using graph paper and expressed as percent of original wound size.

OBSERVATIONS AND RESULTS

Table 1: The model of analgesic action

1) Acetic acid induced writhing

Drugs	Onset of writhes (min)	Number of writhes in 10 min	P value
Normal saline	2.6±0.85	32.85±9.37	
Aspirin	6.73±1.2	4.12±1.65	P<0.01
Berberis aristata	5.7±1.35	8.5±2.82	P<0.05

Acetic acid induced writhing is significantly reduced in barbaris aristata and aspirin group as compared to normal saline group.

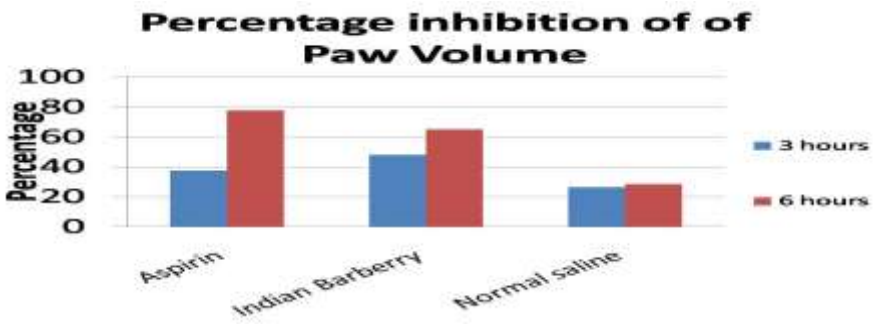
Table 2: Carrageenan Induced Paw Edema

Average Volume of Paw in (ml)

Drug	0 hours	3 hours	6 hours	P value
Normal Saline(control)	4.37±1.65	3.22±1.89	3.11±1.65	P>0.05
Aspirin	4.51±1.28	2.82±1.11	1.01±1.32	P<0.05
Indian Barberry(500mg/kg)	4.48±1.25	2.36±1.17	1.57±1.14	P<0.05

Table 3: Percent inhibition of Paw Volume

Drug	3 hours	6 hours
Normal saline	26.31%	28.33%
Aspirin	37.47%	77.60%
Indian Barberry	47.83%	64.95%



Graph Percent inhibition of Paw Volume

The Average paw volume is as given in the table 2. When the groups were individually compared with the group receiving normal saline there were significant reduction of inflammation in both the groups. Percent inhibition of Paw Volume at 3 hrs is 37.47%, 47.83% and at 6 hrs is 77.60% 64.95% for aspirin and Barbaris aristata respectively.

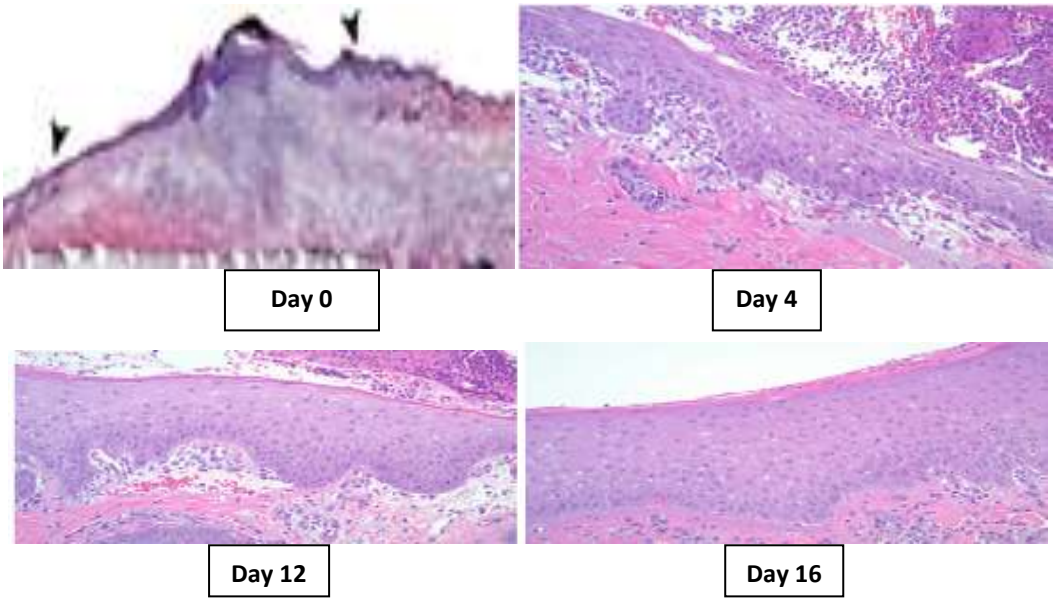
2) Wound Healing model

When tested for wound healing out of the 18 rat chosen 4 died before the completion of the experiment. Out of three from NS group, one rat from the NS group died on day 1 and two others from NS group died on day 6. The reason for the first rat's death was either due to bleeding or shock. The other rats may be died because of infection at the wound site. The fourth rat was from the Indian Barberry group and died due to infection on the fourth day.

Granulation tissue appeared by the 3rd to 6th day in all animals which survived. The granulation tissue was healthy. Sections were taken after anesthetizing the animal on Day 0,4,12 and on day 16. In general healing with healthy scar formation occurred by day 13 to 16 in animals receiving active drugs.

Table 4: % Area closer (Reduction in wound size)

Average size of wounds (sq mm)%area Closer (mean±S.D.)	Day 0	Day 4	Day 8	Day 12	Day 16
Normal Saline	378.12±7.56	310.76±18.43	292.54±16.10	240.73±71.06	199.29±23.46
Indian Barberry(500mg/kg)	369.25±11.1	286.18±78.91 ***	233.09±0.53* **	179.88±11.85 ***	123.15±25.24 ***



Photographs: Showing wound healing process on various days

After histological examination of the wounds it was seen that the neutrophill infiltration started within 2 days in all groups.

In the Indian Barberry group the proportion of fibroblasts increased after the second day and wound healing was apparent by the 4th day. Visible scar tissue was seen on the 10th -12th day. The healing was faster compared to the group receiving normal saline. The group also showed an increase in the amount of collagen deposited. However one rat succumbed to infection on the fourth day.

Discussion: The results of the present study clearly demonstrated the protective effect of the herb as analgesic, anti inflammatory and wound healing. The duration of the inflammatory phase was decreased in the Indian Barberry treatment group compared with their control groups ($p < 0.05$). Also the rate of infection and mortality was significantly lower than that of the control group.

The drug used in our study has demonstrated a significant anti-inflammatory effect as in other studies.[9,10] Many studies are being done to evaluate the potential of plants like aloe vera, Neem, turmeric, etc. Neem has been shown to have many active ingredients.[11] In a study by Neetu Rajput[5] it was shown that wound contraction, epithelialisation and percentage of healing were excellent in wounds treated with the alcoholic extract of *B. aristata* and aqueous extract *B. aristata*.[9] One of the mechanisms for this is the blocking of the creation of products needed for synthesis of inflammatory cytokines. For instance, it appears to inhibit the release of arachidonic acid from cell membrane phospholipids which is required for the creation of inflammatory prostaglandins.[12] Berberine may also modulate levels of prostacyclin. On evaluation of the isolates it was found that the roots of *B. aristata* contain significant amounts of the isoquinoline alkaloid berberine.[10] In our study we found that Indian Barberry extracts proved effective in reducing pain, acute inflammation and in promoting healing of wounds.

The inflammatory response following tissue injury plays important roles both in normal and pathological healing. Immediately after injury, the innate immune system is activated, setting in motion a local inflammatory response that includes the recruitment of inflammatory cells from the circulation.[13] This suggests that anti-inflammatory activity of Barbaris aristata could have hamper or slowed down the wound healing but results of this study shown that wound healing is hastened due to Barbaris aristata. This suggests that wound healing seen is

due to some other reasons like Barbaris aristata may have antibacterial activity or by some other mechanism it may have wound healing action.

When we searched for references of wound healing activity it is found that Berberis aristata (Dauhaldi) is a potential antioxidant agent. It's an antibacterial agent and acts through DNA damage during cellular proliferation of bacteria .This antioxidant and antibacterial property of Daruhaldi might play important role in the wound healing process. It has been communicated that flavonoid derived from plant source has anti-lipidperoxidation potential which helps in improving vascularity and decreasing the cell necrosis.[14] Berberine present in the *Berberis aristata* might promote the wound healing process.[15]

However further studies on more complicated wound models for longer durations with active principles of Indian barberry are required. Since modern allopathic medicine lacks effective drugs that promote healing the use of Berberis aristata plant extracts could be viewed as an exciting option.

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