Volume 5 • Issue 1 • January - March 2011

ISSN 0973-8258

IJGP

International Journal of Green Pharmacy

Official publication of
TIFAC CORE in Green Pharmacy

BRNSS Institutions | Learning Innovations Entrepreneurship
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Original Article

Wound-healing potential of aqueous and ethanolic extracts of apamarga leaves

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The present research work was aimed at exploring the wound-healing activity of alcoholic and an aqueous *Achyranthes aspera* Linn (apamarga) leaf extract. Leaf extracts (aqueous and ethanolic) were examined for its wound-healing activity in the form of ointment (1% w/w) in Excision model and Incision model in rats. The evaluation was made in terms of wound contractibility and wound closure time. *A. aspera* Linn leaf extract showed significant (P<0.001) wound-healing activity when compared with control and was as effective as soframycin (standard cream for comparison). The wound-healing potential of ethanolic extract was slightly more compared with aqueous extract. The present study showed the wound-healing potential of apamarga leaves.

Key words: Apamarga, extract, wound

INTRODUCTION

Traditionally, green leaves of the plant *Achyranthes aspera Linn (Apamarga)* are commonly used in treatment of vomiting, bronchitis, heart disease, piles, abdominal pains, ascites, dyspepsia, dysentery, blood diseases and stomach ache. Other uses and properties of *A. aspera* Linn. are antiperoxidative properties;[1] as abortifacient;[2] as antibacterial agent[3] and immunomodulator.[4] *A. aspera* leaves have been assessed for chemopreventive activity in a wide range of conditions. The methanolic extract, alkaloid, non-alkaloid and saponin fractions exhibited significant inhibitory effects on the Epstein-Barr virus. *In vivo* two-stage mouse skin carcinogenesis test revealed that the total methanolic extract possessed a pronounced anticarcinogenic effect. The study suggested that *A. aspera* leaf extract and the non-alkaloid fraction are valuable antitumour promotors in carcinogenesis.[5] The usefulness of the plant extracts in fish was also studied in *Labeo rohita*, rohu. The results showed the immunostimulatory activity of the prepared diet containing root extract of *A. aspera*.[4] *A. aspera is* a well-known Indian medicinal plant used in the indigenous systems of medicine for the treatment of inflammatory conditions. *A. aspera* inhibited the inflammatory responses at doses of 100 to 200 mg/kg. The study reveal that the ethanolic extract of *A. aspera* possess anti-inflammatory and -arthritic activity and support the rationale behind the traditional use of the plant in inflammatory conditions.[6]

A. aspera has different types of therapeutic activities including antibacterial, immunomodulatory and antioxidant activity. By virtue of having the above-mentioned activities, it was hypothesised that the plant could also possess wound-healing activity. However, information about wound-healing activity of *A. aspera* is sparse. Keeping above in view, attempts were made to evaluate the wound-healing potential of aqueous and ethanolic extract of leaves of *A. aspera*.

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MATERIALS AND METHODS

Preparation of Apamarga Extract and Preliminary Phytochemical Screening

Extraction: Leaves of *A. aspera* were cleaned and washed with distilled water to remove any kind of dust particle and dried in shade. Leaves were powdered in a mixer grinder and passed through sieve no. 10 for extraction. Leaves powder was packed in soxhlet assembly and after that defatted with petroleum ether (60–80°C). Completion of defatting was tested by spot test and the compact mass of powdered leaves was dried at room temperature. Then, extraction was carried out with ethanol (90%) or distilled water for 72 hours.[7]

Preliminary Photochemical Screening

Aqueous and ethanolic extracts were subjected for detection of alkaloids, carbohydrates, glycosides,

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Received: 31-10-2010; **Accepted:** 26-11-2010

phytosterols, phenols compound and tannins, proteins, saponins and flavonoids by methods described in literature.[7,8]

Preparation of Ointment from the Extracts

Method for the preparation of ointment

The stearic acid was melted on a water bath. Potassium hydroxide was dissolved in water and glycerin was added and heated to 85°C. Extract was added slowly to melted stearic acid with constant stirring and cooled. The formulas are given in the Table 1.

Acute toxicity determination of the extracts

It was performed according to OECD guideline No 434. Starting dose of 2 000 mg/kg of extract was given to randomly selected group of five animals by oral and intraperitoneal route.

Wound-Healing Activity

Twenty-four albino rats (100–150 g) were randomly selected and divided in four groups. These were kept under NIH guidelines and fed water and rat chow *ad libitum*. Two weeks after procurement, each animal was anaesthetised with ketamine (60 mg/kg intraperitoneally). A full thickness wound through the surgical seizer was formed on the dorsal midline of each animal from a circular template 500 mm^2 in area. Immediately after making wounds, the animals were placed under a fixed camera and photographed. All wounds were left open to heal and the animals were randomly placed into one of four groups. The wounds in Group 1 animals were dressed daily with 0.9% normal saline (control). Aqueous extract of apamarga 1% (in an ointment base) was applied topically in an amount of 0.5 g/cm^2 of wound surface to the wounds of Group 2 animals. In Group 3, ethanolic extract of apamarga 1 (in an ointment base) was applied

Table 1: Composition of aqueous and ethanolic extract ointment

Chemical used	Quantity used
Aqueous/ethanolic extract	1.0 g
Stearic acid	24.0 g
Potassium hydroxide	0.700 g
Glycerin	10.0 g
Water	q. s.

Table 2: Phytochemical screening of extracts of *A. aspera*

Chemical test	Aqueous extract	Ethanolic extract
Alkaloids		
Mayer test	Positive	Positive
Wagner's test	Negative	Negative
Hager's test	Positive	Positive
Dragondraff's test	Positive	Positive
Carbohydrates and glycosides test		
Molisch's test	Positive	Positive
Fehling's test	Positive	Positive
Legal's test	Positive	Positive
Phytosterol		
Salkowski reaction	Negative	Negative
Fixed oil		
Spot test	Negative	Negative
Phenolic compound and tannins		
Ferric chloride test	Positive	Positive
Lead acetate test	Positive	Positive
Proteins		
Million's reagent	Positive	Positive
Flavonoids detection	Positive	Positive
Saponins		
Foam test	Positive	Positive

Table 3: Effect of *A. aspera* Linn. extract on excision wound model in albino rat when applied topically

Group	Treatment	Remaining of original excision wound area (mm^2) Mean±SEM		
		0 day	8th day	16th day
1	Control (Group 1)	488.4±4.823	287.2±11.015	133.6±9.127
2	Standard (soframycin) Group 4	490.8±5.333	219.2±4.067	32.4±3.544***
3	Group A (aqueous extract) Group 2	492.2±5.826	168.6±5.591	23.4±1.860***
4	Group B (ethanolic extract) Group 3	493.4±5.955	148.4±11.957	11±3.536***

Value are mean±SEM, one way ANNOVA, n=6; ***P<0.001, **P<0.01, *P<0.05, P>0.05 ns *Vs* control

Table 4: Effect of *A. aspera* Linn. extract on excision wound model in rats when applied topically

Group	Treatment	% closure of original excision wound area (mm^2) Mean±SEM	
		8th day	16th day
1	Group 1 (control)	41.20	72.65
2	Group 4 (soframycin)	55.34	93.40
3	Group 2 (aqueous extract)	65.75	95.25
4	Group 3 (ethanolic extract)	69.92	97.77

Value are mean±SEM, one way ANNOVA, n=6; ***P<0.001, **P<0.01, *P<0.05, P>0.05 ns *Vs* control

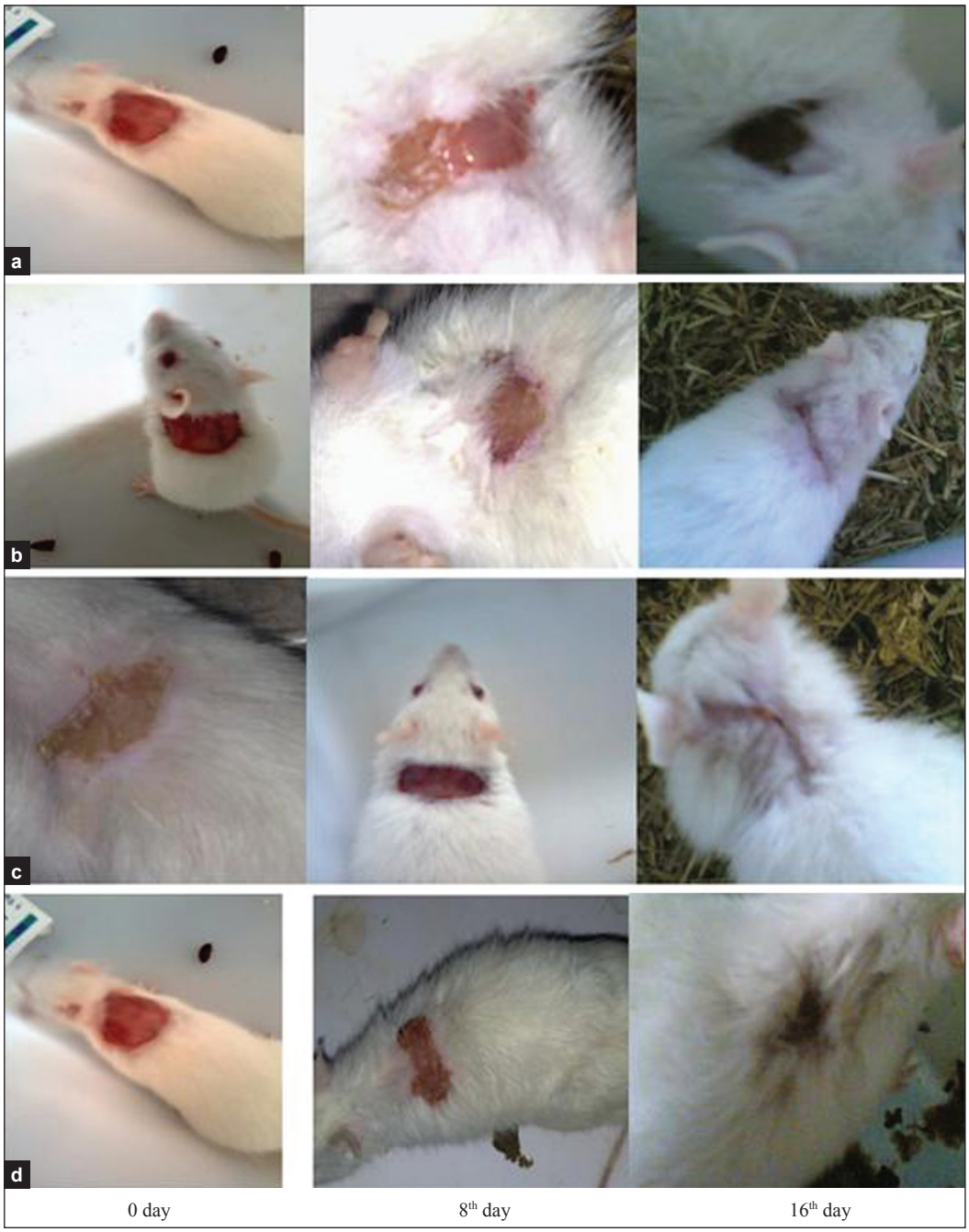

Figure 1: Photographs of rats. (a) Group 1 -Control, (b) Group 2 -Aqueous extract, (c) Group 3 -Ethanolic extract, (d) Group 4 -Soframycin

topically in amount of 0.5 g/cm^2. Wounds of group 4 animals were treated with soframycin (1%w/w cream) 0.5 g/cm^2 topically. The area of each wound was measured on tracing paper, calculating the area with the help of graph paper on days 0, 4, 8, 12, 16 and the mean wound size of each treatment group was compared with control at each time interval.

RESULTS AND DISCUSSION

Both aqueous and ethanolic extracts of apamarga leaves showed positive tests for alkaloids, carbohydrates, phenolic compounds and tannins, flavonoids and saponins. For phytosterols and fixed oils, the extracts showed negative results [Table 2].

The ointment prepared with extracts, according to composition showed in Table 1, were uniform and homogenous in appearance.

Acute studies were conducted twice in animal with a dose mentioned above. Animals were observed for behavioural change and death. No animal was found dead after 14 days. The study was repeated with same dose, and again

no death was observed. The results indicate that the extract is safe and nontoxic.

The results of wound-healing activity are shown in Figure 1, Tables 3 and 4. At 4, 8, 12 and 16 days, the treatment groups exhibited significantly smaller wounds when compared with control (P<0.001 each group) [Table 3 and Figure 1]. In all animals of Groups 2 and 3 above 90%, healing was observed and the animals in Group 4 had healed wounds at 16 day, approximately 90% as compared with control group [Table 4]. Thus, from these studies, it is evident that apamarga leaf extract (aqueous and ethanolic) on topical application enhanced wound contraction and healing in this model and are as effective as soframycin.

The crude extract showed very good wound-healing properties; however, to reveal due to which fraction of the extract it is so, fractionation studies and their wound-healing screening studies are needed.

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Source of Support: Nil, **Conflict of Interest:** None declared.