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PROTECTIVE EFFECTS OF *EUPHORBIA HELIOSCOPIA* ON DIFFERENT WOUND MODELS IN RATS

N Jabeen[1], R Khanum[2], S Shakuat[2], S Mehmood[2] and *M Gulfraz[2]
[1]Quaid-i-Azam, University, Islamabad
[2]Department of Biochemistry, University of Arid Agriculture, Rawalpindi

ABSTRACT

The wound healing effect of aqueous and ethanol extracts of *Euphorbia helioscopia* was studied on incision, excision and dead space models of wound in rats. After application of both extracts it was observed that the area of epithelialisation increased, followed by an increase in wound contraction, skin breaking strength, tissue granulation, dry weight and hydroxylproline contents. Histopathological studies of the granulation tissue also indicated that there was an increase in collagen formation in those rats treated with the ethanol extract, compared to the control group animals. The ethanol extract was more effective than the aqueous extract, but both showed significant results, compared to the control. Results indicate that plant extracts are an interesting source of phytochemicals that are effective against different phases of wounds.

Keywords: *Euphorbia helioscopia,* phytochemicals, alternative medicine, wound healing.

INTRODUCTION

Wounds are physical injuries and healing or repair consists of several processes which regenerate dermal and epidermal tissue**.** Proper healing of a wound is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. When tissue is first wounded, blood comes into contact with collagen, triggering blood platelets to begin secreting inflammatory factors. Platelets also express glycoproteins on their cell membranes that allow them to aggregate (Patil *et al.,* 2001). Fibrin and fibronectin then cross-link to form a plug or matrix which traps proteins and particles, preventing further blood loss, and acting as the main structural support for the wound until collagen is deposited. Migratory cells use this matrix to move across, and platelets adhere to it and secrete clotting factors. The clot is eventually lysed and replaced with granulation tissue, and then later with collagen (Porras *et al*., 1993; Pieper and Galiri, 2003). Furthermore, the abundance and diversity of microorganisms in any wound will be influenced by factors such as wound type, depth location, quality, the level of tissue perfusion and the antimicrobial efficacy of the host immune response. The presence of foreign material and devitalized tissue in a traumatic wound likely to facilitate microbial proliferation unless treatment is implemented (Bowler and Duerden, 2001).

Euphorbia helioscopia Linn (Euphorbiaceae) is an annual herb, found in hilly areas of Pakistan and other countries of the region. The herb is widely used in traditional medicines for treatment of skin infections (wounds, burns, ulcers) especially effective on wounds microflora (Ikram *et al*., 1987). The literature on phytochemicals studies show that plant extracts containing alkaloids, flavonoids, ellagic acid, gallic acid and flavellagic acid are useful for treatment of different phases of wounds and wound microflora (Salah *et al*., 1995 and Kapoor *et al*., 2004).

A survey of the ethnobotanical studies carried out in the North East of Pakistan, indicated the use of *Euphorbia helioscopia* is commonby the inhabitants of the area for wound healing purposes. The common way of treatment is direct application of fresh/ dried extract. Despite widespread use, no systematic studies have been carried-out on the clinical evaluation of the wound healing potency of *Euphorbia helioscopia*. In the present study we have investigated effects of *Euphorbia helioscopia* on different wound models in rats.

MATERIALS AND METHODS

Plant material and extracts Preparation

Samples of *Euphorbia helioscopia* were collected from hilly areas of Kotli Sattian, District Rawalpindi, during May- June 2006. A voucher specimen (No. 113) is stored in the herbarium, University of Arid Agriculture, Rawalpindi. The dried and powdered form of sample (30g) added to 300 ml of ethanol and refluxed by soxhlet for 1 hour, filtered and concentrated under reduced pressure. The aqueous extract was made by macerating 30 g powder form of sample with 1000 ml of distilled water for three days, filtered and concentrated under reduced pressure.

Drug formulation

For topical administration a 5% w/w gel was made in 2 % sodium alginate solution, and for oral administration, a suspension of 30 mg/ml of the extracts in 1 % gum tragacanth was prepared.

*Corresponding author email: m.gulfraz_satti@hotmial.com
Present address: School of Pharmacy, Reading University UK - m.gulfraz@reading.ac.uk

Experimental Animals

Swiss Wister rats of either sex (170-200g) were maintained under standard animal house conditions, fed with commercial rat chow (Feed Mills, Islamabad) and allowed water *ad libitum*. All the animals were carefully monitored and maintained in accordance with ethical recommendations of Pakistan Veterinary Science.

Acute toxicity and selection of dose

Acute toxicity studies were conducted for both extracts (Sairpurana *et al*., 1995) in order to select a suitable dose for evaluation of wound healing activity. The LD_{50} of both aqueous and ethanol extracts were 300 mg/kg, so a dose of 30 mg/kg was selected for testing.

Excision wounds

A circular wound of about 50 sq mm was made on the depilated, ethanol-sterilized dorsal thoracic region of rats under light ether anesthesia. The animals were divided into 4 groups of 6. Group 1 was untreated as the control; group 2 was treated with 1% w/w nitrofurazone ointment and served as a reference standard (positive control); group 3 was treated topically with the gel prepared from aqueous extract of *Euphorbia helioscopia* and group 4 was treated with the gel prepared from the ethanol extract. The gel was topically applied once daily until epithelialisation was complete. The parameters studied were wound closure and epithelialisation, by tracing the outline of each wound on graph paper on the 3rd, 6th, 9th, 12th and 15th days, until healing was completed. The percentage of wound closure and the area of epithelialisation were recorded.

Incision wounds

The animals were divided into 4 groups of 6 animals each and the treatment of the experimental animals was similar to that for the excision wound experiments. Incision wounds 6 cm long, through the full thickness of the skin on either side of the vertebral column of rats (paravertebral incisions), were made. The wounds were closed with interrupted sutures 1 cm apart. The ointment gel was applied topically once daily. The sutures were removed on the 8th day post incision. The skin breaking strength of the wound was measured on the 10th day after treatment with a continuous water flow technique (Suh *et al*., 1998).

Dead wound space

Animals were divided into 3 groups of 6 rats. Group 1, the control, received 1 ml of vehicle (1% gum tragacanth) per kg). Animals in groups 2 and 3 received the oral suspensions of the aqueous and ethanol extracts of *Euphorbia helioscopia* in doses of 30 mg/kg respectively. Under light ether anesthesia, dead space wounds were created by subcutaneous implantation of a sterilized, shallow metallic ring, 2.5 cm x 0.3 cm (known as a cylindrical pith), on each side of the dorsal paravertebral skin surface. Granulation tissue formed on the outside and inside of the pith and was excised on the 10th day post wounding. The dry weight of the granulation tissue and the breaking strength were measured, and the amount of hydroxyproline, which indicates collagen turnover, was estimated using a colorimeter. Histo-pathological examination was used to assess the extent of collagen formation (Udupa *et al*., 1995).

Phytochemical study of *E. helioscopia*

Plant extract was applied to pre coated TLC silica gel plates (Sigma) developed in appropriate solvent systems. Chromatograms were examined before and after spraying under UV and day light to detect the presence of alkaloids, flavonoids, gallic acids egallic acid and flavellagic acid (Wagner and Bladt, 1996).

Table 1. Effect of topical application of the aqueous and ethanol extracts of *euphorbia helioscopia* on epithelialisation (mm^2) in the excision wound repair model.

Group/day	0	3	6	9	12	15	Epithelialisation area (mm^2) at 15 days
Control	50.5 ±0.6	48.2 ±1.4	40.4. ± 1.3	32.5±0.8	17.2 ±0.8	8.1 ±0.3	21.2 ±0.1
Standard drug	50.1 ±1.5	40.7 ±6.0	32.3 ± 1.3	24.3 ±0.4	11.6 ±0.4	0*	11.2 ±0.4
Aqueous Extract	47.6 ±2.3	46.5 ±1.5	34.0 ± 0.5	22.3 ±0.5	10.5 ±0.3	8.8 ±0.7	16.5 ± 0.3
Ethanol Extract	46.8 ±7.2	44.1 ± 1.3	32.8 ± 0.7	15.2 ±1.5	6.3 ±0.6	0*	12.2±0.5
Probability P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Values indicate as mean SEM, n=6 animals in each group. All results are given as percentage of wound contraction p≤ 0.001 as compared to control * = Highly significant

Analysis of Data

Data obtained were subjected to statistical analysis using ANOVA; values of p< 0.001were considered as statistically significant.

RESULTS AND DISCUSSION

Excision wound model

Significant promotion of wound-healing activity was observed with both the aqueous and ethanol extracts in all three wound repair models. In the excision wound repair model (Table 1), the animals treated with the ethanol extract showed faster epithelialisation (12.2 ± 0.5 mm ^{2}) than those treated with the aqueous extract (16.5 ± 0.3 mm^2). The positive control (1% w/w nitrofurazone gel) produced an epithelialisation area of 11.2 ± 0.4 mm^2.

Incision wound model

In the incision wound repair model (Table 2), the animals treated with both the ethanol and aqueous extracts showed an increase in breaking strength (383.7 ± 3.6 g), (348.1 ± 3.2 g) respectively, when compared to the control (235.4 ± 2.8 g).

Dead space wound model

The mean breaking strength of the animals treated with the positive control was also significant. In the dead space wound model, the ethanol extract treated animals showed a more significant increase in dry weight of granulation tissue (168.5 ± 0.6 mg/kg) compared to the aqueous extract and control group (Table 2).

Histological studies

Estimation of hydroxyproline in the granulation tissue (Table 2) revealed that the animal groups treated with the ethanol extract had the highest content (2158.0 ± 0.7 mg/100g), followed by the aqueous extract treated group (1985.4 ± 0.6 mg/100g). The control group showed a significantly lower hydroxyproline content (1415.6 ± 1.5 mg/100g).

Phytochemical analysis

Table 3 shows the results of the TLC screening of ethanol extract of *E. helioscopia*

Wounding healing is a fundamental physiological response to tissue injury that results in the restoration of integrity by the synthesis of a connective tissue matrix (Suh *et al*., 1998 and Leite *et al*., 2002) Collagen is the major protein of the extracellular matrix and ultimately contributes to wound strength. In the present study, the increase in the hydroxyproline content of the granulation tissue, the increase in tensile strength together with the enhancement of collagen maturation shown by increased cross linking, and the increase in dry granulation weight, shows that the extracts of *Euphorbia helioscopia* are promoting wound healing (Martin, 1996 and Phillips *et al*., 1991).

Histological studies of the granulation tissue of the control group of animals showed more aggregation of macrophages with fewer collagen fibres than the treated groups. In the case of the group treated with the aqueous extract, moderate collagen deposition, macrophages and fibroblasts were noticed, whereas the group treated with

Table 2. Effects of the aqueous and ethanol extracts of *Euphorbia helioscopia* on wound breaking strength in the incision model, and granulation in the dead space model.

Group	Weight of granulation tissue (mg/kg)	Breaking strength (g)	Hydroxyproline (mg/100g)
Control (Vehicle)	87.6 ± 0.7	235.4 ± 2.8	1415.6 ± 1.5
Aqueous extract	146.5 ± 0.8	348.1 ± 3.2	1985.4 ±0.6
Ethanol extract	168.5 ±0.6	383.7 ± 3.6	2158.0 ± 0.7
Probability P	<0.001	<0.001	<0.001

Values are expressed as mean, SEM, n=6 in each group, p≤ 0.001 compared to control.

Table 3. Analysis of *Euphorbia helioscopia* with thin layer chromatography

Samples	Alkaloids	Flavonoids	Gallic acid	Egallic acid	Flavellagic acid
Euphorbia helioscopia	–	++	++	++	+

Analysis on the basis of 10g dried weight of sample
(-) not detected; (+) positive; (++) strongly positive reaction

the ethanol extract evidenced significant increase in collagen deposition showing lesser macrophages and fibroblasts (Mahara and Sushma, 2003).

These extracts probably due to presence of effective phytochemicals (Table 3) improved the quality of the wound healing process, reduced the scar formation and increased the rate of epithelialisation. Moreover, Phytochemicals, like flavonoids, tannins and their other allied compounds promote wound healing due to their antioxidants and antimicrobial activities (Ahmad *et al*., 1998 and Bairy, 2002). Many phytochemical and biological studies have been carried out in this area but *Euphorbia helioscopia* has been ignored. Commonly it has been applied in traditional medicine as fresh or dried directly on the wound for healing purposes. To imitate the traditional use of this herb in the present study the dried samples were extracted in ethanol and aqueous media and assessed for its wound healing activity. There are no published data available neither for phytochemical composition nor for the biological activity of *Euphorbia helioscopia*

We conclude that ethanol and aqueous extracts of *Euphorbia helioscopia* have promoted the wound healing process as compared to standards. The findings confirm the therapeutic value of *Euphorbia helioscopia* in the traditional system of medicine. However, more scientific studies are recommended to highlight uses of this valuable herb.

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