Ahmed et al., Afr J Tradit Complement Altern Med. (2016) 13(5):145-152

doi:10.21010/ajtcam.v13i5.19

STUDIES ON WOUND HEALING ACTIVITY OF SOME EUPHORBIA SPECIES ON EXPERIMENTAL RATS

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Abstract

Background: Plants of Euphorbiaceae are used in folkloric medicines in variety of ailments and well known for chemical diversity of their isoprenoid constituents. This study was carried out to explore the preliminary wound healing potential of four Euphorbia species (E. consobrina 1, E. consobrina 2, E. inarticulata, E. balsamifera and E. schimperi).

Materials and Methods: Excision wound surface of the animals were topically treated with ethyl acetate and methanol extracts of plants at a dose of 400 mg/kg body weight for twenty days. Povidone-iodine ointment was used as a reference drug. Wound contraction measurement and period of epithelialization were used to assess the effect of plants extracts on wound repairing.

Results: The groups treated with methanol extracts of E. balsamifera and E. schimperi showed profound effects, high rate of wound contraction (100%) and decrease in epithelization period 19.00±0.40 and 18.50±0.64 respectively, followed by methanol extracts of E. consobrina 2, ethyl acetate extract of E. inarticulata and ethyl acetate extracts of E. consobrina 2 which showed significant (P<0.001) wound contraction and decrease in epithelization period. Conversely ethyl acetate extract of E. consobrina 1, E. balsamifera and E. schimperi and methanol extract of E. Consobrina 1 and E. Inarticulata treated groups was not showing significant wound healing. Methanol extracts of E. balsamifera and E. schimperi were also tested for their safety margin and found safe up to dose of 2000mg/kg body weight.

Conclusion: Topical application of methanol extracts of E. balsamifera and E. schimperi have potential wound healing activity which is identical with standard drug Povidone-iodine.

Key words: Wound healing, excision wounds, Euphorbia, extracts.

Introduction

Wound healing is a process of tissues restoration and the re-establishment of the damaged skin and tissues (Dash and Murthy, 2011). It comprises a systematic movement of actions i.e. inflammation, angiogenesis, proliferation and synthesis of collagen for final healing (Hemamalinet al., 2011). Wound contraction involving movement of fibroblast to the injured tissue followed by shrinkage of the wound zone play an important part in wound healing procedure (Iodigweet al., 2012). However, wound healing is a natural procedure and have the capacity to heal on its own, to restore the integrity and to avoid severe damage to the body; rapid wound healing is required (Thringnet al., 2009).

Many research data revealed that plants may worked as healing and restoration of the injured tissue by various mechanisms (Das, 2013). There are many reports available which states that plant extractives have been used for the treatment and management of wounds not because they are easily accessible and inexpensive but also considered safe as hypersensitivity reactions are rarely come across with the use of these naturally occurring agents (Stephen et al., 2010; Pirbaloutiet al., 2010; Subhashini and Arunachalam, 2011; Dewanganet al., 2012; Rajinder et al., 2008).

The genus Euphorbia is the largest in the plant family Euphorbiaceae, consisting about 2000 known species (Jassbi, 2006) and plants of the family is well acknowledged for the chemical diversity of their isoprenoid constituents (Shi et al., 2008). The latex of these plants contains numerous natural compounds, some of which are of therapeutic importance or of commercial use. The latex usually protects these plants from browsing animals because of its unpleasantness or toxic nature (AL-Sultan and Hussein, 2006). Euphorbia species are reported to have triterpene alcohols in their latex used as chemotaxonomic markers (Giner and Schroeder 2015). In addition, the genus is reported to have cerebrosides, phloracetophenones, glycerols, sesquiterpenoids, steroids, and flavonoids (Shi et al., 2008).

Some species of the genus Euphorbia are used in folkloric medicines to cure skin disorders, gonorrea, intestinal parasites, migraine and wart (Singla and Pathak, 1990). Few Euphorbia species have also been reported for wound healing potential (Goyal et al., 2012; Pattanaik et al., 2014; Bigoniya et al., 2013).

In this paper, we are reporting the preliminary wound healing potential of four Euphorbia species (Euphorbia consobrina N.E.Br., Euphorbia inarticulata Schweinf, Euphorbia balsamifera Aiton and Euphorbia schimperi C. Presl) growing in Saudi Arabia.
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Materials and Methods

Collection of Plant

Four Euphorbia species (E. consorbina 1, E. consorbina 2, E. inarticulata, E. balsamifera and E. schimperi) were collected from different parts of Saudi Arabia (Table 1) and were authenticated by Dr. M. Yusuf (taxonomist) and voucher specimen of each plant was logged in herbarium of College of Pharmacy, King Saud University (KSU), Riyadh, Saudi Arabia.

Chemicals

Solvents used for the extraction were ethyl acetate and methanol obtained from Sigma-Aldrich, USA. Prior to use, solvents were distilled in laboratory. Povidone-Iodine U.S.P. 5% w/w was purchased from local pharmacy and used as a reference drug.

Extraction Procedure

The shade dried aerial parts of the plants were coarsely grounded with blender. The 100 g grounded material of each were extracted in ethyl acetate (300 mL × 3) followed by methanol (300 mL × 3) at room temperature for 72 h (24 h × 3). The extracts were filtered through Whatman no.1 filter paper and solvent was evaporated to dryness at 40 °C in vacuo using Buchi Rota vapour yielded syrupy mass. The percent yields of extracts were depicted in Table 1.

Animals

Wistar albino rats of either sex and of approximately similar age, weighing roughly 180-200 g b.w. and Swiss albino mice (25-30 g b.w.) were obtained from experimental animal house, College of Pharmacy, KSU, Riyadh and were used in these experiments. The experimental animals were housed under standard laboratory conditions, temperature (22 ± 2°C), humidity (55%) and light-dark environment (12/12 hrs light/dark). The animals were fed with Purina chow and free access to drinking water ad libitum. The rats were anesthetized prior to and throughout the infliction of experimental wounds. The surgical procedure was carried out under sterile condition using ketamine anesthesia (120 mg/kg b.w.) (Nayak et al., 2010). All study animals were keenly observed for any infection, if they showed sign of infection, they were excluded from the study and substituted. The excision wound model was used to assess the wound healing capability of plant extracts. All the experimental procedure exercised was in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, Institute for laboratory animals (NIH publication 8th edition, 2011). The study was approved by Ethical Committee College of Pharmacy (No. EACP7315), KSU, Riyadh, Saudi Arabia.

Excision Wound Creation

The rats were anesthetized and inflicted with excision wounds as designed previously (Morton and Malone, 1972). In brief, the dorsal skin fur of the rats was shaved with an electric shaver, and the zone of the wound to be created was defined on the back of the animals with methylene blue using a circular stainless steel stencil. A full thickness of the excision wound of circular area of 254 mm² and 2 mm depth was created along the markings with a surgical blade and pointed scissor.

Grouping of the Animals

The wounded animals were divided into twelve groups of four animals in each group. Group 1 animals were left untreated (control group). Group 2 served as reference standard and treated topically with Povidone-Iodine (5% w/w), group 3 to 7 were treated topically with ethyl acetate extracts of E. consorbina1, E.consorbina2, E. Inarticulata, E. balsamifera and E. schimperi, respectively, at a dose of 400mg/kg b.w. and group 8 to 12 were treated topically with methanol extracts of E. consorbina1, E. consorbina2, E. Inarticulata, E. balsamifera and E. schimperi, respectively, at same dose.

The extracts and standard drugs were topically applied once a day, starting from the day of wound creation, till complete epithelialization. The animals were housed individually in cages. The wounds were traced on mm² graph paper on the day of 4, 8, 12, 16 and 20 post wounding days. The percentage of wound closure (% contraction), and period of epithelialization were calculated.

Acute Toxicity

The acute toxicity test was performed on the mice using oral route. Methanol extract of E. schimperi and E. balsamifera dissolved in distilled water and administered at dose up to 2000 mg/kg b.w., to different groups of mice (each group contains six animals). The animals were observed for 24 hrs for any behavioral change, symptoms of toxicity and mortality. The animals did not show any noticeable sign of acute toxicity.

Statistical Analysis

Values are given as arithmetic means ± standard error of the mean (S.E.M.). Data was statistically analyzed by using one-way analysis of variance (ANOVA) followed by Student’s t-test. The data were considered significant at P < 0.001.
Results

The yield of ethyl acetate and methanol extracts is depicted in Table 1 and the wound healing potential of the ethyl acetate and methanol extract used in the study is summarized in Table 2, 3 and in Figure 1. In excision wound model the parameters studied were percentage wound closure and mean epithelialization time. It was observed that the wound contracting ability of the extracts (E. consorbina 2 ethyl acetate extracts, E. consorbina 2 methanol extract, E. inarticulate ethyl acetate extract, E. balsamifera methanol extract and E. schimperi methanol extract) treated groups showed significant wound healing from the fourth day onwards by showing a decrease in the epithelialization period and increased percentage of wound contraction, when compared with standard drug treated group of animals. The average of percent closure of wound region was measured on the 0, 4, 8, 12, 16, and 20 days. The results of the study indicated that wound healing process in the standard drug treated group and group 11 and 12 (E. balsamifera methanol extract and E. schimperi methanol extract) proceeded almost identically by showing 100 % wound contraction and minimum period of epithelialization on the last day of experiment. On day 4, the extract treated animals, group 11 and 12, showed wound contraction by 34.36 % and 28.78 % respectively which was almost double as compared to that of the standard drug treated animals on the same day, on the day 8 extracts treated animals, groups 11 and 12, wound contraction became almost double 66.55 and 61.25% respectively which was also almost double to that of standard drug on the same day. After that on days 12, 16 and 20 wound contraction gradually increased and was same as that of standard drug treated animals on the 20th day. While the extracts (E. consorbina 2 ethyl acetate extract, E. consorbina 2 methanol extract, E. inarticulate ethyl acetate extract) found to possess significant wound-healing property over other extracts by showing 99.12, 99.53 and 99.29% wound contraction respectively and minimum epithelialization time which was comparable to that of group of animals treated with standard drugs.

On the other hand data obtained from E. consorbina 1 ethyl acetate extract, E. balsamifera ethyl acetate extract and ethyl acetate extract of E. schimperi treated groups was not appreciable and that of E. Consorbina 1 methanol extract and E. Inarticulata methanol extract treated groups was poor and hence not significant compared to that of standard reference. Since the wound healing effects of methanol extract of E.schimperi and E. balsamifera was found most promising (100 % wound contraction) prompted us to investigate the safety margin/toxicity of these two extracts. In the acute toxicity assay, no deaths were observed during the tested dose of up to 2000 mg/kg b.w. of methanol extract of E. schimperi and E. balsamifera.

Table 1: Yield of ethyl acetate and methanol extracts.

| Plant            | Voucher No. | Area of collection | Wt. of dry plant (g) | Ethyl acetate extract | Methanol extract |
|------------------|-------------|--------------------|----------------------|-----------------------|------------------|
| Euphorbia consorbina 1 | 16386       | Wadi Gama          | 100                  | 10.3272               | 6.5243           |
| Euphorbia consorbina 2 | 16387       | Wargan mountains   | 100                  | 8.2792                | 4.5              |
| Euphorbia inarticulate | 16284       | Way to JabalShada  | 100                  | 4.5848                | 2.7343           |
| Euphorbia balsamifera | 16046       | On the road Alkhamees to Najran | 100 | 8.9099  | 5.4903 |
| Euphorbia schimperi | 16322       | Wadi Gama          | 100                  | 4.8525                | 7.04             |
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doi:10.21010/ajtcam.v13i5.19

Table 2: Effect of extracts on excision wound healing potential in rats (wound area mm\(^2\)).
Data are mean of 4 male in each group ± SD; *P<0.05, **P<0.01, ***P<0.001

Student's t-test

| GROUPS | Dose mg/kg | 0 Days | 4th Day | 8th Day | 12th Day | 16th Day | 20th Day | Period of epithelization(days) |
|--------|------------|--------|---------|---------|----------|----------|---------|-----------------------------|
| 1      |            | 326.65±6.79 | 309.8±7.63 | 274.6±10.85** | 166.17±6.99*** | 113.25±10.0*** | 73.47±5.94*** | 23.50±0.64 |
| 2      | Topical    | 331.85±4.09 | 281.95±4.09*** | 235.47±5.45*** | 116.87±5.93*** | 9.95±1.13*** | - | 17.00±0.40*** |
| 3      | 400        | 309.07±7.30 | 270.12±18.00** | 241.57±9.88*** | 142.42±8.73*** | 41.67±3.69*** | 6.27±1.99*** | 22.75±0.62 |
| 4      | 400        | 318.32±4.93 | 298.10±6.78 | 226.82±8.55*** | 116.57±2.31*** | 21.42±3.41*** | 2.80±0.54*** | 20.00±0.91* |
| 5      | 400        | 317.02±8.43 | 282.42±10.68* | 244.12±17.17** | 178.80±13.65*** | 13.95±1.81*** | 2.25±0.64*** | 21.00±0.91 |
| 6      | 400        | 321.25±5.44 | 273.92±3.26*** | 255.90±4.60*** | 193.65±4.71*** | 42.30±2.94*** | 10.57±0.75*** | 22.00±0.40 |
| 7      | 400        | 317.15±10.71 | 253.55±14.91** | 174.42±15.54*** | 108.67±5.17 | 65.30±3.54*** | 7.6±1.15*** | 21.75±0.85 |
| 8      | 400        | 318.57±4.43 | 275.95±9.94** | 215.47±10.31*** | 167.95±9.86*** | 109.35±5.28*** | 57.67±9.19*** | 23.00±0.91 |
| 9      | 400        | 321.30±6.74 | 231.25±5.42*** | 231.25±11.04*** | 89.87±5.51*** | 51.92±5.77*** | 1.50±0.22*** | 20.75±0.75* |
| 10     | 400        | 311.47±3.72 | 274.62±4.65*** | 238.35±13.86*** | 197.17±5.41*** | 158.72±9.30*** | 78.75±4.98*** | 23.25±0.85 |
| 11     | 400        | 314.82±3.99 | 206.62±3.96*** | 105.30±3.51*** | 63.95±7.28** | 24.80±3.34*** | - | 19.00±0.40*** |
| 12     | 400        | 310.25±8.12 | 221.00±15.31*** | 120.25±11.31*** | 65.92±2.56*** | 34.62±2.50*** | - | 18.50±0.64*** |

Group 7: Animals treated with ethyl acetate extract of E. schimperi
Group 8: Animals treated with methanol extract of E. consorbina 1
Group 9: Animals treated with methanol extract of E. consorbina 2
Group 10: Animals treated with methanol extract of E. inarticulata
Group 11: Animals treated with methanol extract of E. balsamifera
Group 12: Animals treated with methanol extract of E. schimperi
Table 3: Effect of extracts on percent wound contraction in excision wound in rats.

| GROUPS | Dose mg/kg | 0 Days | 4th | 8th | 12th | 16th | 20th |
|--------|------------|--------|-----|-----|------|------|------|
| 1      | 00         | 5.15   | 15.93 | 49.12 | 65.33 | 73.47 |
| 2      | Topical    | 00     | 15.03 | 29.04 | 64.78 | 97.00 | 100  |
| 3      | 400        | 00     | 12.60 | 21.83 | 53.91 | 86.51 | 97.96 |
| 4      | 400        | 00     | 6.35  | 28.74 | 63.37 | 92.26 | 99.12 |
| 5      | 400        | 00     | 10.91 | 22.99 | 43.60 | 95.59 | 99.29 |
| 6      | 400        | 00     | 14.72 | 20.33 | 39.71 | 86.83 | 96.70 |
| 7      | 400        | 00     | 20.05 | 45.00 | 65.73 | 79.41 | 97.60 |
| 8      | 400        | 00     | 13.37 | 32.36 | 47.28 | 65.67 | 81.89 |
| 9      | 400        | 00     | 28.02 | 55.15 | 72.02 | 83.83 | 99.53 |
| 10     | 400        | 00     | 11.81 | 23.47 | 36.69 | 49.04 | 74.71 |
| 11     | 400        | 00     | 34.36 | 66.55 | 79.68 | 92.12 | 100  |
| 12     | 400        | 00     | 28.78 | 61.25 | 78.75 | 88.84 | 100  |

Group 1 Untreated animals
Group 2 Animals treated with povidone iodine
Group 3 Animals treated with ethyl acetate extract of *E. consorbina* 1
Group 4 Animals treated with ethyl acetate extract of *E. consorbina* 2
Group 5 Animals treated with ethyl acetate extract of *E. inarticulata*
Group 6 Animals treated with ethyl acetate extract of *E. balsamifera*
Group 7 Animals treated with ethyl acetate extract of *E. schimperi*
Group 8 Animals treated with methanol extract of *E. consorbina* 1
Group 9 Animals treated with methanol extract of *E. consorbina* 2
Group 10 Animals treated with methanol extract of *E. inarticulata*
Group 11 Animals treated with methanol extract of *E. balsamifera*
Group 12 Animals treated with methanol extract of *E. schimperi*
Figure 1: Effect of topical application of extracts on 8th, 16th, and 20th day on wound healing.

- **Group 1:** Untreated animals
- **Group 2:** Animals treated with povidone iodine
- **Group 3:** Animals treated with ethyl acetate extract of *E. consorbina* 1
- **Group 4:** Animals treated with ethyl acetate extract of *E. consorbina* 2
- **Group 5:** Animals treated with ethyl acetate extract of *E. inarticulate*
- **Group 6:** Animals treated with ethyl acetate extract of *E. balsamifera*
- **Group 7:** Animals treated with ethyl acetate extract of *E. schimperi*
- **Group 8:** Animals treated with methanol extract of *E. consorbina* 1
- **Group 9:** Animals treated with methanol extract of *E. consorbina* 2
- **Group 10:** Animals treated with methanol extract of *E. inarticulata*
- **Group 11:** Animals treated with methanol extract of *E. balsamifera*
- **Group 12:** Animals treated with methanol extract of *E. schimperi*
The present work was carried out to investigate the wound healing efficacy of extracts of four Euphorbia species (E. consorbina 1, E. consorbina 2, E. inarticulata, E. balsamifera and E. schimperi) on experimentally induced wounds in experimental rats. The utmost symptoms associated with wounds are discharge of blood, redness and pain full swelling around the wound, puss and water discharge accumulation beneath the skin or drainage from the wounds and obscene odor from the wounds (Phillips et al., 1991). Wound repairing is a procedure by which injured tissues restores its texture and shape to its normal conditions, involving contraction of area of wounds and mainly relies on the nature and degree of injury, capability of tissues to repair and general state of health (Pirbalouti et al., 2010).

Although pharmaceutical industries have developed tremendously but still, the availability of substances with capability to evoke the wound healing process is restricted (Udupa et al., 1995). Furthermore, treatment of chronic wounds is also a big difficulty owing to expensive treatment and of many adverse effects (Porras-Reyeset et al., 1993; Suh et al., 1998).

Any substance which accelerates healing processes is referred to as wound healing promoter. The use of remedial cock-tails obtained from medicinal plants for the treatment injured skin particularly, wounds and burns had a very long practice. The wound healing potential of numerous plants has been studied in many laboratory animal models to disclose the presence of most prominent chemical entities (Rashed et al., 2003).

Results of this work suggested that topical application of ethyl acetate and methanol extracts of Euphorbia species on experimentally induced excision wounds showed enhanced rate of wound shrinkage and healing. The animals treated with E. consorbina 2 ethyl acetate extract, E. consorbina 2 methanol extract, E. inarticulata ethyl acetate extract, E. balsamifera methanol extract and E. schimperi methanol extract showed significant results comparable to standard drug treated groups and control. The treated wounds after 4 days itself display noticeable dryness of wound margins with tissue restoration.

The results obtained are similar to the wound healing potential of latex of Euphorbia caducifolia which showed an acceleration of wound closure with higher fibroblasts and collagen content in treated animals (Goyal et al., 2012). Same type of wound healing effect is also observed in Euphorbia nerifolia (Pattanaik et al., 2014).

The results are also in agreement with the effect of Aloe vera on collagen content and its characteristics in healing of dermal wound. It has been experimentally observed that Aloe vera enhanced the collagen content of the skin which eventually contributed to wound strength (Chithra et al., 1998). Similar types of effects have been observed with the alcoholic extract of Centella asiatica on wound repair by improving the tensile strength and increasing the rate of wound repairing process. (Suguna et al., 1996). Previous studies on Artemisia herba-alba, Anchusastrigosa, Punicagranatum, Nigella sativa, and Trigonellafoenum-graecum have suggested a strong relationship between the collagen fiber formation and increase rate of wound healing process (Rashed et al., 2003). Thus the results obtained from this study suggested that the topical administration of extracts (E. consorbina 2 ethyl acetate extract, E. consorbina2 methanol extract, E. inarticulata ethyl acetate extract, E. balsamifera methanol extract and E. schimperi methanol extract) have significant therapeutic effects on the various phases involving in the wound contraction and healing process. Phytochemicals like alkaloids, triterpenoids, tannins and flavonoids are known to encourage the process of wound healing most and increase rate of wound healing process (Rashed et al., 2003). Thus the results obtained from this study suggested that the topical administration of extracts of four Euphorbia species (E. consorbina 1, E. consorbina 2, E. inarticulata, E. balsamifera and E. schimperi) on experimentally induced wounds in experimental rats. The utmost symptoms associated with wounds are discharge of blood, redness and pain full swelling around the wound, puss and water discharge accumulation beneath the skin or drainage from the wounds and obscene odor from the wounds (Phillips et al., 1991).

In conclusion, the current study revealed that wounds dressed with methanol extract of E. balsamifera and E. schimperi was topical application significantly accelerate the rate of wound healing process and showed 100% wound contraction. Conversely, E. consorbina 2 ethyl acetate extracts, E. consorbina 2 methanol extract and E. inarticulate ethyl acetate extract showed more than 99.0% wound contraction. There is a need for further studies on these active extracts in order to isolate the phytochemical entities responsible to increase the wound healing process and to expose the mechanisms of actions.

Acknowledgements

The authors are grateful to acknowledge the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. (RGP-073). The assistance of Mr. Malik Sawood Ahmed is also thankfully acknowledged.

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