Available Online through
www.ijpbs.com (or) www.ijpbsonline.com

IJPBS |Volume 3| Issue 4 |OCT-DEC|2013|212-215

Research Article
Pharmaceutical Sciences

EFFECT OF *ALKANNA FRIGID* EXTRACTS ON 3T3 FIBROBLAST CELL PROLIFERATION

Alireza Yazdinezhad[*1], Hamidreza Monsef-Esfahani[2], Mohammad Hussein Ghahremani[3]*

[1] Department of Pharmacognosy, Faculty of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.
[2] Department of Pharmacognosy, Faculty of Pharmacy, Medical Sciences/ University of Tehran, Tehran, Iran
[3] Pharmacology Research Laboratory, Department of Toxicology-Pharmacology, Faculty of Pharmacy, Medical Sciences/ University of Tehran, Tehran, Iran

*Corresponding Author Email: yazd1387@gmail.com

ABSTRACT

Fibroblasts are the major cell types found in the granulation of wound tissue and play a key role in wound healing. In this study we examined the effects of the sequential root extracts of Alkanna frigida on the stimulation of the growth of fibroblasts as an aspect of promotion of wound healing by an in vitro study on Embryonic Swiss Mouse Fibroblast (3T3) Cells. Results showed that all the tested extracts were able to increase proliferation of 3t3 cells. N-hexan and chloroform extracts had the most effect on fibroblast proliferation.

KEY WORDS

Alkanna frigid, fibroblast, 3T3, proliferation

INTRODUCTION

Wound healing is a complex, sequential cascade of biological process involving a variety of different cells, proteins, chemo attractants, proteases and growth factors. It follows a closely regulated process including the activation and proliferation of fibroblastic cells [1].

Fibroblasts are the main cell types involved in the regulation of angiogenesis that occurs during wound healing process [2, 3]. A specialized fibroblast type, involved in wound contraction, is the myofibroblast. These cells play an important role in wound contraction, whereby the wound size is reduced to limit water vapor loss and the entrance of foreign material, as well as reduce the amount of extracellular tissue that needs to be synthesized to fill the wound [4, 7]. It has been demonstrated that increasing the number of fibroblasts in an artificial dermal substitute leads to improved healing in experimental wounds [8].

Alkanna frigida Boiss, a shrub which grows wild in high and frigid mountains in the northern and western areas of Iran, is one of the five species of the genus Alkanna (Boraginaceae) which are indigenous to Iran [9,11]. In the rural parts of Tabriz (Azerbaijan Sharghi Province, Iran), its roots are considered useful in wound healing and burns.

The roots of *Alkanna tinctoria*, European species of Alkanna, have been well-known for wound healing effects [12, 13]. In addition, its root extract shows several pharmacological actions such as anti-inflammatory, antibacterial, antifungal, radical scavenging [14, 15] and antioxidant [16] effects which can accelerate the wound healing process.

The aim of the present study was to assay the effects of the sequential root extracts of A. frigida on the stimulation of the growth of fibroblasts as an aspect of promotion of wound healing by an in vitro study on Embryonic Swiss Mouse Fibroblast (3T3) Cells.

MATERIAL AND METHODS

Plant materials

A. frigida (root parts) was collected in July 2011 from the north of Iran, Mazandaran province, Road to Azadbar, ca. 2800m and identified by Dr. A.

Yazdinezhad. Voucher specimen was deposited at The Faculty of Pharmacy Herbarium (ZUMS), Zanjan University of Medical Sciences, Zanjan, Iran (accession No.1027)

Total extraction

Root parts of the plant were dried, powdered (100g) and macerated with an 80% methanol solution for 3 days with three changes of solution. The resulting extract was filtered and evaporated under vacuum into a dried powder extract (12.3g, 12.3%).

Sequential extracts

Different extracts were sequentially prepared using 500g dried and powdered root parts of the plant with increasingly polar solvents: n-hexane (5.7g dry weight corresponding to 1.1 %), chloroform (3.7g dry weight corresponding to 0.7 %), ethyl acetate (2.1g dry weight corresponding to 0.4 %) and 80% methanol solution (1.2g dry weight corresponding to 0.2 %).

Cell culture

3t3 cells obtained from National Cell Bank of Iran (NCBI), Pasteur Institute of Iran, Tehran, Iran, were kept at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium consisted of DMEM (PAN-Austria), supplemented with 10% of fetal calf serum (FCS; Gibco, USA), 100U/ml penicillin (Gibco, USA) and 100µg/ml streptomycin (Gibco, USA).

Cell proliferation-MTT assay

3t3 cells were seeded in 96-well sterile microtitre plates at a density of 1×10^4 cells/well containing 200µl of the growth medium. After 24h, the culture medium was removed and the cells were exposed to serial dilutions (0.05, 0.1, 0.5, 1.0, 5.0, 10 and 50 µg/ml of each extract in a fresh medium supplement with 10%FCS).

Each group was arranged in three duplicates and untreated cells were used as controls. After 72 h, the 3-(4, 5 dimethyl thiazol-2yl)-2, 5-diphenyltetrazoliumbromide (MTT) method was carried out to evaluate cell proliferation. MTT was dissolved in PBS (phosphate buffered saline) at a concentration of 5.0 mg/ml. 50µl of MTT solution was added to each well of the culture plate and incubated at 37°C for 4h. Supernatant was then removed carefully without disturbing the dark blue from azon crystals. DMSO 100µl was added to each well and mixed thoroughly to ensure that all crystals were dissolved. The optic-metric density (OD) was read on ELISA microplate reader at test wavelength of 570 nm and referent wavelength of 630nm.

Statistical analysis

Results were presented as the mean value and standard error of the mean. Statistical analysis was performed using the ANOVA test. Significance was accepted at $p<0.05$.

RESULTS

The present study evaluated the 3t3 cells growth stimulation effect of different extracts of root parts of A. frigida in vitro at doses of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 and 50.0 µg/ml using MTT assay.

Results are shown in **Fig. 1** and are given as sample absorbance/control absorbance versus concentrations (µg/ml). Those with sample absorbance/control absorbance values higher than 1 were accepted to have growth stimulant activity.

The results showed that n-hexan and chloroform extracts increased proliferation of 3t3 cells at concentrations of 0.5 and 0.1µg/ml (**Fig.1**). Et/OAc and total extracts showed in vitro 3t3 cells stimulatory effect at 0.1µg/ml (**Fig.1**). The 80% Methanol extract had no obvious stimulatory effect (**Fig.1**).

All five extracts had cytotoxicity effect at concentrations more than 5µg/ml. Et/OAc and total extracts showed cytotoxicity at 1µg/ml concentration. 80% Methanol extract had the least cytotoxicity at 50 and 10 µg/ml concentrations (**Fig.1**).

DISCUSSION

Fibroblasts are the major cell type found in the granulation of wound tissue and play a key role in

wound healing [3, 17 and 18]. Fibroblasts are responsible for the secretion of a series of growth factors, i.e. VEGF, interleukin and TGF-β to promote wound healing events including angiogenesis, cell proliferation and matrix deposition [19].

There are a lot of studies on wound healing effect of root extracts of some Boraginaceae species.Papageorgiou has studied the wound healing effect of an n-hexane extract of *Alkanna tinctoria* roots in a clinical study on 72 patients with ulcus cruis and has reported that the fraction containing esteric pigments showed excellent wound healing properties [12]. The ether extract of Boraginaceae species, *Lithospermum erythrorhizon* roots and Macrotomia euchroma roots have an accelerative effect on proliferation of granulation tissue in rats [14].

Furthermore, Lithospermum erythrorhizon root extract is useful for wound healing in diabetic mice [20].Ogurtan et al. have reported that an olive oil extract of *Alkanna tinctoria* showed wound healing effect on partial thickness and olive oil burn wounds in rabbits [13].

Senel *et al*. showed that antioxidant compounds improve healing in ischemic skin wounds [21]. Radical scavengering and antioxidant activities of some Alkanna species suggest that *Alkanna frigida* root extracts may also possess antioxidant activity which can enhance wound healing [15, 16, and 22].

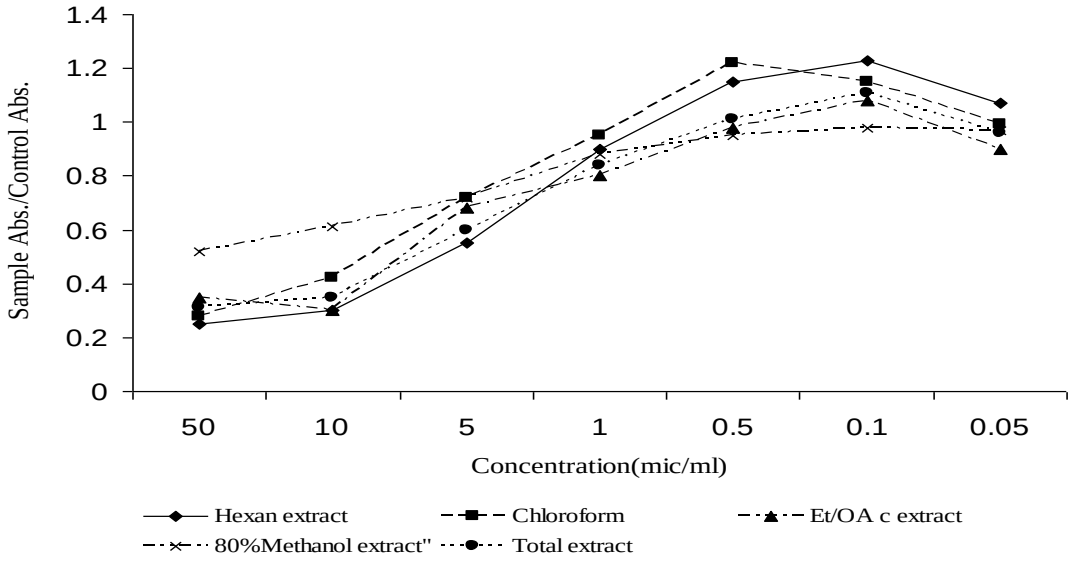

Figure 1. Effect of n-hexane, Chloroform, Ethyl acetate, 80% Methanol solution and total root extracts of A. frigida on the growth of Embryonic Swiss Mouse Fibroblast Cells using MTT assay ($p<0.05$).

CONCLUSION

The observed results of this study suggest that the accelerative effect induced by the total extract might be due to an additive effect of n-hexan and chloroform root extracts compounds of *Alkanna frigida*. The conclusions support scientific basis for the utilization of the plant in Iranian folk medicine for the treatment of a variety of wound conditions. Discussion, speculation and detailed interpretation of data should not be included in the results but should be put into the discussion section. The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

REFERENCES

[1] Mohd J., Shah Y., Omar E., Pai D., Sood S., Cellular events and biomarkers of wound healing. Indian J of Plastic Surgery. 2012;45(2), 220–228

[2] Kon K., Fujiwara T., Transformation of fibroblasts into endothelial cells during angiogenesis, Cell Tissue Res. 1994; 278:625-628,

[3] Gu S., DiPietro LA., Factors Affecting Wound Healing. J of dent research, 2010; 89 (3) 219-229.
[4] Saunders KB., D'Amore PA., An *in vitro* model for cell-cell interactions. *In Vitro* Cell Dev. Biol. 1992; 28:521-528
[5] Clark RAF. The molecular and cellular boiolgy of Wound Repair. New York: Plenium press; 1996.
[6] Schultz GS, Wysocki A. Interactions between extracellular matrix and growth factors in wound healing. Wound Repair and Regeneration. 2009; 17: 153–162.
[7] Swaim SF, Henderson RA. Wound Dressing Materials and Topical Medication. Lea and Febiger. 1997; 120:37-42.
[8] Lamme EN, Van Leeuwen RT, Brandsma K, Van Marie J, Middelkoop E. Higher numbers of autologous fibroblasts in an artificial dermal substitute improve tissue regeneration and modulate scar tissue formation. J Pathol. 2000; 190: 595-603.
[9] Ghahreman A. Plant Systematic, Cormophytes of Iran. vol 3. Tehran: University Press; 1994.
[10] Khatamsaz M. Flora of Iran, No.39: Boraginaceae. Tehran: Research Institute of Forests and Rangelands Press; 2002.
[11] Mozaffarian V. A Dictionary of Iranian Plant Names. Tehran: Farhang Moaser Publication;2008.
[12] Papageorgiou VP. Wound healing properties of naphthaquinone pigments from *Alkanna tinctoria*. Experientia. 1978; 34: 1499-1501.
[13] Ogurtan Z, Hatipoglu F, Ceylan C. The effect of *Alkanna tinctoria* Tausch on burn wound healing in rabbits. Dtsch Tierärztl Wochenschr. 2002; 109: 481-5.
[14] Papageorgiou VP, Assimopoulou AN, Couladouros EA, Hepworth D, Nicolaou KC. The Chemistry and biology of alkannin, shikonin, and related naphthazarin natural products, Angewandte Chemie International. 1999;38:270-300.
[15] Assimopoulou AN, Papageorgiou VP. Radical scavenging activity of *Alkanna tinctoria* root extracts and their main constituents, hydroxynaphthoquinones. Phytother Res. 2005; 19,141-147.
[16] Assimopoulou AN, Papageorgiou, VP. Antioxidant activities of alkannin, shikonin and *Alkanna tinctoria* root extracts in oil substrates. Food Chem. 2004;87,433-8.
[17] Dudnikova GN. Stimulatio of fibroblast proliferation during experimental wound healing. Bull Exp Biol Med. 1979;88:906-908.
[18] Adolphe B, Pointet M, Ronot Y, Wepierre J. Use of fibroblast cell culture for the study of wound healing drugs. Inter J Cosme Sci.1984;61, 55-58.
[19] Mansbridge JN, Liu K, Pinney RE, Patch R, Ratcliffe A, Naughton GK. Growth factors secreted by fibroblasts: role in healing diabetic foot ulcers. Diabetes, Obesity and Metabolism. 2001; 1: 265 – 279.
[20] Fujita N, Sakaguchi I, Kobayashi H, Ikeda N, Kato Y, Minamino M, Ishii M. An extract of the root of Lithospermun erythrorhison accelerates wound healing in diabetic mice. Biol Pharm Bull. 2003;26: 329-35.
[21] Senel O, Cetinkale O, Ozbay G, Ahcioglu F, Bulan R. Oxygen free radicals impair wound healing in ischemic rat skin. Annals of Plastic Surgery. 1997; 39: 516–523.
[22] Kaliora AC, Andrikopoulos NK.. Effect of *Alkanna Albugam* root on LDL oxidation. A comparative study with species of the Lamiaceae family. Phytother Research. 2005;19: 1077-1079.

***Corresponding Author:**
Alireza Yazdinezhad
(PharmD, PhD in Medicinal pharmacognosy)
Assistant Professor,
yazd1387@gmail.com,
Tel: +98-241-4273638; Fax: +98-241-4273639