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Geometric and Histopathologic Assessment of Lavender Extracts (*Lavandula stoechas*) on Healing of Experimental Skin Wounds and its Comparison with Zinc Oxide on Rats

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Abstract: Acceleration in healing of surgical skin wounds, in order to reduction of post-operation consequences is most important. One of the most popular compounds used ever for skin care is lavender oil or *Lavandula stoechas* essential oil. The aim of this study was evaluate the healing effects of *Lavandula stoechas* extract in comparison with zinc oxide ointment on full thickness experimental open surgical skin wound healing in the rats. For this purpose, 75 female Wistar rats were randomly assigned in 5 groups of 15 animals each including: Control, treated by Eucerin as the placebo, treated by zinc oxide topical ointment and two remaining as test groups which received 10 and 20% *Lavandula stoechas* extracts. Under the general anesthesia and analgesia circumstances, a full thickness incisional open wound with 23 mm diameter was made on the skin of the back part of the rats. The healing process was studied macroscopically and microscopically at 0 (the day of surgery) 3, 7, 14, 21 and 28th days of experiment in all of the rats. The greatest degree of wound contraction was considered in *Lavandula stoechas* extract (10%) treated rats while this change was slighter in control group. In microscopic examination, the overall healing process of *Lavandula stoechas* extract (10%) treated rats was significantly better than the other experimental groups ($p<0.05$). The results obtained showed that *Lavandula stoechas* extract (10%) has more effective healing properties on full thickness open skin wounds compared to zinc oxide topical ointment.

Key words: *Lavandula stoechas*, zinc oxide, healing, skin wounds, rats, Iran

INTRODUCTION

In recent years, more public attention to plant originated drugs has been paid, mainly due to side effects of chemicals and more trending of humans to maintaining a healthy condition. Problems in modern drug systems such as high costs, the use of non-renewable resources such as fossil fuels and environmental pollution in the pharmaceutical industries and ultimately the human inability to make some drugs that normally exist in medicinal plants have doubled the importance of this subject (Ahmad *et al*., 2006; Akbarinia *et al*., 2007a, b; Evandri *et al*., 2005; Jones, 2011; Niroumanesh *et al*., 2008; Niyaki *et al*., 2011; Azadbakht, 1999; Duke, 2002; Gordon, 1990; Howard, 1987; Zargari, 1981; Vernon, 1987; Ebadi and Hisoriev, 2011). Materials and drugs derived from natural sources, especially from flowers and whole plants have a long history. In fact, flowers and whole plants are the main sources of many drugs for a large part of the world's population, particularly in developing countries. Despite the rise of pharmaceutical chemistry in the early 20th century, producing many types of pharmaceutical addiction drug molecules extracted from plants began and allowed treatment of incurable or life-threatening diseases. The strategies used by traditional doctors to prevent the progression of diseases or in holding the healthy conditions of patients were varying but the effects of herbal medicines used in the human body were the same. Now hundreds of species of medicinal plants with power and precision are used mostly acting on a specific operating system and are appropriate to treating certain types of diseases within the body (Ahmad *et al*., 2006; Akbarinia *et al*., 2007a, b; Evandri *et al*., 2005; Jones, 2011; Niroumanesh *et al*., 2008; Niyaki *et al*., 2011).

Lavender (*Davendula vera officinals*) grown in most parts of the world as a wild plant, especially in the South of France, the Mediterranean area and in Toronto, Canada. Growing conditions are largely dependent on environmental conditions and different types of soil. Lavender is a perennial plant of low height with narrow long leaves. Lavender (Lavandula) is such a romantic

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flower that every gardener eventually succumbs to the urge to grow it. Undeterred by the fact that it is a native of the Mediterranean and a lover of dry, sunny, rocky habitats and researchers try it anyway hoping it will adapt. After all, England can hardly be considered dry or particularly sunny yet English gardeners are renowned for growing lavender plants (Ahmad *et al*., 2006; Akbarinia *et al*., 2007a, b; Evandri *et al*., 2005; Jones, 2011; Niroumanesh *et al*., 2008; Niyaki *et al*., 2011). Lavender scent is very pleasant. It has a bitter taste and smell and due to its conditioning is used in perfumery. Distillation of lavender essential oil from flowers that come from this plant has yellow or greenish yellow fluid which has a pleasant smell. Lavender essential oil is most commonly and is used in aromatherapy. However, lavender flowers also provide a fragrant aromatic herbal remedy in tea or tincture form which is useful for nausea, motion sickness, flatulence, colic, bloating, gut dysbiosis and irritable bowel syndrome.

Lavender is also well known for its soothing and calming properties. It is useful with other herbs for mild anxiety, depression, tension, restlessness and for insomnia as it helps improve sleep quality. In this study, researchers review some main uses of lavender plant in Iranian traditional medicine and instructions of usage by mainly rural peoples (Azadbakht, 1999; Duke, 2002; Gordon, 1990; Howard, 1987; Zargari, 1981; Vernon, 1987; Ebadi and Hisoriev, 2011).

Numerous herbs have a range of sedative actions, encompassing analgesic, hypnotic, anti-depressant, anxiolytic activities, often combining two or more actions. Western herbalists designate them generically as nervines. Unlike most centrally acting pharmaceutical drugs, nervine herbs are mild and gentle in activity with complex and poorly understood multiple pharmacological effects. Lavender is a wonderful and miraculous plant that makes the body healthy. This plant can affect the effects of burns, insect bites rather than relieves and more affects such as sleep brings, helps digestion and is considered as an antiseptic drug (Azadbakht, 1999; Zargari, 1981). When a part of the body is burned Lavender was used in Iranian tradition medicine. Lavender oil plant performed an important role in the process of skin recovery. Zinc oxide is an inorganic compound with the formula ZnO. It is a white powder that is insoluble in water. Zinc is an essential trace element of which about 2 g is found in the adult human body. At least 200 enzymes in different biological systems are dependent on the presence of the zinc ion. Among these zinc-dependent enzymes, DNA and RNA polymerases are crucial during tissue repair as they affect cell proliferation and protein synthesis. In accordance with the biochemical role of zinc a reduced synthesis of DNA, reduced deposition of granulation tissue, decreased tensile strengths in skin incisions and delayed closure rates in excised wounds in zinc-deficient rats have been demonstrated (Prasad and Oberleas, 1974; Sandstead *et al*., 1970). Zinc supplementation restored to normal the tensile strengths of the incisional and healing rates of the excisional wounds (Sandstead *et al*., 1970). It has been clinically shown that the healing of leg ulcers is delayed in patients with subnormal serum-zinc levels (Haley, 1979). Zinc given as oral and topical zinc sulfate or as topical zinc oxide normalizes impaired healing ability in these patients (Haley, 1979; Stromberg and Agren, 1984; Golden *et al*., 1980). The aim of the present study was to investigate the *in vivo* wound healing activity of lavender in order to elucidate traditional use of this plant from the scientific point of view.

MATERIALS AND METHODS

Animals: In this study, 75 female Wistar rats weighted 200±20 g and aged 8 weeks old were selected. All animals were kept in same situation (temperature 24°C and humanity 70%) and food and water were provided *ad libitum*.

Pre-operation measures: The operation (induction wound in the skin) required general anesthesia, analgesia and muscle relaxation. In term, researchers used of ketamine (10%, 65 mg kg^{-1}) and xylazine (2%, 10 mg kg^{-1}) through IM injection to induction of anesthesia and pre-operation drugs, respectively. To prevention of drugs side effects, liquid therapy with dextrose 5% at the dose of 50-100 mg/kg/day was exerted immediately after induction of anesthesia.

Operation measures: After preparation the dorsal skin of rats (distinct between scapula to ischial tuberosity), a wound in circle shaped with 7 mm in diameter and by biopsy punch were inducted. In this study, rout of wounding was excisional wounding that in way epidermis, dermis, hypoderm and *Panniculus carnosus* completely were removed. After wounding, rats were divided into five groups of fifteen:

- In group 1, control not received any drug
- In group 2, positive control group received zinc oxide 20%
- In group 3, negative control group received eucerin
- In group 4, received low doses (10%) of herbal extract
- In group 5, received high doses (20%) of herbal extract

Samples were fixed in the formalin 10% and sent to pathology laboratory.

Post-operation measures: After biopsy and washing wound area with normal saline, all drugs were administrated as local way by an applicator in the wound area. This administration continued for 21 days.

Sampling: On days 0, 3, 7, 14, 21 and 28 of research, samples as tissue specimens from biopsy areas were collected and sent to pathology laboratory. Sampling was done under anesthesia condition and this anesthesia was induced by ketamine and rampon. Sampling was exerted by scalpel. Samples were fixed into formalin 10%. In lab after processing and staining to H and E method slides were achieved. Slides were investigated by a light microscope.

Statistical analysis: The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), Version 17.0 was used for statistical analysis. All data are presented as mean±SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. $p<0.05$ was considered statistically significant.

RESULTS

Geometric findings: On the 1st day of trial, ulcer size in all five groups showed a significant increase compared to day zero. On the 2nd day, wound size in high dose treatment group was reduced significantly. So that this finding was observed in low dose and zinc oxide groups, control group and eucerin group on days 3-5, respectively. About 7 days until day 7 according to the size of the wound, the highest rate of wound shrinkage was observed in high dose, low dose, zinc oxide, eucerin and control group, respectively. On day 21, maximum and minimum shrinkage was observed in low dose and control groups, respectively (Table 1).

Histopathologic findings: On day 3 in high dose treatment group, pustule covered the wound but still retains its

Table 1: comparison of wound area variation in groups

Groups	Schedule (days)	No.	Mean	SD	SE	Mean square between groups	Mean square within groups	F-value	p-values
Control group	0	5	72.220	0.000	0.000	6719.1	0.416	16136.69	0.000
	3	5	100.680	0.221	0.099	-	-	-	-
	7	5	62.880	0.905	0.404	-	-	-	-
	14	5	30.750	1.115	0.498	-	-	-	-
	21	5	12.570	0.437	0.195	-	-	-	-
	28	5	8.210	0.439	0.196	-	-	-	-
	Total	30	47.890	34.040	6.215	-	-	-	-
Zinc oxide group	0	5	72.220	0.000	0.000	7505.93	0.259	29020.61	0.000
	3	5	100.540	0.093	0.041	-	-	-	-
	7	5	61.530	1.120	0.504	-	-	-	-
	14	5	24.160	0.376	0.168	-	-	-	-
	21	5	10.130	0.309	0.138	-	-	-	-
	28	5	3.070	0.179	0.080	-	-	-	-
	Total	30	45.270	35.970	6.560	-	-	-	-
Eucerin group	0	5	72.220	0.000	0.000	7226.16	0.317	22784.33	0.000
	3	5	100.630	0.181	0.081	-	-	-	-
	7	5	61.590	1.171	0.524	-	-	-	-
	14	5	24.280	0.417	0.186	-	-	-	-
	21	5	11.400	0.407	0.182	-	-	-	-
	28	5	5.470	0.395	0.176	-	-	-	-
	Total	30	45.930	35.300	6.440	-	-	-	-
Low dose herbal extract (10%)	0	5	72.220	0.000	0.000	8474.89	0.313	27101.59	0.000
	3	5	100.510	0.139	0.062	-	-	-	-
	7	5	60.700	0.942	0.421	-	-	-	-
	14	5	22.770	0.442	0.197	-	-	-	-
	21	5	1.260	0.879	0.393	-	-	-	-
	28	5	0.000	0.000	0.000	-	-	-	-
	Total	30	42.910	38.220	6.970	-	-	-	-
High dose herbal extract (20%)	0	5	72.220	0.000	0.000	8292.90	0.248	33407.91	0.000
	3	5	100.560	0.203	0.090	-	-	-	-
	7	5	61.430	1.090	0.488	-	-	-	-
	14	5	24.020	0.320	0.143	-	-	-	-
	21	5	3.260	0.389	0.174	-	-	-	-
	28	5	0.000	0.000	0.000	-	-	-	-
	Total	30	43.580	37.810	6.900	-	-	-	-

moisture. Re-epithelialization is seen from wound sides. Inflammatory cells also are existed. Infiltration of fibroblasts into the connective tissue was obvious. In low dose treatment group, wound was covered by pustule consist of fibrin and blood cells and purulent materials such as neutrophils and RBC remnants. Clod on the wound had more and low inflammatory cells than high dose and zinc oxide groups, respectively. In zinc oxide group, wound was covered by thick and keratinous pustule. Wound was filled with granular connective tissue and hyperemia was obvious. In eucerin group, hemorrhage in the profound layers was obvious and was not seen any pustule and healing has not been started. On day 7 in high dose treatment group in some cases, pustules on the wound still have not been completely dried but wound area has been filled with multicellular and vascular granular tissue. Epithelial regeneration continues and the amount of inflammatory cells is also greatly reduced. In low dose treatment group, wound surface covered by pustules and internal space of wound in the middle parts filled by fibrinous and granular connective tissue and inflammatory and purulent cells are seen between fibrin and connective tissue and granular texture is full of newly built vessels. In zinc oxide group, situation is entirely like with low dose treatment group. In eucerin treatment group, wound surface is covered by pustules and regenerative epithelial cells starts to expanding on to the wound surface from sides. Inflammatory cells are purulent and infiltration of fibroblasts and existence of newly built vessels indicates formation of new granular tissue. In control group, granular tissue as vascular and low filament tissue are seen. Hemorrhage and hyperemia is also seen.

On day 14, in high dose treatment group granular tissue is existed in the wound area and newly formed vessels are low than previous days. The intensity of inflammatory cells is reduced. Collagen fibers are more delicate and have more organization. Hydropic degeneration is also seen in some of the epithelial cells. In low dose treatment group, more thickly epithelial covered wound surface. Collagen is thicker and has more organization. Coagulum is not existed. In zinc oxide group, fibroblasts start to synthesis of collagen. Inflammatory cells reduced and newly formed vessels increased. Lining tissue is seen in margin of the wound but clot is seen in some places.

Blood clot on the wound contains large amounts of acute inflammatory cells were neutrophils and RBC has penetrated into the clot. In eucerin group, space of wound around is occupied by young and multicellular tissue and regeneration of the lining tissue starts from sides. Wound surface covered by pustules that follows contains hyperemia granular tissue. In control group, marginal parts of the wound are completely covered by epithelial tissue.

Also, new and hyper cellular connective tissue covered dermal layer. On day 21 in high dose treatment group in some cases wound surface is covered by lining tissue but in some others this is not occurred completely. The severity of inflammation and hydropic degeneration is reduced.

In low dose treatment group, partial edema and hyperemia is still seen and collagen fibers were thicker and condensed and were more organized than day 14. In zinc oxide group, dermal accessories and hair follicles increased in the treated tissues. Collagen fibers increased and have more organization. In the eucerin and control groups, situations were same with day 14 with exception hydropic degeneration. On day 28, treatment was seen in about all groups.

DISCUSSION

Zinc oxide ointment is among the most widely used topical ointments to treat ulcers is that content is 20% zinc oxide powder. Protect the surface, being astringent, antiseptic and nontoxic relative of the outstanding characteristics that make the drug as an active ingredient in health and pharmaceutical compounds widely used. In this study were used of this ointment as positive control group too. When a wound occurs and is exposed to external environment, it is more prone to attack by microbes which invade through the skin and delay the natural wound healing process. Reactive Oxygen Species (ROS) are vital part of healing and serve as cellular messengers that drive numerous aspects of molecular and cell biology.

ROS can trigger the various beneficial pathways of wound healing for example at micromolar concentrations of hydrogen peroxide can promote Vascular Endothelial Growth Factor (VEGF) expression in keratinocytes (Prasad and Oberleas, 1974; Nayak *et al*., 2009; Khanna *et al*., 2001). During the inflammation phase of healing neutrophils and macrophages are attracted into the injured tissue by various chemotactic factors. They locate, identify, phagocytize, kill and digest microorganisms and eliminate wound debris through their characteristic respiratory burst activity and phagocytosis (Clark and Moon, 1999). At high concentrations, ROS can induce severe tissue damage and even lead to neoplastic transformation which further impede the healing process by causing damage to cellular membranes, DNA, proteins and lipids as well (Martin, 1996). Hence if a compound or a plant extract having antioxidant potentials and

antimicrobial activity additionally, it can be a good therapeutic agent for accelerating the wound-healing process.

CONCLUSION

Several preparations containing *A. millefolium* extract was quite successfully healed the wounds and scars. The liniment containing hiperisin oil and *A. millefolium* extract patented by Motogna accelerates the healing of wounds and gives esthetic scars. Since, the liniment is applied as a spray it is easily applied and painless. The activiy most probably comes from the synergistic effect of compounds present in the extract and also additive effect of hiperisin. According to results reported here yarrow extracts was found to have better activity on the wound healing experimental models compared to the other extracts.

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