



Histomorphometric study on the effects of *Artemisia sieberi* extract in mice skin

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ABSTRACT

Introduction: Skin as the biggest single body organ is always exposing to various injuries, therefore health and healing of its injuries is vital. *Artemisia sieberi* is a valuable medicinal plant with a long history of indication in folk and modern medicine. Due to different chemical components and antioxidant, anti-inflammatory, antimicrobial and cytoprotective properties of *Artemisia*, this study was conducted to study the histomorphometric effects of *Artemisia sieberi* (*A. sieberi*) extract on mice skin.

Methods: Ninety adult mice were randomly divided in 3 groups. In the treatment group *A. sieberi* extract dissolved in ethanol & distilled water, in the positive control ethanol & distilled water, and in negative control only distilled water were applied on the shaved dorsum twice daily for 21 days. Mean thickness of epidermis, hypodermis & dermis layers, percentage of collagen fibers and histological evaluation of skin layers were studied in 1, 3, 5, 14 and 21 days post treatment. Data were presented as mean \pm SD and analyzed using one way ANOVA and LSD post hoc tests. The P<0.05 was considered as statistically significant.

Results: *A. sieberi* extract significantly increased epidermis thickness in day 1, hypodermis, dermis and percentage of collagen fibers in day 3 in comparison to positive and negative control groups. Histology study revealed normal structure of skin and no abnormality was seen.

Conclusion: *A. sieberi* extract can be effective for health and healing of skin injuries by increasing thickness of the skin layers and amount of collagen fibers.

Implication for health policy/practice/research/medical education:

It seems that *A. sieberi* extract via antimicrobial, anti-inflammatory and anti-oxidant properties and increasing fibroblast activity increases thickness of skin layers and improves integrity and healing of its injuries. Therefore, it might be beneficial for skin health.

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Introduction

Skin involves about 16% of body weight and is the first physical defense barrier between internal and external body environment against microbial, physical and chemical infectious agents and also via its lymphatic system participates in the primary immune reactions (1). Skin has basic role in the homeostasis and thermoregulation and as a sensory organ it has numerous nerve terminals and sensory receptors. Skin especially facial skin is very important in beauty and social-emotional communications and due to the extensive area and different functions, it is

always exposing to various injuries, therefore skin health and integrity is vital (1). So from ancient times human had great attention toward remedies and medications for beauty and repairing skin injuries e.g. dressings made of milk of mothers of baby boys, honey, tea leaves, wine and myrrh, pig fat and vinegar, aloe vera, red Ginseng root, and henna (2).

Medicinal plants existed long before human life and although it is not known that when does human discover their therapeutic property, but in all civilizations trace of their indication has been seen. Since medicinal plants

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possess suitable therapeutic efficacy and lesser side effects than chemical drugs, there is increasing tendency toward them so that nowadays they are valuable source of natural health keeping products and about 80% of people in developed countries use remedies derived from medicinal plants (3). *Artemisia* is one of these valuable medicinal plants with long history of indication in the traditional medicine especially in China as tonic, appetizer, disinfectant, analgesics, antipyretic, anti-rheumatoid and anti-helminthes (4-7). Chemical analyses have shown presence of active compounds e.g. flavonoids, coumarins, caffeoylquinic acids, sterols, saponins, sesquiterpene lactones (artemisinin & dehydroleucodine) in different species of *Artemisia*, thus, antimicrobial, antifungal, antiviral, anti-diabetic, analgesic, anti-inflammatory, anti-parasitic, anti-oxidant, antipyretic, antispasmodic, anti-tumor, anti-complementary and anti-ulcerogenic properties have been attributed to *Artemisia* plant in the modern medicine (3,8-18). In the 1990 decade anti-ulcerogenic activity of *A. annua* in peptic ulcers was shown for the first time (19). Anti-ulcerogenic effects of ethanolic extract and some component of aerial part of *Artemisia* has also been shown (20). Biochemical and histopathological study of *A. sieberi* extract has shown its suitable effects on wound healing in experimental dermal injuries (21). Hydro alcoholic extract of *A. aucheri* can accelerate wound healing and decrease healing time in experimental dermal injuries (22). Also suitable therapeutic effects of *A. sieberi* extract on healing of second degree burn injuries have been shown (23). So, due to antioxidant, anti-inflammatory, antimicrobial and cytoprotective efficacies of *Artemisia* and its therapeutic effects on healing of dermal injuries, this study was conducted to evaluate the effects of *A. sieberi* extract on histomorphometric parameters of skin in mice.

Material and Methods

Animals

Ninety adult healthy mice (20-30 g BW) were prepared and housed under standard laboratory conditions in accordance with the European community guidelines for laboratory animals (12 h. light/dark cycle, ambient temperature of $22\pm1^{\circ}\text{C}$, 60% humidity) and under supervision of the Ethical Committee of the Shahrekord University. During experiments chow pellet and water were freely available.

Plant extraction

Aerial parts of *A. Sieberi* were collected from Yazd province of Iran, in the late summer and beginning of autumn, dried at room temperature, grounded and sieved. Artemisinin extraction was done by using petroleum ether based on the previously published method (21-25).

Animal experiment

After one week for acclimatization, animals were randomly divided into three groups. In the negative control

group only distilled water and in the positive control group distilled water plus ethanol were administered. In the treatment group, *A. sieberi* extract dissolved in distilled water and ethanol was administered. Before any experiment, animals dorsum were shaved and depicted carefully and local medication was done twice daily with sterile swab. In the days 1, 3, 5, 14 and 21 post treatments, 5 animals of each group were randomly selected and after euthanasia, a full thickness skin flap of the experimental area was taken and fixed in the 10% buffer formalin. Tissue processing was done by Autotechnicon tissue processor (Citadel 1000, Thermoshanon, UK), 20 slides were prepared from each paraffin block and Hematoxylin - Eosin (H&E) staining was done. By using an ocular micrometer lens, tackiness of epidermis, dermis and hypodermis layers were measured and registered. Percentage of collagen fibers was determined by using a gridding graticule as a percentage of tissue matrixes. Histological changes of skin were studied on different sampling days by using light microscope.

Statistical analysis

Data were demonstrated as mean \pm SD. One-way analysis of variance (ANOVA) followed by LSD post hoc test were used for analysis of data ($p<0.05$).

Results

Epidermis layer

As the Table 1 shows, while there was no significant difference in the average thickness of epidermis layer between positive and negative control groups ($p>0.05$), in the treatment group, it was significantly higher than control groups at day 1 post treatment ($p<0.05$), meanwhile this difference in the rest of days was not significant ($p>0.05$).

Dermis and hypodermis layers

Despite that there was no significant difference in the average thickness of dermis & hypodermis layers between positive and negative groups ($p>0.05$), but in the treatment group it was significantly higher than control groups at day 3 post treatment ($p<0.05$), although in other days this difference was not significant ($p>0.05$; Table 1).

Percentage of collagen fibers

Comparison between negative and positive control and treatment groups revealed that only in the day 3 post treatment, percentage of collagen fiber in the treatment groups was significantly higher than that of control groups ($p>0.05$), but in the other days such a significant difference was not seen ($p>0.05$; Table 1).

Histological survey

Histological survey showed that in the control and treatment groups, skin tissue had natural structure and no injury or abnormality was seen. Epidermis was composed of stratified squamous cells with acidophil cytoplasm and basophil nucleus (Figure 1A & D). In the dermis, a loss

Table 1. Mean \pm standard deviation of thickness of skin layers (Micrometer) and percentage of collagen fibers in the control and treatment groups during different days.

	Sampling day	Group		
		Negative control	Positive control	Treatment
Mean thickness of epidermis	1	2.49 \pm 0.41	2.48 \pm 0.24	3.46 \pm 0.14 ^a
	3	2.71 \pm 0.32	2.68 \pm 0.31	2.81 \pm 0.36
	5	2.84 \pm 0.18	2.63 \pm 0.28	2.21 \pm 0.23
	14	2.12 \pm 0.24	2.21 \pm 0.32	2.72 \pm 0.48
	21	2.36 \pm 0.72	2.30 \pm 0.36	2.29 \pm 0.64
Mean thickness of dermis	1	25.24 \pm 2.23	28.74 \pm 1.24	29.56 \pm 5.4
	3	24.16 \pm 4.1	25.19 \pm 3.04	65.24 \pm 8.13 ^a
	5	28.36 \pm 3.4	26.47 \pm 3.81	32.18 \pm 2.5
	14	31.18 \pm 1.4	30.24 \pm 2.73	38.11 \pm 4.2
	21	35.47 \pm 5.2	31.21 \pm 4.29	40.02 \pm 2.61
Mean thickness of Hypodermis	1	24.74 \pm 6.4	22.36 \pm 6.4	21.02 \pm 4.3
	3	31.42 \pm 7.1	28.13 \pm 7.1	46.65 \pm 8.6 ^a
	5	24.68 \pm 5.1	23.76 \pm 5.1	24.58 \pm 7.2
	14	27.45 \pm 2.7	25.27 \pm 2.7	22.50 \pm 3.5
	21	21.58 \pm 4.9	21.17 \pm 4.9	23.75 \pm 6.7
Percentage of collagen fibers	1	86.35 \pm 7.2	89.41 \pm 7.2	91.69 \pm 4.3
	3	90.43 \pm 6.5	88.59 \pm 6.5	108.42 \pm 4.2 ^a
	5	96.4 \pm 3.9	93.62 \pm 3.9	89.76 \pm 6.1
	14	87.28 \pm 6.8	89.48 \pm 6.8	93.21 \pm 5.3
	21	92.73 \pm 5.4	90.15 \pm 5.4	92.57 \pm 4.5

^aSignificantly different at ($P<0.05$) in comparison to control groups (LSD test).

connective tissue was seen that support the epidermis and connected to the underlying tissue (Figure 1B and C). Papillary layer invaginated between dermis papilla and fibroblasts and other cells could be seen. In the connective tissue blood vessels and hair follicles were seen clearly (Figure 1A). Hypodermis with its loss connective tissue and adipocytes was directly connected with underlying muscular structure, and it had properties of connective tissue (Figure 1A).

Discussion

Different *Artemisia* species are widely distributed in Iran which among them *A. sieberi* is the most valuable (7). Various indications of *Artemisia* have been reported in the folk and modern medicine, so that nowadays, Artemisinin isolated form *Artemisia* is the best medication for malaria especially cerebral and drug resistant with excellent efficacy, high therapeutic index and rapid action (6,26,27). Additionally, some researches indicated suitable effects of *Artemisia* extract on the healing of dermal injuries and burns (19-23). Results of this research showed that *A. sieberi* extract increases thickness of the epidermis, dermis and hypodermis layers and also percentage of collagen fibers.

Oil essence of *Artemisia* is composed of a mixture of terpenes (7). Chemical analysis of *Artemisia* extract revealed presence of compounds such as verbenol, camphor, α & β thujone, cineole, linaleole, borneol, cymene, sabinene and 1, 8-Cineole, thus it has anti-bacterial activity against bacteria such as: *Staphylococcus aureus*, *streptococcus*, *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *dermatophyte* fungi (3,10,11,14,16,17,28).

Wound infection is one the major reasons of delaying wound healing, so it seems that *Artemisia* can prevent growth of bacteria and fungi on the skin, hinder, and control infection following skin injuries, boosts skin defense against microorganisms' invasion and colonization, therefore accelerates wound healing. In addition anti-oxidant and anti-inflammatory properties of many *Artemisia* species have been shown (15,18). It is reported that sesquiterpene lactone compound dehydroleucodine interacts with production of nuclear factor κ B(NF- κ B) and inhibits release of inflammatory mediators and thus possess anti-inflammatory activity (9,15). Additionally, scoparone of the plant extract inhibits nitric oxide synthase and cyclooxygenase II and thus inhibits production of inflammatory mediators in macrophages (18,21). *Artemisia* extract hinders proliferation and activation of T lymphocytes and exerts immunosuppressive activity, so it can be effective in the inflammatory conditions such as contact hyper sensitivity (18,21). *Artemisia* extract contains some compounds e.g. flavonoids, coumarins, tannin, xanthines, copper, zinc and manganese with high anti-oxidant and frees radical scavenger potential which prevent lipids peroxidation (21). Beside this, it is reported that aqueous extract of *Artemisia* increases superoxide dismutase, catalase and glutathione S-transferase activity which are among the most important anti-oxidant mechanisms of the body (18,21). Therefore, it seems that anti-oxidant and anti-inflammatory activity of *Artemisia* decrease the impact of free radicals and inflammatory reactions and associated diseases on the skin and also during skin injuries predispose healing process. In this study similar to previous studies significant increase in

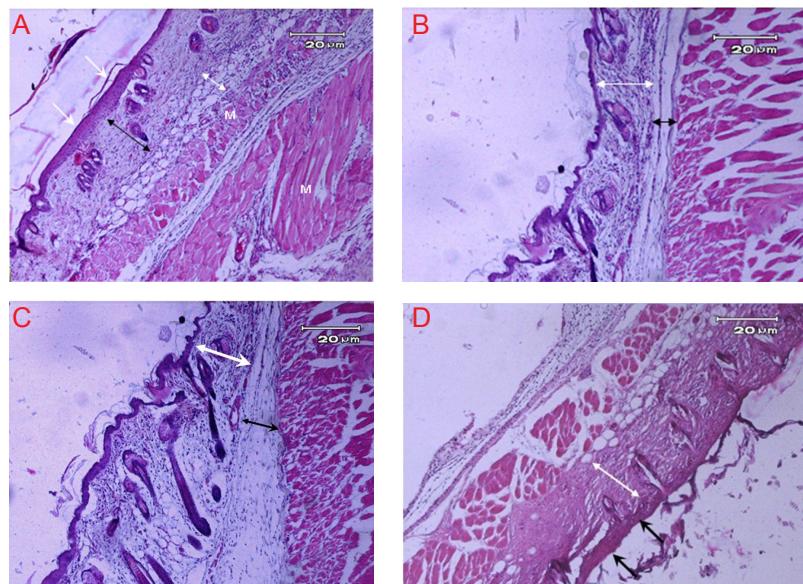


Figure 1. A) Light microscope structure of the skin in the control group. Epidermis (white arrow) Dermis (black double head arrow), hypodermis (white double head arrow), Muscle layers (M). H&E staining, magnification $\times 20$. B) Light microscope structure of the skin day 1 after treatment. Dermis structure is completely seen. (White double head arrow), muscle layer (M). H&E staining, magnification $\times 20$. C) Light microscope structure of the skin days 3 after treatment. Attention to the different thickness of dermis (white double head arrow) which is located on the hypodermis (black double head arrow). Muscle layer (M). H&E staining, magnification $\times 20$. D) Light microscope structure of the skin days 14 after treatment. Attention to the hypodermis (white double head arrow) and dermis (black arrows); H&E staining, magnification $\times 20$.

the percentage of collagen fibers were seen. Significant increase of collagen fibers and capillary buds following treatment with *A. sieberi* extract has previously been reported (21). In another study significant improvement in healing of second degree burn healing and increase of hydroxyproline as an indicator for collagen production has been reported (23). Collagen has a basic role in the remodeling stage of healing process and increases tensile of skin tissue and its resistance against pressure and tension (1). Increased number of fibroblast may be due to mitogenic activity of the *Artemisia* plant leading to increase synthesis of collagen and further durability of healing tissue. Also increasing capillary buds can accelerate granulation tissue formation and thus decrease healing time (21).

Conclusion

It seems that *Artemisia* by utilizing different mechanisms such as antimicrobial, anti-inflammatory and anti-oxidant properties, decreasing inflammation, increasing defense against free radicals, free radical scavenging, preventing bacteria and fungi colonization and infection, increasing fibroblast activity and collagen synthesis boosts skin health and improve its healing following injuries.

Authors' contributions

JK and MSH: designing study; RF: histological examination and statistical analysis; SR: performing animal experiment and histological examination; JK: manuscript preparation.

Conflict of interests

All authors declare that they have no conflict of interests.

Ethical considerations

Authors have been entirely regarded ethical issues such as: plagiarisms, data assembly, fraud, double publication or submission. Also all animal experiments had been approved by the institutional ethical committee and carried out according to the International Guide for the Care and Use of Laboratory Animals.

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