THE EFFESCT OF ALCHOHOLIC AND AQUEOUS EXTRACTS OF *PLANTAGO LANCEOLATA L.* ON BURNED SKIN IN RAT

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Summary

The aim of this study was to investigate the effects of aqueous and alcoholic extracts of *Plantago lanceolata* (PL) on burned wounds.
Fourty eight Wistar rats were divided into six groups. About 28 cm^2 of the back skin was exposed to actively boiled water resulting in third degree burns. Negative and positive control groups (1 and 2) were topically treated twice daily with normal saline and silver sulfadiazine respectively. Experimental groups (3 and 4) were treated with aqueous and ethanolic extract of PL. After 14 days burned surface area in each rat was calculated, microscopic slides were prepared and stained with HE. Pathological parameters including; epithelialization, granulation, fibrotic tissues and inflammation were detected and expressed in semi-quantitative scale. Morphological observations and pathologic results demonstrated that the burned surface area and inflammation were significantly reduced with 10% aqueous extract. Repairmen of epidermal tissue and hair follicles only were demonstrated in animals treated with 10% aqueous extract. The effects of this extract on all studied parameters were equal or greater than of silver sulfadiazine; therefore it might be used in human burned injuries.

Keywords: *Plantago lanceolata,* Burned skin, Aqueous and ethanolic extracts, Epithelialization, Granulation, Fibrotic tissue.

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Introduction

Burn wounds, which caused by coming into contact with fire, chemicals and electricity, are among the very serious illnesses of human beings with high morbidity and mortality (1). The depth of thermal injury is the most important determinant of healing capacity and mechanism in burn wound patients (2). Whereas superficial burns involve only the topmost layers of skin, partial thickness and deep partial thickness burn wounds, named third and fourth degree burns, involve destruction of the underlying dermal elements (3). The process of wound healing can be divided into three overlapping phases including inflammation, granulation, and matrix formation, which interweaving form tissue remodeling (4).

A growing number of scientific concerns are focusing on the significance of natural compounds that can act as wound healers. Plantago is the most important genus of Plantaginaceae family and is used in traditional medicine around the world for different purposes (5, 6). *plantago lanceolata L.*, a perennial plant species with a worldwide distribution and large ecological amplitude, has several therapeutic effects described in Iranian ancient medical books (7). Different pharmacological effects have been reported for the *Plantago lanceolata* including growth hormone like effect (8)**,** anti-pollen (9), anti-oxidant (10), anti-inflammatory (11, 12), anti-tussive (13), antispasmodic (14) and therapeutic effect on asthma (15).

In the present study the effects of alcoholic and aqueous extract of *Plantago lanceolata* on third degree burn injuries in a rat model was investigated.

Methods

Plantago lanceolata was collected from Sangsefid, a region near mashhad in Khorasan Province Iran; The hole plant was recognizied by botanists in a herbarium in Ferdowsi University. The plant extracts were prepared as follows: Areal parts of the plant were drided and used in this experiment. For aqueous soxhlet extract, 50 g of the chopped, dried powder was extracted with 500 ml water by soxhlet apparatus and the outcome of the extract was calculated 26.4%. For ethanolic extract the same amount of the dried powder was extracted with 500 ml 70% ethanol by soxhlet apparatus and outcome of the extract was calculated 26.8%. The solvent of both extracts were removed and various concentrations of aqueous and ethanolic extracts of *Plantago lanceolata* were prepared.

All animal procedures were carried out in according with the institute of Laboratory Animal Research guide for the care and use of laboratory animals. Forty eight Wistar rats weighted 200±20g were housed at 25±2°C and 12h light/dark cycle. They were randomly divided into six groups and anaesthetized by ip injection of sodium tiopental (100mg/kg BW). About 28 cm^2 of the back skin on both sides was exposed to actively boiled water for 20 seconds, resulting in third degree burns. One rat from each group was killed and burned skin was removed for detection of the degree of burns by pathological methods. Negative and positive control groups (1 and 2) were topically treated twice daily with normal saline and silver sulfadiazine oinment respectively. Groups 3 and 4 were treated respectively with 5% and 10% of aqueous extract while groups 5 and 6 were treated respectively with 5% and 10% of ethanolic extract of *Plantago lanceolata* applied twice/day topically. All animals were treated for 14 days. At the end of experiment (day 15) all rats were killed and burned surface areas in each rat was calculated. Then the burned skins were discorded and kept in formalin for further pathological examinations. Five micrometer sections were prepared with routine

pathological methods, stained with hematoxylin eosin (HE) and were examined under light microscope. Pathological parameters including: epithelialization, granulation, fibrotic tissues and inflammation were detected and expressed in a semi-quantitative scale (Table 1). Data were expressed as Mean± SEM and were analyzed firstly by Kruskal-Wallis test to find the significant differences between groups; then one way ANOVA and subsequently Tukey-Krammer testes were used; $p<0.05$ was considered to be significant.

Table 1: Semi-quantative pathological scales.

score	Inflammation	Fibrotic tissue	Granulation	Epithelialization
1	Very weak-acute	Is not present	Is not present	Is not present
2	Mild-acute	Just started from the margin	Present around the margin and over the wound surface	Started from the margin of the wound
3	Sever-acute	Present at margin	Moderately present	A few spots at margin of the wound
4	Chronic inflammation	Moderate	Complete granulation	Moderate
5	Moderate to weak-chronic	Completed fibrotic tissue	Regressed granular tissue	Complete epithelialization
6	Week	Not present	Completely regressed	Not present

Results

As it is shown in table 2, two weeks after treatment with 10% aqueous extract of *Plantago lanceolata,* the burned surface area in group 4 significantly decreased in comparison with groups 2 ($p=0.021$) and 3 ($p=0.026$).

Table 2: Burned surface area (mm^2) in different groups at the end of experiment.

Groups	1	2	3	4	5	6
burned surface area (mm^2)	759.357 ± 217.38	1818.9 ± 537.28	1778.52 ± 211.69	269.875 ± 96.21 * ◘	772.7± 163.86	1079.8 ± 267.37

Data are express as Mean±SEM
N=7 rats in each group
* $p=0.021$ the different between group 2 and 4.
◘ $p=0.026$ the different between group 3 and 4.

Furthermore epithelialization in group 4 was significantly higher when compared with groups 1, 3 and 6 (all $p=0.046$, Table 3). Our results also indicate that in group 4 the amount of granulation was significantly higher than group 3 ($p=0.046$, Table 4).
As it is shown in table 5, not only the formation of fibrotic tissue in group 1 was significantly higher than groups 3 ($p=0.021$) and 6 ($p=0.00$), but also it was higher in group 2 compared with groups 3 and 6 ($p=0.00$). Moreover fibrotic tissue formation was significantly higher in group 4 compared with groups 3 ($p=0.023$) and 6 ($p=0.00$).

Table 3: Comparison of epithelialization in different groups at the end of experiment.

Groups	1	2	3	4	5	6
Epithelialization	3.14± 0.26	3.60± 0.5	3.0± 0.0	4.20± 0.2*	3.2± 0.20	3.00 ± 0.03

Data are express as Mean±SEM
N=7 rats in each group
* p=0.046 the different between groups 1, 3 and 6 with 4.

Table 4: Comparison of granulation in different groups at the end of experiment.

Groups	1	2	3	4	5	6
Granulation	4.14± 0.1	4.2± 0.2	3.4± 0.25 *◘	4.4± 0.25	4± 0. 0	4± 0.0

Data are express as Mean±SEM
N=7 rats in each group
* p=0.037 the different between groups 1 and 2 with 3.
◘ p=0.046 the different between group 3 and 4.

Table 5: Comparison of fibrotic tissue in different groups at the end of experiment.

Groups	1	2	3	4	5	6
Fibrotic tissue	4± 0.0	4.2± 0.2	3.1± 0.14 ΔΔ * ●● ◘◘	4± 0.0	4± 0. 0	3± 0.0 *Δ ● ◘

Data are express as Mean±SEM
N=7 rats in each group
Δ p=0.00 the different between group 1 and 6.
ΔΔ p=0.012 the different between group 1 and 3.
* p=0.00 the different between groups 3 and 6 with 2.
● p=0.00 the different between group 4 and 6.
●● p=0.023 the different between group 4 and 3.
◘ p=0.00 the different between group 5 and 6.
◘◘ p=0.023 the different between group 5 and 3.

Comparing the scale of inflammation in experimental groups indicate that there was a significant difference between group 4 and groups 1, 3, 5 and 6 (p=0.03, Table 6). Morphological studies, which took place everyday, revealed that the morphology of burn wound changed in all groups but changes happened rapidly in group 4 compared with other groups. As it is shown in figure 1, the formation of hair follicles just happened in group 4 after 14 days treatment by 10% aqueous extract of *Plantago lanceolata*.

Table 6: Comparison of inflammation in different groups at the end of experiment.

Groups	1	2	3	4	5	6
Inflammation	3.43± 0.20	4.0± 0.32	3.0± 0.0	4.4± 0.4 *	3.0± 0. 0	3.0± 0.0

Data are express as Mean±SEM
N=7 rats in each group
Significant increase in compare with groups 1, 3, 5 and 6 (p=0.003).*

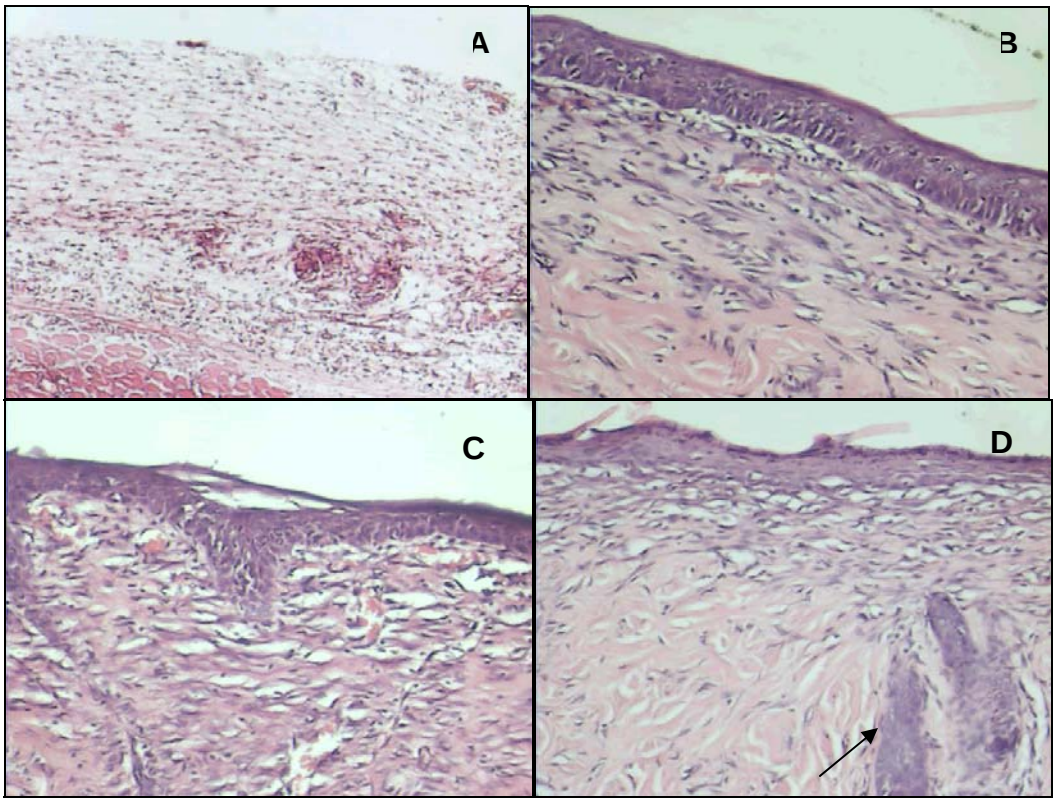

Figure 1: Microscopic sections of burned areas in different groups of rats stained with HE (×400). **A:** Third degree burn after 48h. **B:** normal salin treatment (day 15). **C:** silver sulfadiazine treatment (day 15). **D:** treatment with 10% aqueous extract of *Plantago lanceolata* (day 15). The arrow shows hair follicle formation).

Discussion

Burn injuries had been treated in different ways with topical products or systemic drugs for several centuries. About one third of medicinal plants are used in burn wound healing (16). The main finding of this research indicate that 10% aqueous extract of *Plantago lanceolata* has positive effects on wound healing parameters. Our results also show that 10% aqueous extract of *Plantago lanceolata* significantly decrease the burned surface area when compared with silver sulfadiazine (Table 2). It has been reported that *Plantago lanceolata* extract has mitogenic effects on epidermal cells and promote granulation (18). It may be suggested that the effect of 10% aqueous extract of *Plantago lanceolata* on reducing the burned skin area and other parameters such as inflammation may be due to anti-microbial, anti-inflammation or mitogenic effects of the extract on granular tissue which cause a rapid healing of the wound.

Tannin and Aucubine, two important components of *Plantago lanceolata* which have anti-microbial and anti-inflammatory effects, have beneficial effects in wound healing (16, 17).

As it is shown in tables 3 and 4, there is no significant difference between the effects of 10% aqueous extract of *Plantago lanceolata* and silver sulfadiazine on epithelialization and granulation, whereas 10% aqueous extract significantly increase epithelialization and granulation compared with 5% aqueous extract of *Plantago lanceolata*.

It was reported that *Plantago lanceolata* contain Epidermal Growth Factor (EGF), which cause collagen synthesis and angiogenesis during wound healing (17), therefore the positive effects of 10% aqueous extract of *Plantago lanceolata* on epithelialization may be due to presence of EGF and its effects on epithelial cells. Another plant, *Datura alba* also has been reported to have mitogenic and angiogenic compounds which increases granulation in wound site (19); therefore, the positive effect of *Plantago lanceolata* on granulation may be due to mitogenic and angiogenic compounds in the extract.

As it is shown in tables 5 and 6, 10% aqueous extract of *Plantago lanceolata* significantly increase fibrotic tissue and decrease inflammation when compared with 5% aqueous extract and 10% ethanolic extract of the plant. On the other hand, the difference between the effects of 10% aqueous extract of *Plantago lanceolata* and silver sulfadiazine on above parameters are insignificant, which means that the effect of the extract is in the rank of silver sulfadiazine for these parameters.

The effects of *Aloe vera* on wound healing arise from its anti-inflammatory compounds which prevents inflammation in wound site (20). It was shown that Catapol is an anti-inflammation compound present in *Plantago lanceolata* extrct (17), therefore the effect of *Plantago lanceolata* aqueous extrct on inflammation, observed in this research, may arise from catapol.

As it is shown in figure 1, repairment of hair follicles only were demonstrated in animals treated with 10% aqueous extract.

We conclude that the effects of 10% aqueous extract of *Plantago lanceolata* on all studied parameters were equal or greater than that of silver sulfadiazain; therefore, it may be suggested that 10% aqueous extract of *Plantago lanceolata* could be used for treatment of human burned injuries.

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