Original Article

Interleukin-1 and Keratinocyte Growth Factor/Fibroblast Growth Factor-7 Gene Expression in Skin Experimental Irritant Contact Dermatitis Mouse Model Treated with Aqueous Extract of *Trachyspermum copticum (L.) Link* Seeds

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Abstract

Background: In our previous study, the extract of *Trachyspermum copticum (L.) Link* seeds on gene expression of IFN-γ and TGF-β1 in mouse model with irritant contact dermatitis (ICD), in comparison with cutaneous corticosteroids were evaluated. In that study, in addition to significantly increase of IFN-γ and TGF-β1 genes expression levels in skin samples of "mice with ICD" groups treated with extract in comparison to other groups, histopathologic findings showed substantial improvement of skin color, texture and thickness, and also significant increase in hair follicle number. Therefore, we have decided to study the levels of Interleukin-1 (IL-1) gene expression, which plays a major role in inflammation responses, and Keratinocyte Growth Factor/Fibroblast Growth Factor-7 (KGF/FGF-7), which has growth effect on cells and is an important endogenous mediator of hair follicle growth and development.

Materials and Methods: We used autopsy samples of skin lesions obtained from "mice model with irritant contact dermatitis (ICD)" from the previous study. In that study, "mice with ICD" divided in 9 groups and were treated with three concentrations of *Trachyspermum Copticum (L.) Link* dried seeds, cutaneous hydrocortisone, and fluocinolone acetonide. Then from the first day until the 10th day of treatment, clinical signs and histopathologic investigations were investigated. In the present study, using Real-Time PCR, the levels of IL-1 and KGF/FGF-7 genes expression in skin samples of inflammation site in above mice groups were studied. Statistical analysis, using one -way ANOVA, were performed. Level of significance was set at 0.05.

Results: The IL-1 gene expression showed a significant difference between groups: IL-1 gene expression levels in mice with ICD treated with extract and corticosteroids were higher than the other groups (p=0.0001). While in untreated "mice with ICD", no significant differences were observed. Also, during the treatment, there was a considerable increase in levels of IL-1 gene expression in groups treated with the extract at a rate of at least 2 to 3-fold in comparison with the "healthy untreated mice" group. The levels of KGF/FGF-7 gene expression in "mice with ICD" groups treated with the extract showed significance difference (p=0.014); also there was a meaningful difference in "mice with ICD" groups treated with cutaneous corticosteroids (p=0.004). While, in "untreated mice with ICD" group there were a significant decrease in the levels of KGF/FGF-7 gene expression in comparison with "healthy untreated mice" group (p=0.0001). Also, changes in the levels IL-1 and KGF/FGF-7 gene expressions in each group in different days were seen.

Conclusion: In this study, significant changes in the IL-1 and KGF/FGF-7 genes expression levels in the skin samples with inflammation, were associated with an increase in the rate and speed of improvement of contact dermatitis, more favorable conditions of the healed skin (in terms of color, consistency, and thickness), and a remarkable increase in the number of hair grown on the site of dermatitis (compared with control groups, and even groups with corticosteroid therapy).

Keywords: Animal Model of Experimental Irritant Contact Dermatitis; Treatment of Irritant Contact Dermatitis; Ajwain; Zenian; 2, 4-Dinitrochlorobenzene (DNCB); Gene Expression Profiling.

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Introduction

Contact dermatitis is inflammation of the skin, as a result of exposure to an exogenous agent, and constitutes a significant portion of occupational disorders in industrialized societies. Contact dermatitis is divided into irritant contact dermatitis and allergic contact dermatitis, based on their mechanistic differences: Irritant contact dermatitis (ICD) is caused by direct tissue damage following a single exposure or multiple exposures to an irritant while allergic contact dermatitis (ACD) is a delayed (type IV) hypersensitivity reaction, mediated by T cells and requiring prior sensitization[1].

Contact irritants in the workplace, are one of the causes of the dermatitis. Importance of the disease will be determined according to the International Labor Organization in 2010, which rates it as the second important of all occupational skin diseases that cause job disorders. In addition, it has been suggested to be rated as the first skin disease causing job disorder and is considered as a major health problem[1]. Irritant contact dermatitis (ICD) is an inflammatory response of the skin to various external stimuli. It arises because of activated innate immunity to direct injury of the skin without prior sensitization[2-4]. To treat dermatitis, various pharmacological groups used but corticosteroids considered as the main group. But these drugs in all cases, not only do not have sufficient efficacy but also long-term use of these drugs (due to the nature and type of the disease), has side effects, so researches to find new drugs with greater efficacy and fewer side effects continues. Studies have shown some of the herb's healing effects on the inflammation of the skin and wounds. In this regard, the effectiveness of materials such as olive tree extract in healing of skin wounds in rats were evaluated and confirmed[5]. An annual plant that grows in Iran that is called *Trachyspermum copticum L. link*, traditionally were used as diuretic, carminative, and antihelmentic[6]. Some biological effects of ajowan such as antiviral[7], anti-inflammatory[8], antifungal[9], antipyretic[10], antifilarial[11], analgesic[12,13], antinociceptive[14] and antioxidant activity[15] have been confirmed.

Malekpour *et al*, studied the effects of aqueous extract of *Trachyspermum copticum (L.) Link* seeds on levels of IFN-γ and TGF-β1 genes expression in mouse model with irritant contact dermatitis (ICD), in comparison with cutaneous corticosteroids as well as its effect on healing process. In that study, in addition to significantly increase of IFN-γ and TGF-β1 genes expression levels in skin samples of "mice with ICD" groups treated with extract in comparison to other groups, clinical and histopathologic findings showed substantial improvement of skin color, texture and thickness, and also significant increase in hair follicle number[16].

In this study, we planned additional investigations on aqueous extracts non-cytotoxic concentrations in experimental contact dermatitis mouse model. Considering that in the process of contact dermatitis, immune cells involved in tissue location can be influenced by factors such as cytokines and growth factors and there may be changes may be changes in tissue inflammation by *Trachyspermum copticum L.*

link extract contacted with the tissue in different concentrations. Thus, the macroscopic and microscopicchanges in tissue, as well as changes in gene expression of these cytokines can be a result of treatment of tissue with extract.

Actually, we decided to study the gene expression of interleukin-1 (IL-1), which plays a major role in inflammation responses. Its role proved in ICD[17,18]. Keratinocyte Growth Factor/Fibroblast Growth Factor-7 (KGF/FGF-7), which is mitogenic, cell survival, cell growth, morphogenesis, tissue repair, tumor growth, and invasion activities is an important endogenous mediator of hair follicle growth, development, and differentiation[18-23].

Methods

We used post-mortem samples of mice skin lesions with ICD from study of Malekpour *et al,*[16]. In that study, 3 weeks female BALB/c mice were divided into 9 groups: healthy control group (n=6), healthy controls treated with the solvent (used for the solution of the DNCB that is a solution of 4:1 acetone and olive oil) (n=6), control group with ICD without treatment (n=6), control group with ICD treated with distilled water (n=6), mice with ICD treated with concentrations of 200, 1000 and 7000 µg/mL of dried seed aqueous extract of *Trachyspermum copticum (L.) Link* (each group: n=10), mice with ICD treated with cutaneous hydrocortisone (n=6) and fluocinolone acetonide (n=6), 4 times a day. During the study, all clinical, macroscopic, and microscopic changes in mice were examined and monitored.

After a duration of 10 days of treatment and humanely killing of mice, autopsy skin samples were prepared and ICD were confirmed by histopathological examination [hematoxylin and eosin (H&E) staining] and comparing the results with clinical signs.

Real-Time PCR: The expression of IL-1 and KGF/FGF-7 genes, and GAPDH (as a housekeeping gene) in skin samples of the lesions after RNA extraction and cDNA synthesis were measured using Real-Time PCR.

Statistical analysis: We performed statistical analysis, using one-way ANOVA. Level of significance was set at 0.05.

Results

You can find groups orientation and interventions in Malekpour *et al,* study[16].

IL-1 gene expression: The IL-1 gene expression showed a significant difference between groups: IL-1 gene expression levels in mice with ICD treated with extract and corticosteroids were higher than the other groups (p=0.0001). While in untreated mice with ICD, no significant differences were observed. In addition, during the treatment, there was a considerable increase in levels of IL-1 gene expression in groups treated with the extract in the rate of at least 2 to 3-fold in comparison with the healthy untreated mice group (Figure 1a).

KGF/FGF-7 gene expression: The levels of KGF/FGF-7 gene expression in mice with ICD groups treated with the extract showed significance difference (p=0.014); also there was a meaningful difference in mice with ICD groups treated with cutaneous corticosteroids (p=0.004). While, in "untreated mice with ICD" group there were a significant decrease in the levels of KGF/FGF-7 gene expression in comparison with healthy untreated mice group (p=0.0001). In addition, changes were seen in the levels of IL-1 and KGF/FGF-7 gene expressions in each group in different days (Figure 1b).

Discussion

Untreated mice with ICD group: Skin samples of untreated mice with ICD group showed an increased in the levels of IL-1 gene expression on the second to the 6th day of treatment phase, and then, its expression was reduced; so that from the 7th to the 10th day of treatment phase, the levels of IL-1 gene expression were lower than its expression in the skin samples of healthy untreated mice group (almost the half). These are some commonly expected changes in an inflammation period (first phase: inflammation and tissue damage, and the second phase: tissue repair and healing).

In addition, its elevation peak was on the 4th day of inflammation, about 1.5-fold in comparison with that of healthy untreated mice group.

On the other hand, at the first days of treatment phase, the KGF/FGF-7 gene expression levels in the skin samples of untreated mice with ICD was about 0.3 of

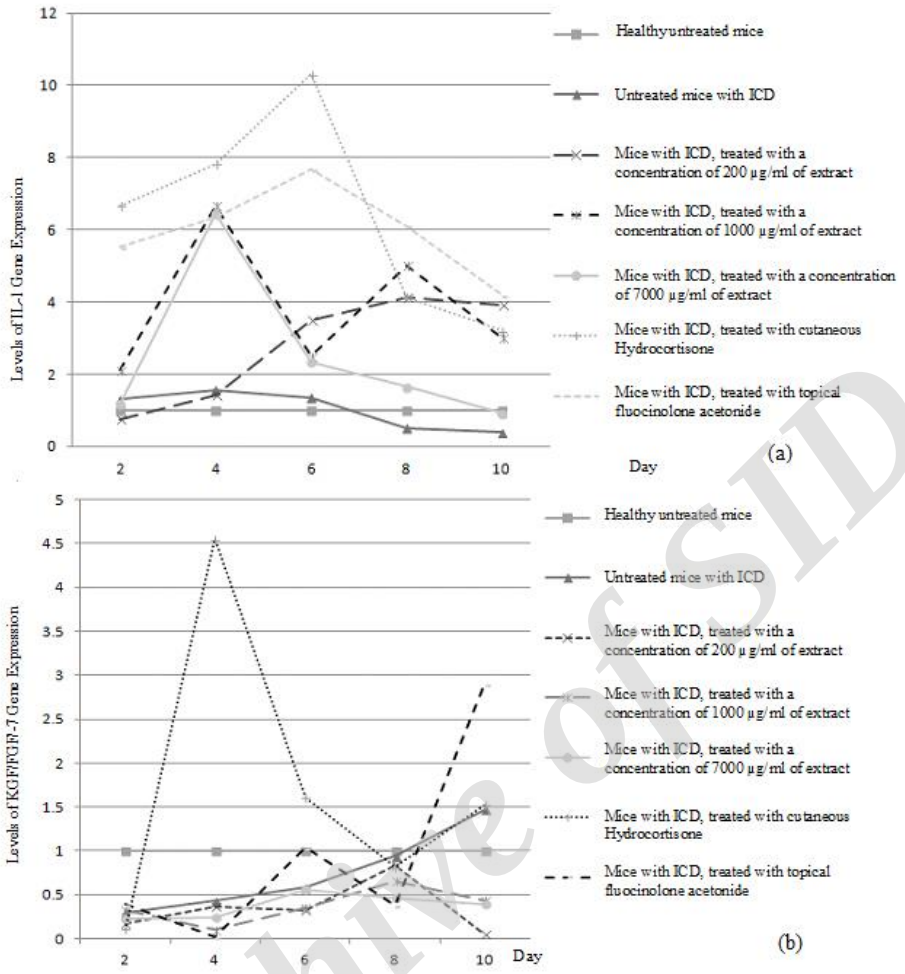

Figure 1. Changes in the levels of IL-1(a) and KGF/FGF-7(b) gene expression during treatment phase.

its expression in healthy untreated mice group, and then with decreasing inflammation and gradual healing of the tissue (as detailed reported in study of Malekpour et al[16]), its levels gradually increased; so that on the 10th day of treatment phase, a 1.5-fold increase in its expression in the healthy untreated mice group were seen.

Regarding the above mentioned findings, it seems that in the skin samples of untreated mice with ICD, the following changes are associated with an inflammatory state: multifold increase in levels of IL-1 gene expression; and a significant decrease in the levels of KGF/FGF-7 gene expression in the early days of treatment phase and an increase in its expression in the end days of the treatment phase (*i.e.*, recovery period of the inflammation).

Report of Malekpour et al[16] on the clinical and histopathologic status of the skin of the untreated mice with ICD group at the end of the ten-day treatment phase, suggesting that, despite the clinical signs of inflammation reported in the skin samples of untreated mice with ICD, the appearance of the skin was thin and fragile, and the number of raised hair was very small. Also appearance of the hair was thin and fragile (poor quality and quantity of hair).

It seems that the above mentioned changes in the levels of IL-1 and KGF/FGF-7 genes expression are a part of the molecular and microenvironment conditions of the skin in the mice with ICD:

Mice with ICD, treated with Distilled water group: Levels of IL-1 and KGF/FGF-7 in the skin samples of this group showed a relatively similar changes to the untreated mice with ICD group. This results also indicates that the use of water for treatment of ICD in

these mice did not change the levels of IL-1 and KGF/FGF-7 gene expressions; and also, according to the clinical quality of the skin of the mice and their histopathologic status, as reported by Malekpour et al[16]., had no effect on the reduction of dermatitis and tissue healing.

The above observations provide the expected overall format of changes in the IL-1 and KGF/FGF-7 gene expressions in the skin samples obtained from mice with ICD in this study.

Mice with ICD group treated with extract: The levels of IL-1 and KGF-FGF-7 genes expression in the skin samples of mice with ICD treated with different concentrations of the aqueous extract of *Trachyspermum Copticum (L.) Link* dried seeds were accompanied with considerable changes compared to their expression in the skin of "healthy untreated mice" and it could be said that largely, it followed a particular pattern. These changes were as follows:

- In mice treated at a concentration of 200 µg/mL of extract, gradual increase in the levels of IL-1 gene expression continued until the 8th day of treatment phase was observed, and on the 8th day of treatment, levels of the gene expression reached to more than 4-fold of its expression in the sample skins of healthy untreated mice group. The levels of gene expression on the last day of treatment (on the day 10) was also slightly less than 4-fold of the amount expressed in the skin samples of healthy untreated mice group.
- In mice treated with a concentration of 1000 µg/mL of the extract, on the 4th day of treatment phase, an increase in levels of IL-1 gene expression was observed at a rate of 6.5-fold of that of the "healthy untreated mice" group.

On the 6th day of treatment phase, a drop in the expression of this gene made it about 2.5-fold as much as expressed in the skin of "healthy untreated mice" group.

On the 8th day of treatment phase, again, an increase in the levels of IL-1 gene expression was observed at 5 times that of its expression in "healthy untreated mice" group; and on the 10th day, while the levels of gene expression decreased, but its expression was 3-fold of its expression in skin samples of healthy untreated mice group.

- In mice with ICD, treated at a concentration of 7000 µg/mL, an increase in the levels of IL-1 gene expression was observed: on the 4th day of treatment phase, it was 6.5-fold greater than that of the "healthy untreated mice". This was similar to the observed IL-1 gene expression levels in the skin samples of "mice with ICD" treated with a concentration of 1000 µg/mL of extract.

Subsequently, the levels of IL-1 gene expression showed a decrease, and on the 6th day it was about 2.5-fold of that of the healthy untreated mice group; which was similar to that of the mice with ICD" group treated with a concentration of 1000 µg/mL of extract.

After the 6th day of treatment phase, the level of IL-1 gene expression continued to decrease, but less rapidly than the interval between treatment days 4th and 6th. On the 10th day of treatment phase, its expression was approximately the same as the expression of this gene in the skin samples of healthy untreated mice group.

After the 6th day of treatment phase, the levels of IL-1 gene expression continued to decrease, but less rapidly than the interval between treatment days of 4th and 6th. On the 10th day of treatment, its expression was approximately the same as the expression of this gene in the skin samples of healthy untreated mice group.

The study of the clinical and histopathologic findings of skin samples reported in the study by Malekpour et al showed that the above mentioned changes were accompanied by a completely improved clinical presentation and also a suitable histopathologic condition at the end of the 10th day of treatment phase; although increased concentrations of the extract were associated by better quality of the healing tissue and the raised hair (both in terms of appearance and number of hair).

In mice with ICD group treated with extract, it seems that there is an association between increase in the levels of IL-1 gene expression in the first half of the course of treatment, and its reduction in the second half of the treatment phase, with the presence and activity of this cytokine in two stages of inflammation (*i.e.*, initial and secondary phase). In other words, increasing the levels of IL-1 gene expression and, subsequently, the possible increase in IL-1 cytokines, triggers inflammatory and defensive reactions in the damaged tissue, and then, decreasing the levels of its expression; and subsequently reducing its potential concentration, leads to removing the inflammation and healing the tissue. The clinical and histopathologic

observations performed on the skin sample of mice (reported in study of Malekpour et al[16]) are consistent with these findings.

In general, study of levels of gene expression in skin samples of "mice with ICD" treated with different concentrations of the aqueous extract, regardless of the concentrations of extract, showed similar changes in the levels of IL-1 gene expression, and the clinical and reported histopathological conditions of the skin. These similar changes were as follows:

• Considerable differences in levels of IL-1 gene expression (severalfold increase, in comparison with the expression in "untreated healthy mice" group;

• A severalfold increase in the levels of IL-1 gene expression, which has been noticeable from the 4th day of the treatment period.

• High rate of elimination of inflammation and healing of the skin samples (reported in study of Malekpour et al[16]) in comparison with the control groups and groups treated with cutaneous corticosteroids;

• Highly desirable skin quality and a large number of hair follicles (observed in clinical and histopathologic examinations). Although the increase in the quality and number of hair follicles was associated with increasing the concentration of the extract);

Also, there were some differences among the levels of IL-1 gene expression in the skin samples of "mice with ICD" treated with different concentrations of the extract, as follows:

Although levels of IL-1 gene expression increased dramatically in some days in "mice with ICD" groups treated with extract with a relatively similar pattern, but in the treated group at a concentration of 1000 μg/mL, a decreased levels of IL-1 gene expression on the 6th day of the treatment, and again an increased levels of IL-1 gene expression by up to 5-fold of its expression in the sample of skins of heathy untreated mice group were observed. This difference in the fluctuation in levels of IL-1 gene expression in "mice with ICD" groups treated with different concentrations of the extract can be due to:

• Different effects of different concentrations on the levels of gene expression due to changing in amount and concentrations of extract components;

• Experimental error;

• The necessity of examining the gene expression in more samples and lower time intervals;

On the other hand, changes in levels of KGF/FGF-7 gene expression in mice with ICD treated with different concentrations of the extract (200, 1000, and 7000 μg/mL of extract), showed a relatively similar pattern of changes in KGF/FGF-7 gene expression in these three groups; *i.e.*: Inflammation in the skin of the mice has been associated with a high reduction in the levels of expression of this gene (about 0.2 of its expression in the skin samples of healthy untreated mice group), and after a gradual increase and decrease in its expression (around the 6th to 8th day), a peak is observed in the expression of this gene, which has reached its expression to about 0.6 to 0.8 that of its expression in the skin samples of the healthy untreated mice group; but its expression has never reached to that of its expression in this group. In fact, its expression at the end of the 10th day of treatment, was at a maximum of 0.4 of its expression in the skin samples of "healthy untreated mice" group.

Comparing the clinical and histopathologic conditions reported in Malekpour et al with the above mentioned results, suggests that increasing the speed of tissue healing and reduction of inflammation, have been associated with gradually increasing levels of KGF/FGF-7 gene expression and then its reduction in the final days of treatment. It is noticeable that during the 10 days of treatment period, the levels of KGF/FGF-7 gene expression has never reached to its expression in the skin sample obtained from the healthy untreated mice group.

In general, it can be said that a healthy, non-inflammatory, and healed skin from 6th to 10th days of treatment with extract in the study of Malekpour et al[16] indicates that the treatment of skin of mice with ICD with extract had a significant effect on the speed and quality of the healing process and removing the inflammatory condition of the skin, as well as the quality and quantity of the raised hair.

It seems that a high increase in the levels of IL-1 gene expression in the first half of the inflammatory process and the gradual increase in KGF/FGF-7 gene expression during treatment, and reaching to about 1.5-fold of its level of expression in the health status, is associated with a faster and higher quality of the process of removing the inflammation, healing the

skin, and the quality and quantity of raised hair, though in a gradual and upward trend.

Therefore, it can be argued that if a drug can provide such an alteration in levels of IL-1 and KGF/FGF-7 gene expression, it can be effective in healing and improving the cutaneous inflammation and increasing the quality and quantity of raised hair.

Considering the role of KGF/FGF-7 in processes such as cell growth and tissue regeneration, a gradual increase in the expression of this gene in this group of mice is expected and suggests that the induction of ICD in mice has been associated with a change in the expression of this gene involved in the process of inflammation and tissue healing. However, it seems that an increase more than that observed in the "mice with ICD" groups treated with extract, has not had a positive effect on the removing of inflammation, and tissue repair in other groups.

Mice with ICD treated with cutaneous corticosteroids: In the "Mice with ICD" group treated with cutaneous hydrocortisone, a rapid initial increase in levels of IL-1 gene expression was observed; so that on the second day of treatment phase, the expression rate was 6-fold, and on the 6th day it was more than 10-fold of its expression in the "healthy untreated mice". Then, with a gradual reduction trend, its expression on the 10th day of treatment phase was 3-fold higher than its in "healthy untreated mice" group. It seems that changes in the expression of this gene under the influence of treatment with cutaneous hydrocortisone follow the same pattern of expression changes in mice with ICD groups treated with the extract, with the exception that its initial rate of increase is much higher than that of the treated groups with extract is.

Also, treatment of the mice with ICD group with cutaneous hydrocortisone was associated with a rapid increase in levels of KGF/FGF-7 gene expression: its gene expression on the 4th day of treatment phase showed a 4.5-fold increase in comparison with its expression in the healthy untreated mice group, and then its expression declined slightly below its levels of that of the health status. While in mice with ICD groups treated with different concentrations of extract, although there was an increase and a decrease in the expression of this gene, but its expression was always lower than the health status.

In the mice with ICD group treated with fluocinolone topical, a rapid initial increase in levels of IL-1 gene expression was observed (similar to that of hydrocortisone-treated group), so that on the second day of treatment phase, the gene expression rate was 5.5-fold and on the 6th day, it showed about 7.8-fold increase in comparison with its expression in the healthy status. Then, with a gradual decline, its expression on the 10th day of treatment phase showed a 4-fold increase in comparison with the healthy status. It seems that changes in the levels of expression of this gene in group treated with topical fluocinolone were very similar to the changes observed in the mice treated with cutaneous hydrocortisone. Also it followed the same pattern of changes in levels of gene expression in mice with ICD groups treated with three different concentrations of extract; with the difference that its initial increasing rate was much higher than that of the groups treated with the extract.

In addition, treatment of mice with cutaneous hydrocortisone was associated with fluctuations in the KGF/FGF-7 gene expression levels. However, the direction of these fluctuations was toward to an increase in the expression of this gene, so that on the 10th day of treatment phase, its expression showed about 3-fold increase in comparison with that in the health status. While in mice treated with different concentrations of the extract, there was an increase and decrease in expression levels of this gene, but levels were lower than that in the healthy status at all times.

The report of Malekpour et al on the apparent and histopathologic conditions of the skin of the mice in the two groups treated with cutaneous corticosteroids suggests that, although at the end of the 10th day of treatment phase, the skins were completely treated, but they were thin and the raised hair was low and fragile. In addition, in terms of quantity and quality, they did not show the favorable conditions of the "mice with ICD" groups treated with extract.

It seems that the pattern of IL-1 and KGF/FGF-7 genes expression levels in the skin samples obtained from the "mice with ICD" groups treated with three concentrations of the aqueous extract of *Trachyspermum copticum (L.) Link* Seeds, especially the concentration of 7000µg/mL, were the best possible state for the treatment of mice model of

irritant contact dermatitis.

Conclusion

Investigating the changes in the IL-1 and KGF/FGF-7 genes expression levels in the skin from mice model of irritant contact dermatitis and comparing them with clinical evidence and histopathologic findings reported in study of Malekpour et al, showed the promising effects of aqueous extract of *Trachyspermum copticum (L.) Link* Seeds in treatment of skin in these mice. In fact, in this study, significant changes in the IL-1 and KGF/FGF-7 genes expression levels in the skin samples with inflammation, were associated with an increase in the rate and speed of improvement of contact dermatitis, more favorable conditions of the healed skin (in terms of color, consistency, and thickness), and a remarkable increase in the number of hair grown on the site of dermatitis (compared with control groups, and even groups with corticosteroid therapy).

A thorough and comprehensive molecular, clinical and histopathological study of anti-inflammatory and healing effect mechanisms of aqueous extract of *Trachyspermum copticum (L.) Link* Seeds and the relationship between changes in the expression of genes associated with inflammation, healing, and hair growth, including IL-1 and KGF/FGF-7 cytokines, is suggested. Also, given that the *Trachyspermum copticum (L.) Link* Seeds is used in traditional medicine in countries such as Iran, India, and Egypt, a clinical trial to investigate the anti-inflammatory and healing effects of this extract on the irritant contact dermatitis in human is suggested.

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