The effect lanolin extract and vaseline with glycerine mixture to treatment by affecting of IL-4 and IL-13 level in induced eczema in rats

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Abstract---The goal of this study was to assess the healing effectiveness of lanoline (also known as wool waxes or wool grease), an oil compound released by skin sebaceous glands for sheep that possesses characteristics against microbial, inflammation, as well as protecting properties for skin. It also has an excellent moisturizing function. The determination of Interleukin-4 and Interleukin-13, it can be determined due to the accumulation of inflammatory cells in the affected area that signal the production of Interleukin-4 and Interleukin-13 in cases of eczema. Both the increase in IL-4 and IL-13, reduces synthesis of essential structural proteins such as filaggrin and lipid components required for effective skin barrier activities, resulting in increased trans epidermal water loss, which is commonly observed and expected in eczema. The study showed a significant increase in the eczema and glycerine group for 24 hours for both IL-4 and IL-13, where there was a slight decrease in the 24-hour lanolin group compared to the control group as well as between groups. The present results showed an optimal tissue recovery in the 72-h lanolin group, so there was a significant decrease in the 72-h lanolin group compared to the control group as well as between groups for IL-4 and when compared to the control group, IL-13 levels increased considerably, simply not in glycerine groups.

Keywords---IL-4, IL-13, lanolin, vaseline, rats.
Introduction

Lanolin, wool grease or wax is a mixture of unconjugated sterols, sterol esters and other fats secreted in the fibres of the woolen coat animals by the sebaceous glands, it is driven from Romani words, Lana means wool and oleum means oil (Jones, et al., 2017). Lanolin is a wax-like material that is light yellow in colour including obvious emollient and soothing properties. It melts at 36 °- 42 °C (Allafi et al., 2020).

Glycerine is a non-volatile, clear, colourless, and odourless viscous liquid with a high hygroscopicity; it can crystallize but rarely does due to its tendency to super cool and the significant effect of tiny amounts of water on lowering the freezing point (Becker, et al., 2019). Atopic dermatitis (eczema) is primarily caused by type 2 helper T (Th2) lymphocytes, with significant contributions from Th17 and Th22 cells that release cytokines, resulting in increased IgE production and skin inflammation (Torres et al., 2019).

Interleukin (IL-13 and IL-4) are pleiotropic cytokines that give 20–25% of the effect of the same functions in type II immune response (May et al. 2015), and since atopic dermatitis is a relapsing, chronic inflammatory skin disease. The underlying inflammation in (AD) is revealed by molecular characterization, with tissue infiltration of T helper (Th) 2 cells and increased IL-4 and IL-13 levels (Brøgger et al. 2020).

The role of Interleukin-4 and Interleukin-13 in Eczema due to the T2 cytokines have a considerable influence on the barrier functions of the skin and immune response by activation of STAT 6, has been demonstrated that IL-13 and IL-4 obviously lower the expression of essential structural proteins such as filaggrin, keratin 1, filaggrin 2, etc., in addition to the composition of lipids required for normal barrier function in skin. This leads to increased trans epidermal water loss (TEWL), which is commonly measured and may even indicate AD (Al-Haidary and Naqi, 2020; Bieber, 2020).

Jointly Interleukins 13 and 4 are considered pruritogen, can stimulate peripheral itch-sensory neurons. The previously discussed mechanistic involvement of IL-13 in skin inflammation, when combined with an increase in IL-13 expression in the skin comparing to IL-4, provides evidence that AD is an IL-13-driven disease (Oetjen et al. 2017).

Materials and methods

Thirty mature female albino rats weighing (160g – 230g), The experimental animals were housed in the animal house in a typical situation in Faculty of Veterinary Medicine - University of Kufa, the animals housed in well ventilated wire-plastic cages with metal covers and containing bedding of sawdust which was changed once per daily and exact circumstances to the normal laboratory nutrition with appreciated diet (pellets) and water provided to animals through the all-time of the experiment, for 2 weeks before the experiment started, to adapt rats to the new environment.
Acetone is used to induce eczema, and it is readily caused. It produces quick effects in 24 hours, with perfect eczema on day six. Also, Interleukin 4 and Interleukin 13 kits were used to evaluate IL-4 and IL-13 level in blood serum (Zhou and Sui, 2019).

An experimental design has been applied to thirty matured Albino rats. Eczema induction was done by 99.9% pure acetone. Apply squad cotton to the back’s hairless skin rats. After six days of acetone application on the hairless backs of rats, the eczema group was randomly divided into three groups, each containing ten rats. The first two groups were treated with glycerin and lanolin. After 24 hours, these ten rats were sacrificed, and the last ten rats were treated with glycerin and lanolin for 72 hours. The third group (eczema positive control) had five rats.

**Statistical analysis**

The statistical analysis of experimental results was concluded according to SPSS version 26 were one way (ANOVA) test to compare between different groups and Paired Sample T test to compare between same group at significant level $p \geq 0.05$ (Hussien and Al-Sharafi, 2020).

**Results**

Determination the level of Interleukin-4 serum samples results that used in this study show there is a (significant increase) after 24 hours of glycerine application compared to the control group. There is also a (significant increase) after 24 hours of lanolin extract application compared to the control group. As well, after 72 hours of glycerine application, there is a significant decrease from the control group, and a significant decrease between groups, whereas after 72 hours of lanolin extract application, there is a significant decrease from the control group and a significant decrease between groups in the level of Interleukin-4.

![Inter-4](image)

Figure 1. Determination of Interleukin 4 levels (pg/ml)
• significant difference from control group $p < 0.05$ with one-way anova test
• significant difference from eczema 24-hour group $p < 0.05$ with paired $t$ test
• significant difference from lanolin extract 24-hour group $p < 0.05$ with paired $t$ test
• significant difference from glycerin 24-hour group $p < 0.05$ with paired $t$ test

Determination the level of Interleukin-13 serum samples results that used in this study show there is a (significant increase) after 24 hours of glycerine application compared to the control group. There is also a (significant increase) after 24 hours of lanolin extract application compared to the control group. While there is a significant decrease from the control group after 72 hours of glycerine application, there is also a significant decrease among groups, while there is a significant increase from the control group after 72 hours of lanolin extract application.

**Figure 2. Determination of Interleukin 13 levels (pg/ml)**

- significant difference from control group $p < 0.05$ with one-way anova test
- significant difference from eczema 24-hour group $p < 0.05$ with paired $t$ test
- significant difference from lanolin extract 24-hour group $p < 0.05$ with paired $t$ test
- significant difference from glycerine 24-hour group $p < 0.05$ with paired $t$ test

**Discussion**

Interleukin-4 is a glycosylated type I cytokine released by T helper 2 cells, type-2 cytokine-secreting CD8 plus T lymphocytes, NK T cells (Natural killer T), γ/δ T cells (Gamma delta T cells), ILC2 (innate lymphoid cells type 2), eosinophils, activated basophils, and masts cells (Paller et al., 2017).
During eczema the present results indicates that there is increase IL-4 level so that IL-4 has the centrality in Atopic Dermatitis pathogenesis, the focus is on its ability to induce all AD histopathological features. The cytokine interleukin 4 is high in patients with atopic eczema. Overexpression of IL-4 induces IgE production, enhances skin inflammation, favors bacterial skin infections, mediates pruritus in addition to its effects on immune cells, IL-4 also affects keratinocyte differentiation and its activation of innate immunity (Chiricozzi et al., 2020).

While after lanolin application the results indicates that there was decease in IL-4 level, because of damage repair by action of lanolin, it will create a moist atmosphere by creating a semi-occlusive barrier, preventing the wound from drying out (water loss) and accelerating re-epithelialization and proliferation of fibroblasts (Akbik et al., 2014). Also, there was expression of gene, because of damage repair by action of lanolin (Landeck, et al., 2014).

Lanolin, with skin protecting, reduce inflammation, antimicrobial, and barrier repairing properties, long history of beneficial use and safety in topical formulations was had (Dennis et al., 2014). In atopic dermatitis, due to action of interleukins which sent a signal to inflammatory cells to accumulate in the affected area in eczema cases (Kilanowicz, et al., 2019). At first period of experiment (24h.) the glycerin had been having higher interleukin level than lanolin at the same period because of the reconstructive action of lanolin on skin tissue and decrease inflammation while, as the present results indicate there are decrease IL-4 level in glycerin group of second period (after 72h.) also there are decrease between groups. When compared to untreated skin, the use of a glycerin-based moisturizer resulted in a quick hydration effect (Purnamawati et al., 2017), but in the lanolin 72h group has decreased the level IL-4 at the same period compared to control group, as well as between groups due to reconstructive effect on destroyed tissues, as well as lanolin reduce the inflammatory cells in veins underneath the skin due to its anti-inflammation activity (Shanazi, et al., 2015).

At first period of experiment (24h.) the glycerin elevation IL-13 level than lanolin at the same period because of the reconstructive action of lanolin on skin tissue and decrease inflammation while, as the present results indicate there are depression IL-13 level in glycerin group of second period (after 72h.) also there are depression between groups. When compared to the untreated skin of rats, the application of a glycerin containing moisturizer resulted in a faster hydrating effect (Purnamawati et al., 2017). But the lanolin has increase at the same period compared to control group, as well as between groups due to reconstructive effect on destroyed tissues, as well as lanolin reduce the inflammatory cells in veins underneath the skin due to its anti-inflammation activity (Shanazi, et al., 2015).

**Conclusions**

Eczema in rats can be induced by 99.9% pure acetone. Lanolin is very useful in the treatment of eczema in rats.
References


