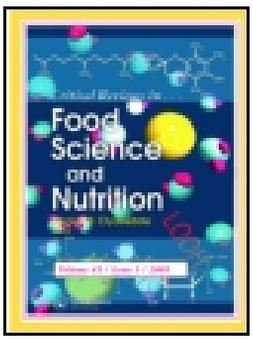

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A Systematic Review of Lactoferrin Use in Dermatology

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Abstract:

Lactoferrin is a glycoprotein widely present in mammalian secretions and possesses documented protective effects, including antimicrobial and anti-inflammatory properties. While its therapeutic use is being investigated for a myriad of diseases, there is increasing interest in its application for skin disease. Our objective was to systematically review the clinical evidence for the use and efficacy of lactoferrin for the treatment of dermatological conditions. Pubmed and Embase databases were searched for clinical studies evaluating lactoferrin for dermatological conditions. A total of 6 studies were reviewed. Of the current clinical trials, there is encouraging evidence to suggest that lactoferrin may be beneficial in acne, psoriasis, and diabetic ulcerations. Although the current evidence is promising, further research is necessary to establish lactoferrin as complementary therapy in the clinical setting.

Keywords: alternative, skin, milk, acne, psoriasis, ulcer

Introduction

Lactoferrin (LF) is a non-heme iron-binding glycoprotein that is part of the transferrin family of proteins. While one of its main functions is to transport iron in blood, LF possesses a range of protective effects(Yalcin 2006). Specifically, LF is produced by mucosal epithelial cells and is present in most biological fluids, including tears, saliva, vaginal fluids, semen, nasal and bronchial secretions, bile, gastrointestinal fluids, urine, and most abundantly in milk and colostrum(Yalcin 2006, Legrand, Pierce et al. 2008). Additionally LF is present in significant amounts in polymorphonuclear granules, and its net positive charge and distribution in various tissues allow it to play a role in several physiological processes. These include regulation of iron absorption in the bowel, immune response, as well as antimicrobial, antioxidant, anti-carcinogenic, and anti-inflammatory properties(Schanbacher, Goodman et al. 1993, Yalcin 2006, Zimecki and Kruzel 2007, Ng, Cheung et al. 2015).

Several in vitro and in vivo studies have demonstrated LF's ability to protect against microbial infections. Specific to bacteria, it is well documented that LF exhibits an inhibitory effect against several Gram-positive and Gram-negative species, including some strains (e.g. *Staphylococcus aureus, Listeria monocytogenes, and Klebsiella pneumonia*) that are antibiotic resistant(Ellison, Giehl et al. 1988). The mechanisms through which LF exerts its therapeutic effects are both bacteriostatic and bactericidal in nature. Its bacteriostatic effect is mediated by its ability to bind the Fe^{3+} ion, which consequently limits the use of this nutrient by bacteria locally at the site of infection as well inhibits their growth systemically and the expression of their virulence factors in the host organism(Coughlin, Tonsager et al. 1983, Ellison, Giehl et al. 1988). On the other hand, LF mediates its bactericidal effect by directly interacting with the

bacterial surface. Specifically, LF damages the external membrane of the Gram-negative bacteria by interacting with the lipopolysaccharide (LPS)(Ellison and Giehl 1991). This interaction then prevents the LPS from binding other bacterial cations (i.e. Mg^{2+} and Ca^{2+}), causing a release of LPS from the cell wall, increased permeability of the cell wall and ultimately damage to the bacteria(Ellison and Giehl 1991). LF's mechanism of action against Gram-positive bacteria involves it binding due to its net positive charge to anionic molecules on the bacterial surface. This causes a reduction in the overall negative charge and facilitates more interaction between lysozyme and the intrinsic peptidoglycan layer over which it exerts an enzymatic effect(Leitch and Willcox 1999). In addition to the respective mechanisms that LF utilizes in destroying Gram-negative and Gram-positive bacteria, in vitro and in vivo studies have also shown that LF has the ability to prevent the attachment of bacteria to host cells (Ellison, Giehl et al. 1988).

Bacteria are not the only class of pathogens that LF has demonstrated activity against. In fact, LF possesses anti-viral activity against a wide range of RNA and DNA viruses that infect both humans and animals(Ng, Cheung et al. 2015). For example, LF exerts strong activity against respiratory syncytial virus, adenoviruses, enteroviruses, and HIV(Viani, Gutteberg et al. 1999, Seganti, Di Biase et al. 2004). While the exact anti-viral mechanisms have not yet been elucidated, one of the leading hypotheses is that LF binds to and blocks glycosaminoglycan viral receptors, most notably heparan sulfate (HS). It is believed that the binding of LF and HS prevents the first contact between the virus and the host cell and therefore prevents infection(Hasegawa, Motsuchi et al. 1994, Marchetti, Superti et al. 1999, Beljaars, van der Strate et al. 2004).

Similar to its effect on bacteria, LF's ability to sequester Fe^{3+} is one of the ways in which

it acts as an anti-fungal and anti-candidal agent(Kirkpatrick, Green et al. 1971). LF alters the membrane permeability in both *Candida albicans* and *Candida krusei(Bellamy, Wakabayashi et al. 1993)*. LF's mechanisms of action take place via direct and indirect interactions with several different fungal pathogens that have been studied including *Aspergillus fumigatus* and *Trichophyton mentagrophytes*(Wakabayashi, Uchida et al. 2000, Zarember, Sugui et al. 2007). For example, it was shown that Fe^{3+} sequestration by neutrophil apo-LF (free of Fe^{3+}) is important for host defense against *Aspergillus fumigatus*(Zarember, Sugui et al. 2007).

Although most of the studies on LF's anti-parasitic effects have been done in vitro, LF does hold promising hope as a therapeutic for parasitic infections, including intestinal amebiasis, caused by *Entamoeba histolytica* and one of the leading causes of dysentery worldwide. Similar to the mechanism LF exerts on bacteria and viruses, apo-LF is the milk protein with the greatest inhibitory effect for *E. histolytica* in vitro, in which it binds to the lipids present on the trophozoite's membrane and consequently causes membrane disruption and parasite death(Leon-Sicairos, Lopez-Soto et al. 2006).

In addition to its antimicrobial properties that it exerts on a broad range of pathogens, LF modulates the innate and acquired immune systems. LF's positive charge allows it to bind to negatively charged molecules on the surface of various cells of the immune system which is thought to trigger different pathways that lead to cellular responses such as activation, differentiation and proliferation(Breton-Gorius, Mason et al. 1980, Legrand, Elass et al. 2006). LF demonstrates anti-inflammatory properties by inhibiting several pro-inflammatory cytokines such as interferon-gamma (INF-γ), tumor necrosis factor-alpha (TNF-α), and interleukin (IL)-1β, IL-2, and IL-6(Crouch, Slater et al. 1992, Griffiths, Cumberbatch et al. 2001).

Similar to inflammation, LF exhibits the ability to modulate the production of cytokines with regards to cancer. Treating tumors in mice with recombinant human LF (rhLF) inhibits their growth by 60% compared with a placebo and increases the levels of anticarcinogenic cytokines such as IL-18, in addition to activating natural killer cells and $CD8^+$ T-lymphocytes(Wang, Iigo et al. 2000). Moreover, in vivo studies demonstrate that oral administration of LF causes an inhibition of T-cell dependent tumors in head and neck squamous cell carcinomas(Wolf, Li et al. 2007).

Due to LF's various functions, it is increasingly being tested and sought out for potential clinical applications. These include being used as a supplement in infant formula to help promote iron absorption and protect the host from harmful bacteria(Saarinen and Siimes 1977, Siimes, Salmenpera et al. 1984), as a second line treatment for *Helicobacter pylori* infection(Tursi, Elisei et al. 2007), and as adjuvant therapy with several different anti-viral drugs including ribavirin, cidofovir, and zidovudine in the treatment of HCV, CMV, and HIV, respectively(Viani, Gutteberg et al. 1999, Kaito, Iwasa et al. 2007). Therefore, there is growing interest in the potential use of LF as medical therapy. While LF holds promise for a variety of medical entities, there is need to better understand the current clinical evidence for its use. Here, we review the scientific evidence for its use in the treatment of dermatological conditions.

Methods

Search Strategy: Pubmed and Embase databases were systematically searched on August 14, 2015 for clinical trials, comparative studies, evaluation studies, observational studies, randomized controlled trials, and validation studies evaluating the use of lactoferrin or lactotransferrin in skin diseases. In Pubmed the Mesh terms used were, "lactoferrin,

lactotransferrin, skin diseases, therapeutic use, and drug therapy." Embase was searched using Emtree terms: "lactoferrin, lactotransferrin, humans, and skin disease." Searches were filtered to return only studies published in English. Two independent investigators evaluated the search results and any discrepancies were discussed.

Study Selection: Abstracts were reviewed based on predefined inclusion and exclusion criteria. When necessary, full texts were retrieved to assess study eligibility. The inclusion criteria were: studies in humans, studies of a skin related condition, and those written in English.

Results/Discussion

The efficacy of LF for dermatological conditions is summarized in Table 1 and a total of 6 studies were eligible for review.

Acne Vulgaris

Two studies have evaluated the use of LF for acne vulgaris. One such study was a randomized clinical trial in which 36 subjects with mild to moderate acne were assigned to drink fermented milk containing probiotics (*L. bulgaricus* and *Streptococcus thermophilus*) supplemented with LF (200 mg) daily or to fermented milk containing probiotics only (placebo group) (Kim, Ko et al. 2010). In order to determine treatment efficacy, inflammatory and total lesion counts (ILC and TLC, respectively) were recorded at baseline and after 4, 8, and 12 weeks of treatment. In addition, clinical assessment of the subjects' acne severity was evaluated at the same time points according to the Leeds revised acne system. Secondary end points included varying measurements of the skin including sebum content, skin hydration and skin pH, as well

as analysis of the lipid profile in the LF group versus placebo group, and their changes if any over time.

At the end of the 12-week study, investigators found that the LF group had a decrease in ILC compared to placebo (69.8% versus 31.2%, p= 0.019), decrease in TLC compared to placebo (56.3% versus 33.2%, p= 0.033), and decrease in acne grade compared to placebo (37.3% versus 17.0%, p= 0.023). Importantly, despite the relative gender imbalance of subjects between the LF group (n=18, 3 men and 15 women) and the placebo group (n=18, 10 men and 8 women), gender was not the significant discriminating factor for the changes of acne lesion count and acne grade in each group, indicating that there was no gender effect on changes of ILC, TLC, and acne grade between the LF and placebo groups over 12 weeks.

Skin hydration and pH of both groups remained unchanged during the study period with no significant differences between the treatment groups. However, the sebum content in the LF group decreased significantly compared to the placebo group (80.5% versus 49.4%, p =0.043).

Epidermal lipids obtained by tape stripping decreased significantly in both the placebo and LF groups. Notably, of the major skin surface lipids, the amounts of triacylglycerols (TGs) and free fatty acids (FFAs) decreased significantly in the LF group, whereas the amount of FFAs decreased only significantly in the placebo group from baseline to the end of the 12-week study. Moreover, the decreased amount of TGs in the LF group was significantly correlated with decreases in sebum content ($r = 0.684$, $p = 0.007$), ILC ($r = 0.573$, $p = 0.032$), TLC ($r = 0.680$, $P = 0.007$), and acne grade ($r = 0.607$, $P = 0.021$).

This study had several limitations. It is not clear how the sebum content correlates with the collected lipids because the authors utilized tape-stripping and likely collected significant

epidermal lipids rather than sebum. This study utilized fermented milk as the control group but a more appropriate control would have been to compare LF against placebo without fermented milk. These findings suggest that the probiotics themselves as part of fermented milk may play a role in ameliorating acne symptoms.

Another study evaluated LF as treatment for mild to moderate acne in an open-label, single arm study with 39 subjects who consumed a chewable tablet formulation of bovine LF (100 mg) twice daily for 8 weeks(Mueller, Trapp et al. 2011). There was a statistically significant decrease in the mean non-inflammatory and mean total lesion count from baseline. Specifically, after 8 weeks of LF supplementation, mean improvements in total lesion counts was 22.5% ($p < 0.001$), and 30 out of 39 subjects (76.9%) had a reduction in total lesion count from baseline. The remaining subjects (9 out of 39) experienced an overall increase in total lesion count ranging from 2 to 53% more lesions. However investigators did not witness a statistically significant decrease in the endpoint. This discrepancy may be due to the fact that the open-label, single arm study was shorter in duration (8 weeks versus 12 weeks), which may have been too short for such an endpoint. Other secondary parameters of skin assessment such as erythema, oiliness, scaling, edema, vesicles, and weeping did not significantly change.

Taken together, these two studies suggest that LF may be useful for acne but future studies are needed for more definitive conclusions. Future studies will need to utilize placebo controls to better isolate the effects of LF and the study should be conducted over at least 12 weeks.

Psoriasis

LF supplementation has been studied for psoriasis, a chronic inflammatory skin condition. In an open label study 5 grams of XP-828L, a protein extract consisting of α-lactalbumin, LF, and immunoglobulins among other growth factors, was orally administered twice daily for 8 weeks in adults with mild to moderate chronic plaque psoriasis(Poulin, Pouliot et al. 2005). The psoriasis severity was followed through the Psoriasis Area Severity Index (PASI). Seven out of 11 subjects experienced a decrease in their Psoriasis Area Severity Index (PASI) score with an overall improvement of approximately 21% (Table 1). Two subjects achieved PASI 50 at 8 weeks while one achieved PASI 75. In addition, 7 out of 11 subjects who completed the 8-week study agreed to participate in an optional 8-week extension treatment period designed to demonstrate the safety and efficacy of long-term LF treatment. One subject maintained PASI 50 from the 8-week study while another subject newly achieved PASI 75.

Secondary endpoints (PGA [physician global assessment], patient's global assessment, pruritus) did not change significantly for the majority of subjects during the study, including the extension period. While XP-828L proved to be well tolerated and there were no clinically significant adverse events during the trial, further double-blinded, randomized clinical trials are necessary in order to evaluate its efficacy and potential use in the treatment of psoriasis.

Another study evaluated the role of topical and oral LF in a 4-week trial that included 30 subjects affected by mild to moderate plaque psoriasis(Saraceno, Gramiccia et al. 2014). They were assigned to two split-body treatment groups: Group A applied 10% LF ointment and vehicle control while Group B applied 20% LF ointment and vehicle control. Both groups received oral bovine LF 100 mg twice a day. The efficacy was evaluated using the Target Lesion Score (TLS), which assessed the parameters of erythema, scaling, and infiltration, each one

ranked on a four point scale (0= none, 1= mild, 2= moderate, 3= severe). At the study end, the mean TLS of the LF treated psoriatic target lesion improved by 23.5% at week 2 and by 37.3% at week 4 in group A and by 25.8% at week 2 and by 35.5% at week 4 in group B. These results were statistically significantly improved compared to the contralateral psoriatic plaques treated with vehicle controls, although the % improvement of the control treated sides were not reported. Moreover, there was no additional improvement seen with increasing the topical concentration from 20% versus 10% topical formulations. Also, there was marked improvement of itch from baseline to week 4 (Visual Analogue Scale [VAS] score: from 5.8 to 3.2) in the target bovine LF treated lesions, compared to the control lesions (VAS score at week 4 was 5.1).

Therefore, despite the relatively small sample size in this pilot study, the investigators noted clinical improvement with the use of topical bovine LF. This study showed that bovine LF at 100 mg twice daily was not effective for psoriasis over the 4-week duration studied. Future studies should evaluate a long duration and consider utilizing a higher dose of LF.

Tinea Pedis

LF has been evaluated for its role in tinea pedis as well. In a randomized, double-blinded placebo-controlled trial, subjects with mild to moderate tinea pedis were given oral doses of either 600 mg or 2000 mg of LF or placebo daily for 8 weeks(Yamauchi, Hiruma et al. 2000). Clinical improvement was assessed by evaluating the parameters of itching, erythema, vesicles/pustules, maceration/erosion and scales, each of which was graded on a four point scale. These individual scores were combined to calculate a total score.

A statistically significant improvement in the clinical score was only observed in the LF treated groups compared to placebo when subjects were limited to having either moderate

vesicular or interdigital tinea pedis ($p < 0.05$). There was no significant difference in clinical improvement between the groups treated with either 600 mg or 2000 mg LF daily. However, a mycological cure was not seen in any of the subjects after the species *Trichophyton rubrum* and *Trichophyton mentagrophytes* were isolated.

The utility of LF for tinea pedis remains unclear. The lack of mycological cure suggests that LF is not likely to have a lasting improvement in tinea pedis.

Diabetic Ulcer

Topical talactoferrin gel has been studied in the treatment of chronic, non-healing diabetic foot ulcers(Lyons, Miller et al. 2007). The study consisted of a first dose ranging phase and the second phase involved the use of 2.5% and 8.5% gels that were compared against placebo. The primary endpoint was ≥75% reduction in ulcer size and the authors conducted a power analysis with significance set at $p<0.1$. A significantly higher proportion of subjects assigned to either the 2.5% or 8.5% study drug (50%) achieved the primary endpoint compared with the placebo group (25%) ($p = .091$). The frequency of patients achieving complete healing of target ulcer at the end of treatment was similar in both the combined study drug and placebo groups were 20% and 19%, respectively. However, at 30 days post-treatment, the incidence of complete healing of the ulcers trended to be higher in the combined study drug groups (33%) compared with placebo (19%), and this remained higher in the study group than placebo at 90 days post treatment, 30% and 19%, respectively (p value reported as not significant). This pilot study shows promising results for talactoferrin and future studies should further investigate its use as adjuvant therapy for diabetic ulcers.

In addition to its study in the context of the aforementioned dermatological conditions, LF may serve a future therapeutic role in other diseases, namely atopic dermatitis. This stems from the fact that LF influences differentiation of CD4-positive T helper lymphocytes (Th cells) and the maturation into subtypes Th1 or Th2 cells. Specific to Th2-mediated atopic diseases, LF is thought to destabilize tryptase release from mast cells, although further studies are needed to elucidate whether LF plays a role in correcting the Th1/Th2 imbalance, a known mechanism by which LF alleviates the symptoms of autoimmune and allergic diseases. (Fischer, Debbabi et al. 2006)

Conclusion

Overall there are only a handful of clinical studies that have evaluated LF for dermatological conditions. Nevertheless, the studies showcase promising results regarding the use of LF for acne, psoriasis, and diabetic ulcerations. The data does not appear to support its use for tinea pedis. Larger randomized clinical trials are necessary in the future to better define the role of oral and topical LF.

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Table 1: Summary of clinical studies evaluating the use of lactoferrin in dermatology

Study	Intervention	Design	Subjects	Comparison	Major Outcome Measures	Major Results	Limitations	Level of evidence
Kim, et al.	Fermented milk containing probiotics *L. bulgaricus* and *Streptococcus thermophilus*) with 200 mg of LF daily	RCT* for 12 weeks	N= 36, with mild to moderate acne vulgaris of the face	Placebo (fermented milk containing probiotics only)	• Inflammatory lesion count • total lesion count • Acne grade (Leeds revised acne grading system) • Sebum content • Skin hydration • Skin pH • Total skin	• In LF group, significant decrease in inflammatory lesion count by 38.6%, total lesion count by 23.1%, and acne grade by 20.3% compared with placebo	Systemic effect and precise mechanism of action of LF on acne remains to be elucidated	1B

					surface lipids	group at 12 wk. • Sebum content in LF group was decreased by 31.1% compared with placebo group. • Of the major lipids, amounts of triacylglycerols and free fatty acids decreased in the LF group, whereas the amount of free fatty acids decreased only in the placebo group.		

Lyons, et al.	Phase 1: 1%, 2.5%, or 8.5% talactoferrin gel twice daily, in a sequential design, in combination with standard would care to diabetic patients with chronic, non-healing foot ulcer for 30 days Phase 2: 2.5% and 8.5% gels in combination with standard wound care, was administered topically twice daily to chronic, non-healing diabetic foot ulcers for 12 weeks.	Phase 1: open-label, sequential, dose-escalation design. Phase 2: single-blind, randomized, stratified, placebo-controlled pilot study to evaluate the efficacy of the 2 highest dose levels (2 highest doses from study phase 1 were	N= 55; adults with diabetes mellitus with an HbA1C between 6% and 13%, and 1 or more diabetic neuropathic foot ulcers at or below the ankle that had not healed or decreased in size (≥30%) within the prior 4 weeks despite appropriate	Phase 1: None Phase 2: placebo	Incidence of ≥75% healing (relative to baseline size).	• On an intent-to-treat basis, the combination of the 2 active groups when compared with the placebo group showed a strong trend toward statistical significance (P = .09).		1B

		chosen) below the maximum tolerated dose (if any, up to 8.5% talactoferrin gel) of topically applied ta-lactoferrin compared with placebo.	standard treatment.					
Mueller, et al.	100 mg chewable tablet formulation of bovine LF twice daily for 8 weeks	Open-label, single arm study	N= 43, adolescents and young adults with mild-moderate acne	None	• Improvement in acne lesion counts compared with baseline • Change in skin status scores compared with baseline	• Mean reduction in inflammatory lesion count of 20.2% (-2.2 ±7.0, p= 0.054), • Mean reduction in non-inflammat	Future randomized, placebo-controlled trials are needed to assess true efficacy	2B

					• Visual ranking results of photographs	ory lesion count of 23.5% (-6.2 ± 9.8, p<0.001), and • Mean reduction in total lesion count of 22.5% (−8.4 ±13.1, p <0.001) was observed as compared with baseline. • 76.9% (30 of 39) of subjects showed a reduction in total lesion count. • Inflammatory acne lesions were variable		

						over the study course.		
Poulin, et al.	Oral administration of 5 grams of XP-828L (a protein extract derived from sweet whey and consisting of α-lactalbumin, LF, and immunoglobulins among other growth factors) twice daily for 56 days (with an 8 week extension treatment phase)	Open-label study	N= 11; adult patients with stable plaque psoriasis on 2% of body surface or more	None	• Psoriasis Area Severity Index (PASI) score • Physician's Global Assessment (PGA) • Percentage of Body Surface Area (BSA) involved by psoriasis	At end of the study, 7 out of 11 subjects had a decrease in PASI score ranging from 9.5% to 81.3%	• No control used • Study evaluated efficacy of XP-828L, a nutraceutical compound containing LF, rather than LF in its pure form	2B
Saracen o, et al.	• All pat ien ts rec eiv	Open-label, bilateral-paired controlle	N= 30; adults with stable and symmetr	All patients applied only ointment vehicle	• Target Lesion Score (TLS) • Psoriasis Area and	• In both groups A and B: improvem ent in elevation,	• Small sample sizes of cases enrolle	2B

	ed oral bovine LF (bLF) 100 mg • 15 patients (group A) were topically treated with 10% LF	d study	ical mild to moderate plaque psoriasis for at least one month and involving < 10% body surface area	on contralateral target lesion as intra-patient side to side control.	Severity Index (PASI) score (for inclusion criteria)	redness and scaling was observed on LF treated psoriatic target lesions comparing to the contralateral controls (P<0.05). • There was no additional efficacy for 20% versus 10% topical applications. • Mean TLS improved by 37.3% in group A and by 35.5% in group B at week 4 (statisticall	d • Absence of control group not taking oral bLF • Short period of the study • Low doses of oral bLF	

	oin tm ent , 15 pat ien ts (gr ou p B) wit h 20 % LF oin tm ent .					y significant $p < 0.05$ Wilcoxon two sample test)		
Yamau chi, et al.	Doses of either 600 mg or 2000 mg of LF, or a placebo was orally administered daily for 8 weeks	RCT	N= 37; adults with mild to moderat e tinea pedis	Placebo	Dermatological improvement (a five-grade scale) and anti-fungal efficacy	No statistically significant differences in dermatological improvement or antifungal efficacy comparing the three groups	Weather may have affected symptom scores in both the placebo and LF-treated groups	1B

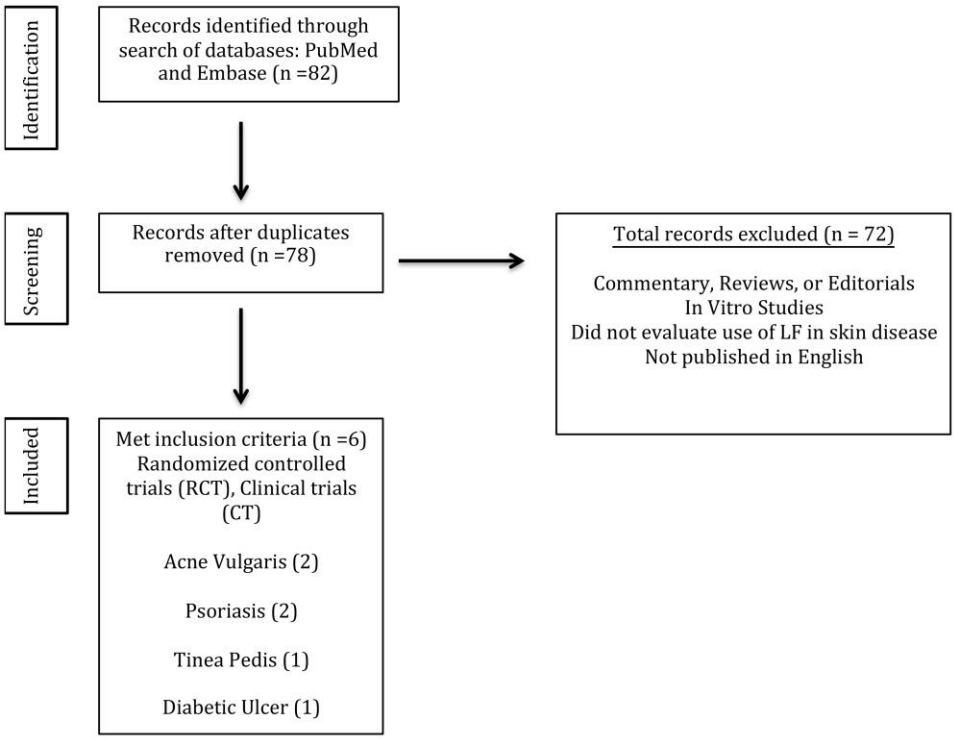

Figure 1: Flow chart of systematic search selection process