A comparative study for the topical treatment of atopic dermatitis with *Aloe ferox* and *Aloe vera* in Balb/c mice.

by
Marike Finberg

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Supervisor: Dr. G. Muntingh

Department of Pharmacology
School of Medicine
Faculty of Health Science
University of Pretoria
Prívate Bag X323
Arcadia
Pretoria
0007

Fax: 012- 319 2411
Tel: 012- 319 2143

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Declaration

I MJ Finberg declare that this dissertation is the product of my own work, that it has not been submitted before for any degree or examination in any other university, and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

Signed…….. Date:…….

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Abstract

Atopic dermatitis (AD) typically develops in patients with a history of allergic ailments, and is characterised by an itchy, inflammatory skin condition with scaling, lichenification, papules, excoriations and pruritus. In AD patients a chronic relapsing inflammatory condition is seen, associated with IgE hyper production. AD flares are largely triggered by environmental factors. However, the exact etiology of AD is unclear and there is a pressing need for new treatment regimens as AD is a chronic condition and requires long term treatment. Historically *Aloe* has been used to treat skin conditions as well as a variety of other diseases. To further explore the pathogenesis and treatment of AD, Balb/c mice were sensitized and challenged with 2,4-dinitrochlorobenzene (DNCB) for atopic dermatitis induction. Thereafter, mice were treated with either *Aloe ferox* or *Aloe vera* applied daily on the dorsal skin for 10 consecutive days. A placebo gel was used for the control mice. Blood was collected at the end of the treatment period and serum IgE levels measured. Serum IgE levels were significantly lowered in the *Aloe ferox* group than in the *Aloe vera* group. This study demonstrated *Aloe's* immunoregulatory potential for alleviating atopic dermatitis through influencing of Th2 cell activation.

Key words: Atopic dermatitis, Balb/c mice, treatment, corticosteroids, Aloe ferox, Aloe vera, Dinitrofluorobenzene, Adverse Event.

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List of abbreviations

AD	Atopic dermatitis
AE	Adverse Event
AEDS	Atopic eczema/dermatitis syndrome
Aloe ferox	*A. ferox*
Aloe vera	*A. vera*
APOC1	Apolipoprotein C1
cAMP	Cyclic adenosine monophosphate
DCs	Dendritic cells
DNFB	Dinitrofluorobenzene
EC	Epicutaneous
FDA	Food and Drug Administration
FLG	Filaggrin gene
GCR	Glucocorticoid receptor
HLA-DR	Human leucocyte antigen-DR
IFN-g	Interferon-gamma
IFN-y	Interferon-y
IgE	Immunoglobulin E

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IL- 1	Interleukin 1
IL-13	Interleukin 13
IL-2	Interleukin 2
IL-4	Interleukin-4
IL-5	Interleukin 5
IL-6	Interleukin-6
ITH	immediate hypersensitivity reaction
ITR	Immediate-type response
LCs	Langerhan cells
LTR	Late-type reaction
NF-Atp	Nuclear factor of activated T cell protein
NF-κB	Nuclear Factor Kappa Beta
PUVA	Psoralen combined with ultraviolet A
RAST	Radioallergosorbent test
rIFN-gamma	Recombinant interferon-gamma
S. aureus	*Staphylococcus aureus*
Th	T-helper cell
TNCB	Trinitrochlorobenzene
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor alpha

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TSLP	Thymic Stromal Lymphopoietin
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B

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Chapter 1
Introduction

1. 1. Problem statement

Atopic dermatitis (AD) is a chronic relapsing cutaneous disease characterized by dry and scaly skin, inflammation, and intense itching. [1] In time, acute spongiotic dermatitis may evolve to a more chronic form in which epidermal hyperplasia and excessive scale, rather than blistering, dominate the clinical and histological picture.

In general, atopic dermatitis occurs mainly from dysregulation in helper T (Th) cell reactivity's.[1, 3] The Th cell is classified into two types, type 1 and type 2, depending on type of cytokine secreted by the cell and immunoregulatory functions driven by the secreted cytokine. [2, 3, 4] Any disruption of the balance between these two types of Th cells may cause a variety of immunological diseases, such as allergic asthma and allergic rhinitis. AD is an immunologic disease induced from an imbalance favouring type-2 helper T (Th2) cell. [5]

The cause of AD is multi-factorial, with genetics, environment, and impaired immune response being the most predominant factors. The role of genetics has been demonstrated in studies of families and twins. [6] AD has also been associated with numerous environmental factors for example; exposure to allergens, irritants, bacteria, and hard water. Socioeconomic status and large family size also contribute to environmental factors. [7] There is evidence that AD is a risk factor for childhood asthma, affecting asthma occurrence, severity, and persistence. AD is likely related to early immunoglobulin E (IgE) production and later allergen/IgE

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reactivity [8] which is caused by an impaired immune response that is characterized by activation of T-helper type 2 cells, leading to increased interleukin-4 production, which promotes IgE production.

AD can have a significant impact on morbidity and quality of life. Children may be affected by itching and associated sleep disturbance, the social stigma of a visible skin condition, and the need for frequent application of topical medications and physician visits. It has been estimated that children with atopic dermatitis lose an average of 1.9 hours of sleep per night, and their parents lose an average of 2.1 hours of sleep per night. [9] Other significant problems reported in children with AD include; irritability, daytime tiredness, dependence, fearfulness, and mood changes.[9]

AD is a common disease, occurring in nearly 1 in 5 children. The estimated prevalence in the United States is 9%. [10] The prevalence of AD appears to be increasing each year. Health care provider visits for contact dermatitis and other eczemas, which include AD, are 7 million per year. [11] 60% of patients will develop AD in the 1st year of life, and 30% between of patients will develop AD between 1 and 5 years of age. A family history of atopy (e.g., allergies, asthma, eczema, or hay fever) is present in 30–70% of patients. The symptoms of AD are usually worse in the winter, but can flare at any time of year. Ill-advisedly no data for South Africa is available, as no similar studies has been conducted yet.

Treatment of AD often requires a combination of home measures and medications and/or other treatments e.g. prescribed drugs that can relieve the rash and itching. As the skin in AD is "leaky," the foundation of treatment is improving the barrier function of the skin. Correspondingly, an atopic dermatitis rash can come and go

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and therefore different medications are often prescribed and used for various stages of the rash witch make the treatment of AD even more difficult.

There is a need for safe and effective therapies for inflammatory skin diseases. Current topical and systemic treatments for AD are reasonably effective but suffer from side-effects or are inconvenient. One of the first line treatments for the symptoms of AD is antihistamines and topical corticosteroids. The older generation antihistamines has various side effects, among other things sedation whereas the newer generation has less side effects but are more costly and as AD is a chronic condition most patient can't afford a life time treatment of these medications.

Topical corticosteroids is used in the treatment of AD to relieve itching and ease scaling but these topical treatments come with varies side effects such as skin thinning and stretch marks, skin irritation, discoloration and skin infection that in turn need to be treated with antibiotics**.** Oral or injected corticosteroids are reserved **f**or more severe cases of AD to reduce inflammation and to control symptoms. These medications are effective when given orally, but can't be used long term because of potential serious side effects, which include cataracts, loss of bone mineral (osteoporosis), and muscle weakness, decreased resistance to infection, high blood pressure and thinning of the skin. The therapeutic armamentarium for AD is clearly very limited and far from satisfactory and thus we need to explore alternative treatment regimens for AD.

Genus Aloe plant has been traditionally applied for medicinal practice over thousands of years in many cultures around world. Physicians prescribed *Aloe* for disturbances of the digestion, anorexia, dyspepsia, flatulent distension, habitual

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costiveness, and obstinate constipation, in jaundice and other diseases of the liver, in affections of the menstrual secretions, and for troublesome affections such as hypochondria, melancholy, and mania. [12] Physicians also believed that *Aloe* affected the genitourinary system, creating hyperemia of the uterus, and augmenting the menstrual flow in women while occasioning erections and increasing the sexual appetite in men. [13] Modern clinical use of the gel began in the 1930s, with reports of successful treatment of X-ray and radium burns, which led to further experimental studies using laboratory animals in the following decades. The reports of these experiments and the numerous favourable case histories did not give conclusive evidence, since although positive results were usually described, much of the work suffered from poor experimental design and insufficiently large test samples. There are at least four *Aloe* species that are reported having therapeutic effects, namely, *Aloe barbadensis Miller* (syn. *Aloe vera*; Liliaceae),[14] *Aloe ferox* (syn. *Cape Aloe*; Liliaceae), [15] *Aloe arborescens* (syn. *Candelabra Aloe*; Liliaceae) [16] and *Aloe perryi baker* (syn. *Perry's Aloe*; Liliaceae), [17] among which *Aloe vera* is the most widely studied species so far for its clinical effectiveness against a variety of skin disorders including burns and wounds. [18, 19, 20]

Aloe ferox is a commonly harvested plant in South Africa for leaf exudate which is suggested to be very similar to the exudate of *Aloe vera* due to the similarity of the phytochemicals present in both extracts. Since *Aloe vera* is not indigenous to South Africa and is imported it would be logical to investigate *Aloe* species that are locally available to the community for possible therapeutic properties or benefits. Communities in different parts of the world use the species of *Aloe* indigenous to their immediate surroundings. In South Africa, for instance, various traditional

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communities and local industries are using a variety of location-specific *Aloe* species as herbal medicine, as laxatives, in creams for skin ailments, and as a treatment for a wide range of diseases, respectively, [21] for example, *Aloe ferox* in the Eastern and Western Cape provinces and *Aloe greatheadiia* in the northern regions of South Africa. [22]

The dried product of the *Aloe ferox* exudate is commercially known as *Cape Aloe*. *Cape Aloe* extract has been demonstrated to possess the following pharmacological effects: anti-inflammatory, anti-bacterial, anti-fungal and protects against liver injury. [23] Cape Aloe contains the enzymes carboxypeptidase and bradykinase, both of which have been shown to relieve pain and decrease inflammation and swelling 24. The anti-inflammatory compound aloeresin-I was also recently isolated from *Aloe ferox.* [25] The presence of two dihydrocoumarins with immunomodulatory and antioxidative properties has also been reported recently. [26]

Cape Aloe is mostly sold locally. However the international market has indicated their interested once the efficacy of this product has been established. Using *Aloe* Species indigenous to South Africa would not only make the products more affordable but also will create work opportunities to the people of South Africa.

Once the activity and efficacy of *Aloe ferox* has been established there is no reason why South Africa could not compete with *Aloe vera* for its huge international market.

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1.2. Study aim

The aim of this study was to investigate and confirm the therapeutic efficacy of *Aloe ferox* versus a comparator *Aloe vera* as a topical treatment for Dinitrofluorobenzene (DNFB)-induced atopic eczema/dermatitis syndrome (AEDS) in Balb/c mice.

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Chapter 2
Literature review

2.1 Atopic Dermatitis (AD)

2.1.1. What is Atopic Dermatitis?

AD is a chronically relapsing, non-contagious, pruritic skin disorder that causes dry and flaky skin, intense itching, lichenification (Fig.1) or in other words, thickening of the skin and an increase in skin markings and a red, raised rash (Fig.2). AD usually starts in early infancy but an adult-onset variant is also recognized. AD can have a significant impact on morbidity and quality of life. For example children may be affected by itching and associated sleep disturbance, the social stigma of a visible skin condition, and the need for frequent application of topical medications and physician visits. As previously mentioned, it has been estimated that children with AD lose an average of 1.9 hours of sleep per night, and their parents lose an average of 2.1 hours of sleep per night. Other significant problems reported in children with AD include irritability, daytime tiredness, fearfulness, mood changes and dependence on parents for treatment and support. [29]

AD may be associated with other atopic IgE diseases (eg, asthma, allergic rhinitis, urticaria, acute allergic reactions to foods). [27] For some people, AD may be a chronic problem that requires more than one treatment. Aggressive therapy with emollients is an important intervention for patients with AD. The condition is characterized by intense pruritus and a course marked by exacerbations and

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remissions. These periods of remission and flare-ups adversely affects the quality of life of these patients and their families.

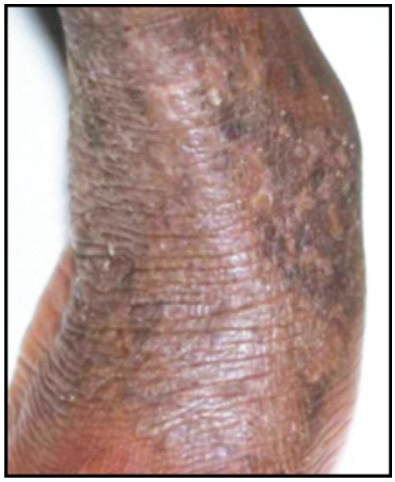

Figure 1: Lichenification (skin thickening and enhancement of skin markings) and scaling on the front of the ankle in an adult with chronic atopic dermatitis.

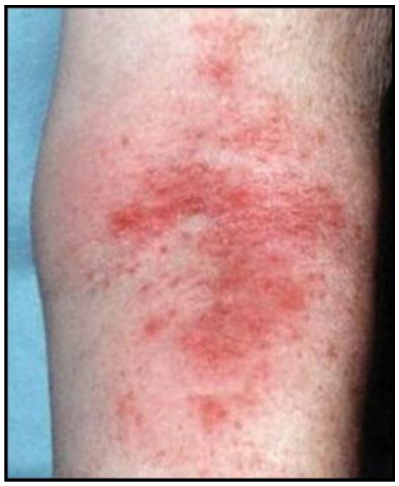

Figure 2: Atopic dermatitis. Flexural areas are common locations for recurrent atopic dermatitis in children and adults.

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2.1.2. Clinical features and Symptoms of AD

The diagnosis of AD is based on the constellation of clinical features. The diagnosis of AD requires the presence of at least three major features and at least three minor features. [31] Table 1 summarizes the diagnostic features of AD. AD usually begins during infancy. In approximately 50% of patients this illness develops by the first year of life, and in an additional 30%, between the ages of 1 and 5 years. In nearly 80% of patients with AD allergic rhinitis or asthma eventually develops later in childhood. Symptoms or signs vary from person to person. The most common symptoms are dry, itchy skin and red sensitive rashes inside the elbows, behind the knees and, on the hands and feet and on the face. Itching is the most imperative symptom of atopic dermatitis. Scratching and rubbing in response to itching irritates the skin, increases inflammation, and actually increases irritation and itchiness. Itching is a particular problem during sleep when conscious control of scratching is lost.

The appearance of the skin affected by atopic dermatitis depends on the amount of scratching and the presence or absence of secondary skin infections for example secondary infections caused by bacteria namely *Staphylococcus aureus*. Secondary infections due to fungi have, comparatively, received less attention, but there is evidence for a role for *Malassezia* spp. as a factor in dermatitis with a head and neck distribution pattern. Viral infections, such as *herpes simplex* virus, and mixed infections of intertriginous spaces, may complicate an underlying AD, but are not perceived as etiologic factors. [28] The skin may be red and scaly, be thick and leathery, contain small raised bumps, or leak fluid and become crusty and infected. AD may also affect the skin around the eyes, the eyelids, and the eyebrows and lashes. Scratching and rubbing the eye area can cause the skin to redden and swell.

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Some people with atopic dermatitis develop an extra fold of skin under their eyes. Patchy loss of eyebrows and eyelashes may also result from scratching or rubbing. Table 2 summarize the skin features associated with AD.

Table 1: The diagnostic features of atopic dermatitis
Major features
Pruritus and excoriations(Characteristic rash in locations typical of the disease)
Typical appearance and distribution of skin lesions
Intense itching
Facial and extensor involvement in infancy and arly childhood
Flexural involvement and lichenification by adolescence
Chronic or frequently relapsing course (duration > 6 weeks)
Personal or family history of atopic disorders, allergic rhinoconjunctivitis, food allergy, asthma or hay fever
Minor features
Increased susceptibility to skin infections, particularly *S. aureus*
Xerosis (dryness of the skin)
Early age of onset
Multiple positive immediate prick skin test results
Ichthyosis, keratosis pilaris, hyperlinearity of palms
Nonspecific hand/foot dermatitis
Scalp dermatitis (e.g., cradle cap)
Elevated serum IgE levels
Inflammation around the lips
Cataracts (anterior subcapsular) Keratoconus
Impaired cell-mediated immunity
Nipple eczema

Adapted from Williams et al. [29]

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Table 2: Skin Features Associated With Atopic Dermatitis	
Keratosis pilaris	small, rough bumps, generally on the face, upper arms, and thighs
Atopic pleat (Dennie-Morgan fold)	an extra fold of skin that develops under the eye
Cheilitis	inflammation of the skin on and around the lips
Hyperlinear palms	increased number of skin creases on the palms
Hyperpigmented eyelids	eyelids that have become darker in colour from inflammation or hay fever
Lichenification	thick, leathery skin resulting from constant scratching and rubbing
Ichthyosis	dry, rectangular scales on the skin
Papules	small raised bumps that may open when scratched and become crusty and infected
Urticaria	hives (red, raised bumps) that may occur after exposure to an allergen, at the beginning of flares, or after exercise or a hot bath

* Adapted from Williams et al [29]

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2.1.3. Pathogenesis and Etiology of AD

The clinical phenotype that characterizes AD is the product of interactions between susceptibility genes, the environment, defective skin barrier function, and immunologic responses. The pathophysiology is still poorly understood but three main hypotheses have been proposed regarding the development of the inflammatory lesions:

1.) The first suggests an immune dysfunction resulting in IgE sensitization,

2.) Secondary epithelial-barrier disturbance or dysfunction which proposes a defect in epithelial cells leading to the defective barrier problem, with the immunological aspects being epiphenomena. [30]

3.) The most current theory is an unidentified genetic abnormality. The role of genetics has been demonstrated in studies of families and twins. [6]

2.1.3.1. Immune dysfunction

Immune dysfunction in AD is likely related to early IgE production and later allergen/IgE reactivity. [31] This impaired immune response is characterized by activation of T-helper type 2 cells. T cells are a group of white blood cells known as lymphocytes, which play central role in cell-mediated immunity. In healthy individuals, balance exists between 2 important subdivisions of T cells (i.e., T_H 1, T_H 2). The immune hypothesis invokes an imbalance in the T lymphocytes, with T_H 2 cells predominating; this results in cytokine production of interleukins [4, 5, 12, 13] and granulocyte macrophage colony-stimulating factor, causing an increase in IgE and lowered interferon gamma (IFN-g) levels. Later, in persons with chronic AD, the T_H 1-type cells predominate. Other cell types are also involved in the process, including eosinophils, Langerhans cells, keratinocytes, and B cell. [32]

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Clinically unaffected skin in AD is not normal. It frequently manifests increased dryness and a greater irritant skin response than healthy controls. Unaffected AD skin contains a sparse perivascular T cell infiltrate not seen in normal healthy skin (Figure 3). Analyses of biopsies from clinically unaffected skin of AD patients, as compared with normal non atopic skin, demonstrated an increased number of Th2 cells expressing chemokine's like IL-4 and IL-13, but not IFN-g. [33]

The evolution of AD skin lesions is orchestrated by the local tissue expression of pro-inflammatory cytokines and chemokine's. Cytokines and chemokine's are soluble mediators that dictate the immune responses. Measurements of these soluble proteins are critical to understanding the extent and direction of immune responses. Cytokines are small cell-signalling protein molecules that are secreted by the glial cells of the nervous system and by numerous cells of the immune system and are a category of signalling molecules used extensively in intercellular communication. Chemokine's are a family of small cytokines, or proteins secreted by cells and they have the ability to induce directed chemotaxis in nearby responsive cells. Cytokines such as TNF-α and IL-1 from resident cells (keratinocytes, mast cells, and dendritic cells) bind to receptors on vascular endothelium, activating cellular signalling including the NF-κB pathway and inducing expression of vascular endothelial cell adhesion molecules. These events initiate the process of tethering, activation, and adhesion to the endothelium followed by extravasation of inflammatory cells. Once the inflammatory cells have infiltrated into the tissue, they respond to chemotactic gradients established by chemoattractant cytokines and chemokine's, which emanate from sites of injury or infection. [34] These molecules play a central role in defining the nature of the inflammatory infiltrate in AD. [35]

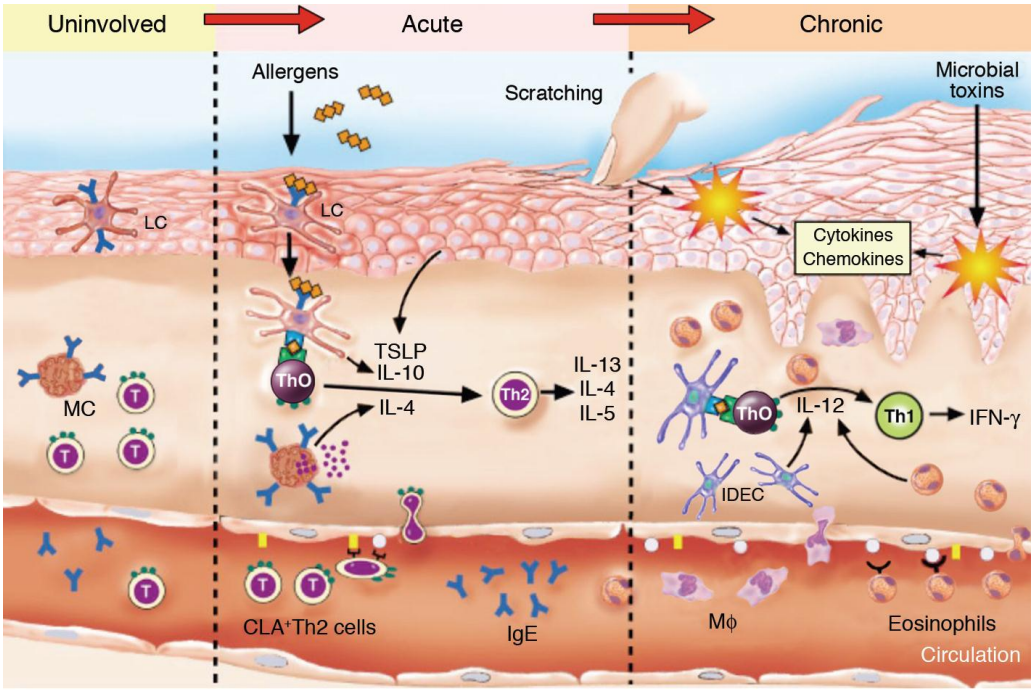

Figure 3: Immunologic pathways in AD. Th2 cells circulating in the peripheral blood of AD patients result in elevated serum IgE and eosinophils. These T cells express the skin homing receptor, CLA, and recirculate through unaffected AD skin where they can engage allergen-triggered IgE+ LCs and mast cells (MCs) that contribute to Th2 cell development. Skin injury by environmental allergens, scratching, or microbial toxins activates keratinocytes to release proinflammatory cytokines and chemokines that induce the expression of adhesion molecules on vascular endothelium and facilitate the extravasation of inflammatory cells into the skin. Keratinocyte-derived thymic stromal lymphopoietin (TSLP) and DC-derived IL-10 also enhance Th2 cell differentiation. AD inflammation is associated with increased Th2 cells in acute skin lesions, but chronic AD results in the infiltration of inflammatory IDECs, macrophages (Mf), and eosinophils. IL-12 production by these various cell types results in the switch to a Th1-type cytokine milieu associated with increased IFN-g expression.

Figure modified with permission from The Journal of Allergy and Clinical Immunology [36]

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2.1.3.2. Epithelial-barrier disturbances

The second hypothesis involves defective barrier function in the stratum corneum of AD patients. Researchers have noted differences in the skin of people with AD that may contribute to the symptoms of the disease. The outer layer of skin, called the epidermis, is divided into two parts: an inner part containing moist, living cells, and an outer part, known as the horny layer or stratum corneum, containing dry, flattened, dead cells. Under normal conditions the stratum corneum acts as a barrier, keeping the rest of the skin from drying out and protecting other layers of skin from damage caused by irritants and infections. When this barrier is damaged, irritants act more intensely on the skin leading to the entry of antigens that result in the production of inflammatory cytokines and trans epidermal water loss is increased. The skin of a person with atopic dermatitis loses moisture from the epidermal layer, allowing the skin to become very dry and reducing its protective abilities. [37] Thus, when combined with the abnormal skin immune system, the person's skin is more likely to become infected by bacteria (for example, *Staphylococcus* and *Streptococcus*) or viruses, such as those that cause warts and cold. [28]

2.1.3.3. Unidentified genetic abnormality

Defective lamellar bodies may be caused by abnormalities of ceramide production. Whether the inflammation causes primary or secondary epidermal barrier breakdown is not known, but with the knowledge that filaggrin is involved in epithelial disruption (and this is where the third hypothesis of genetic variation fits in), it is now thought that this finding leads to increased trans epidermal

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penetration of environmental allergens, increasing inflammation and sensitivity. [38, 39] Some authors question whether the antigens can also be absorbed from the gut (eg, from food) and the lungs (eg, from house dust mites) but evidence has shown multiple loss-of-function mutations in the filaggrin gene (*FLG*) on band 1q21.3 in patients with AD in Europe and other filaggrin mutations in Japanese patients. This gene is mutated in persons with ichthyosis vulgaris; it is associated with early-onset atopic dermatitis and with airway disease in the setting of atopic dermatitis. These changes are only found in 30% of European patients, begging the question of whether other genetic variants may also be responsible for some of the findings in the pathogenesis of AD.

Genetic abnormality also causes increased levels of cyclic adenosine monophosphate (cAMP) phosphodiesterase, which in turn leads to low levels of intracellular cAMP, a secondary messenger controlling cell activity. This change in the level of intracellular cAMP causes basophils and mast cells to be hyperreactive, with the end result being increased histamine and leukotriene production and release. T cells are predominantly Th-2 subtype cells, which predispose to atopy. [40, 41, 42]

2.1.4. Risk factors for AD flares

Atopic dermatitis flares can be triggered by a number of factors for example a moist environment, burns, high stress, anxiety, food allergies, history of alternative allergies or exposure to chemicals and metals. Complicating the risk factors for flares is that not everyone with AD will have the same triggers, so people with the disorder will have to keep track of their particular sensitivities. Identifying triggers

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can be tricky, for example, sometimes there is a delay between eating a certain food and seeing a resulting flare-up.

Foods, chemicals and aeroallergens may play a role in the pathogenesis and exacerbation of AD. However, the exact roles of aeroallergens and food allergy are controversial because of limitations of the in vitro radioallergosorbent test (RAST) and skin prick test. Both tests have a nearly 90% negative predictive value for AD, but their positive predictive value is less than 50% because of frequent false-positive results. [43] Induction of bleeding, irritant reaction or nonspecific enhancement through axon reflex from nearby strong reaction. , may all possibly lead to false-positive results,[43] therefore it is important to place the test atleast 2cm apart.

Placebo-controlled, food challenge studies have demonstrated that food allergens can induce eczematoid skin rashes in nearly 40% of children with moderate to severe AD. In a subset of these patients, urticarial reactions, or non-cutaneous symptoms, are elicited, which can trigger the itch-scratch cycle that flares this skin condition. Children with food allergies generally have positive immediate skin tests or serum IgE directed to various foods, particularly eggs, milk, wheat, soy, and peanuts. Importantly, food allergen–specific T cells have been cloned from the skin lesions of patients with AD, providing direct evidence that foods can contribute to skin inflammation. [44]

Exposure to aeroallergens such as pollens, molds, mites and animal dander appears to play an important role in AD flare in some patients with atopic dermatitis. Substantial clinical improvement may occur when these patients are removed from environments that contain the allergens to which they react. [45]

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Microbes like *S. aureus* also play a role in triggering flares of AD patients. Most patients with AD are colonized with *S. aureus* and suffer relapses of their skin disease following overgrowth of this organism. [44] The magnitude of *S. aureus* in AD is supported by the observation that, in patients with secondary infection of *S. aureus*, treatment with a combination of antistaphylococcal antibiotics and topical corticosteroids results in greater clinical improvement than treatment with topical corticosteroids alone. One strategy by which *S. aureus* exacerbates AD is by secreting toxins called superantigens, which stimulate activation of T cells and macrophages. Most AD patients make specific IgE antibodies directed against staphylococcal superantigens, [46] and these IgE anti-superantigens correlate with skin disease severity. Another complication in the treatment of AD flares is that these toxic superantigens also induce corticosteroid resistance, suggesting that several mechanisms exist by which superantigens increase AD severity. [47]

2.2. Treatment of AD

The treatment of atopic dermatitis mainly targets underlying skin abnormalities such as xerosis, pruritus, super infection and inflammation. Patients should be educated about the chronic nature of the disease, treatment may be required for many months and possibly years. Thus validating the need for continued adherence to proper skin care. Preventing flare-ups with good skin-care practices and avoiding triggers is an important part of the overall treatment of atopic dermatitis. Dry skin is a feature in nearly all patients with the condition for this reason emollients are the mainstay of maintenance therapy for AD. [48,49] Treatment guidelines from the United States and the United Kingdom recommend the use of emollients with or without moisturizers. [49, 50]

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Treatment of AD is characterised by a profusion of treatments aimed at disease control rather than curing AD. The evidential basis of these treatments is often unclear. Lubricants, antihistamines and topical corticosteroids have traditionally been considered the most effective method of treating eczema, but is feared because of the variety of side effects especially with long term use. The immunosuppressants such as tacrolimus and pimecrolimus can be used as a topical preparation in the treatment of severe atopic dermatitis instead of or in addition to traditional steroid creams. There can be unpleasant side effects in some patients such as intense stinging, itching or burning, which mostly get better after the first week of treatment. [51] However, the risk of developing skin cancer from the use of these drugs [52] (especially when combined to UV exposure, such as sunrays) was not ignored by the FDA, which issued a "black box warning". [53]

Systemic treatments for AD is used as last resort for severe resistant AD and only used short term as the side-effects is greater with systemic treatments. Some systemic treatments include oral corticosteroids, oral antihistamines as well as oral antibiotics that are used for secondary skin infections caused by *S.aureus*. [54]

2.2.1. Maintaining the skin barrier

2.2.1.1. Emollients (Moisturizers for AD)

Emollients are one of the most important self-care steps for treating and managing AD (eczema). Ointments are superior to creams and lotions because of their high oil content and higher viscosity. They form a barrier that helps to seal moisture into the skin. But they are greasy and therefore poorly tolerated by the patient and usually only used during the night. Creams are better tolerated than ointments but

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should be applied five to ten times per day in order to be effective. Lotions are least effective because of their alcohol content. [55]

There is no current evidence that emollients improve AD directly. Emollients provide moisture to the skin and help prevent further water loss. Emollients are widely used because they improve the appearance and symptoms like itching of the xerosis associated with this condition. [50, 56, 57] Emollients are a very important part of eczema management, even (especially) when the eczema is well controlled. Emollients should be used in almost everyone with AD in an attempt to restore skin barrier function. One study has shown that emollients may reduce the need for topical corticosteroids by approximately 50%, [58] and another study found that emollients enhanced the response to treatment with topical corticosteroids. [59] There is little basis for suggesting the use of one emollient over another, and the preference of the patient is probably the most important factor. [57]

The FDA has approved products composed of ceramides, cholesterol and fatty acids that aim to mimic the lipids naturally found in the horny layer of the skin and may reduce the need for anti-inflammatory medications such as topical corticosteroids. Ceramide is one of the three key lipids that comprise the skin barrier [60]. The “stratum corneum ceramide deficiency” is possibly “the putative cause of the barrier abnormality” [61] in atopic dermatitis. There are various ceramide based creams available including the prescription drug Epiceram as well as other non-prescription options like Cerave and Aveeno for Eczema. Addition of a “ceramidedominant” emollient to standard therapy results in both clinical improvement and decreased transepidermal water loss and improvement of stratum corneum integrity in children with “stubborn-torecalcitrant”AD. [61]

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2.2.2. Topical treatments for AD

2.2.2.1. Topical corticosteroids

Topical corticosteroids are the mainstay of treatment for mild to moderate atopic dermatitis. Showing efficacy in the control of both acute and chronic skin inflammation. Corticosteroids mediate their anti-inflammatory effects through a cytoplasmic glucocorticoid receptor (GCR) in target cells. [62] Topical corticosteroids are also used to reduce the dose of oral treatments and ultraviolet light treatment in patients with severe AD, but because of their long term side-effects, topical corticosteroids are not very popular in patients suffering from AD. They are however very effective and safe if used correctly. The trick is to use the correct strength of corticosteroid for the severity of the eczema and be prepared to change treatment as the severity of the eczema changes.

There are more than 30 topical corticosteroids available, ranging from low to high potency. Potency of topical corticosteroids is classified by the potential for vasoconstriction — a surrogate for clinical efficacy and skin thinning (Table 3). [63] Most of these agents are available in varying concentrations and doses; nearly all are available in generic formulations. Unfortunately, there is a paucity of clinical trial data to assist in choosing a corticosteroid.

The thickness of human skin varies in different areas of the body. The thinnest skin is found on the face (particularly the eyelids), genitals, body folds and the skin of infants. These areas absorb topical corticosteroids very readily and are more prone to local side effects. Only low-potency, weak or moderate strength (class 6 or 7) agents should be used on the face, groin, and axillae to minimize these local side

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effects such as acne, striae, telangiectasia, and atrophy. Whereas those that have moderate or potent strength are used on other areas of the body [57]. There is no compelling evidence regarding the most appropriate frequency of topical corticosteroid application and the role of the vehicle used to deliver the active ingredient. [64] Evidence that more frequent application of hydrocortisone butyrate 0.1% (Locoid) or fluticasone propionate 0.05% (Cutivate) cream is more effective than once-daily dosing is also lacking. [65, 66] Treatment guidelines do not recommend more than twice-daily application of topical corticosteroids. [48, 67] The use of an emollient is recommended if the skin is dry or irritable.

Low potency agents are favoured in infants because infants have a relatively higher ratio of skin surface area to body mass and thinner skin than older children and adults and and therefore increase the potential for systemic absorption with these topical corticosteroid drugs. Preparations are typically used in bursts of three to seven days in order to achieve control. There is little difference in the outcome between short-term use of potent preparations or longer use of weaker preparations in children with mild-to-moderate disease. [68] In general, the lowest strength and smallest quantity of topical steroid that is effective should be used. Table 4 illustrate the perspective of the appropriate quantity of topical corticosteroids to be used for each body part and age group for the treatment of AD.

AD tends to be persistent and seen as a chronic disease, most people will have to use topical corticosteroids on and off for many years or through their entire life time. If used continuously topical steroids may lose their effectiveness after a few weeks, this is known as tachyphylaxis. Tachyphylaxis can be avoided by reducing the strength and frequency of the topical steroid as the eczema comes under control

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or treatment needs to be tailored to the individual patient. Changing to a different topical steroid of the same strength can also be helpful. Treatment of infection, if present, may again make the topical steroid ineffective. A stronger topical steroid or an additional treatment such as phototherapy or antibiotics may then be required.

The method of application of a corticosteroid can influence potency of the active ingredient. Ointments generally are more potent than creams but may have a greasy appearance. Ointments should be avoided on open or oozing lesions and in intertriginous folds. They also should not be used in hot, moist climates. Creams may contain preservatives that can precipitate contact dermatitis. Lotions generally lack the hydrating properties necessary for treating atopic dermatitis. An adequate supply of a topical corticosteroid is essential for effective treatment and patient adherence to therapy. Patients generally underestimate the appropriate quantity of topical corticosteroids and emollients needed for long-term therapy. [69]

Clinical trials have shown that topical corticosteroids are safe and effective for the treatment of AD flare-ups when used for up to four weeks, although many flare-ups may be adequately controlled with a shorter treatment course. [70, 71, 72] To minimize toxicity; topical corticosteroids should be used for the shortest duration needed to control the flare-up. After the flare-up resolves, maximal preventive strategies should be used to control the disease. It is important to note that topical corticosteroids do not cure AD and that long-term topical corticosteroid use is associated with local and systemic adverse effects, see Table 5, that may lead to the underutilization of these effective agents. [73]

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Topical corticosteroids can further reduce the skin barrier function so are best applied as intermittent courses so it can recover. Systemic absorption and adrenal suppression is only a concern if large amounts of potent topical steroids (e.g. more than 100g/week) are used over an extended area of the body and over a long period of time (months). [74] One thing to remember is that the body surface area relative to body mass is greater in infants (a 10 % higher ratio of body surface area compared to their weight) and thus they can be easily over dosed.These effects are reported infrequently in clinical trials, although trials are primarily designed to assess effectiveness rather than safety and tolerability. Most clinical trials of topical steroids are of short duration and, therefore, are unable to evaluate long-term toxicity. Systemic adverse effects (primarily hypothalamicpituitary- adrenal axis suppression, reduced linear growth in children, and bone density changes in adults) with topical corticosteroids. The most common local adverse effects are skin burning and irritation. [74]

Lichenified AD requires more potent preparations for longer periods of time. Long-term studies of moderate-to-potent preparations in children are scarce. One study of 231 children with stabilized atopic dermatitis randomly assigned to receive twice-weekly 0.05% fluticasone propionate (plus emollients) or vehicle alone plus emollients for 16 weeks showed that patients in the control group were more likely, by a factor of 8, to have a relapse (95% confidence interval, 4.3 to 15.2). [75] Another study showed biochemical evidence of suppression of the hypothalamic– pituitary–adrenal axis only in children with AD who used potent or very potent topical corticosteroids and in those who had received glucocorticoids from other routes, and not in those who had used topical corticosteroids of mild or

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moderate strength for a median of 6.9 years. [76] A four-month trial of persons 12 to 64 years of age with moderate-to severe disease showed that the application of fluticasone to previously active and new sites of AD for two consecutive days each week reduced flares significantly, as compared with a group receiving an emollient only. [62] Reduced efficacy of topical corticosteroids may be related to disease severity rather than to glucocorticoid resistance. [64]

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Table 3:Table of different topical corticosteroids and their relative potency

Therapy	Relative potency class*	Preparation
Alclometasone dipropionate 0.05% (Aclovate)	6	Cream, ointment
Betamethasone dipropionate 0.05% (Diprolene)	3	Cream
Betamethasone dipropionate 0.05%	2	Ointment
Betamethasone valerate 0.1% (Beta-Val)	5	Cream
Betamethasone valerate 0.1%	3	Ointment
Clobetasol propionate 0.05% (Temovate)	1	Cream, ointment
Desonide 0.05% (Desowen)	6	Cream
Desoximetasone 0.25% (Topicort)	2	Cream, ointment
Fluocinolone acetonide 0.01% (Synalar)	6	Cream, solution
Fluocinonide 0.05% (Lidex)	2	Cream, ointment, gel, solution
Fluticasone propionate 0.005% (Cutivate)	3	Ointment
Fluticasone propionate 0.05%	5	Cream
Halobetasol propionate 0.05% (Ultravate)	1	Cream, ointment
Hydrocortisone 0.5 to 2.5%	7	Cream, ointment, lotion
Hydrocortisone butyrate 0.1% (Locoid)	5	Cream, ointment, lotion
Hydrocortisone valerate 0.2% (Westcort)	5	Cream
Hydrocortisone valerate 0.2%	4	Ointment
Mometasone furoate 0.1% (Elocon)	4	Cream, ointment, lotion
Triamcinolone acetonide 0.025 to 0.1% (Kenalog)	5	Cream
Triamcinolone acetonide 0.1%	4	Ointment
Triamcinolone diacetate 0.5% (Aristocort)	3	Cream

*—*Highest potency class is 1 and lowest potency class is 7*
Adapted with permission from Habif TP. [63]

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Table 4: Appropriate Quantity of Topical Corticosteroids for the Treatment of Atopic Dermatitis			
Amount of topical corticosteroids required (g)			
Anatomic	**Infants**	**Children**	**Adults**
Face and Neck	10	15	30
Hand	5	7.5	15
Arm	40	15	30
Leg	20	30	60
Body	100	150	300

Information from reference's [63, 69 and 77].

Table 5:Possible Adverse Effects of Topical Corticosteroid Use	
Systemic Absorption	
Hypothalamic-pituitary-adrenal axis suppression	
Cushing's disease	
Femoral head osteonecrosis	
Local Effects	
Striae distensae	Milia
Cutaneous atrophy	Cataracts
Stellate pseudoscars	Tinea
Telangiectasia	Candidiasis
Purpura	Scabies
Erythema	Hypertrichosis
Rosacea	Hypopigmentation
Acneiform dermatoses	Contact dermatitis
Rebound erythema	Tachyphylaxis
Demodicidosis	

Adapted from [74]

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2.2.2.2. Topical immunomodulators/calcineurin inhibitors.

Topical tacrolimus and pimecrolimus have recently been FDA-approved for the treatment of AD. These topical immunomodulators or also known as calcineurin inhibitors inhibit the release of calcineurin in the skin and are marketed in two forms; pimecrolimus (Elidel) and tacrolimus (Protopic). The distinction between pimecrolimus and tacrolimus is that pimecrolimus is a cream that is somewhat weaker than tacrolimus but less irritating. Tacrolimus is currently marketed as an ointment that is more potent but also more irritating. These are immunosuppressant agents originally developed for systemic administration to prevent allogeneic GRAFT rejection. [78] These agents inhibit calcineurin in the skin, which blocks early T-cell activation and the release of cytokines. Both these agents act by binding to the 12 kDa macrophilin with high affinity and inhibit the phosphatase activity of the calcium-dependent serine/threonine phosphatase, calcineurin. In the presence of these calcineurin inhibitors, the transcription factor, nuclear factor of activated T cell protein (NF-Atp), is not dephosphorylated and therefore cannot translocate into the nucleus to activate transcription of various Th1 and Th2 cytokine genes. Tacrolimus and pimecrolimus inhibit the activation of a number of key effector cells involved in AD, including T cells and mast cells. [79]

The effectiveness of pimecrolimus compared with less potent topical corticosteroids is unknown. Although pimecrolimus has been shown to prevent more flare-ups than vehicle alone, there are no available data comparing low-potency corticosteroids with pimecrolimus to prevent flare-ups [80.] Unlike topical glucocorticoids, topical calcineurin inhibitors are not atrophogenic and have been used safely for facial and eyelid eczema.

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The approval of topical calcineurin inhibitors for the treatment of AD represents a significant advance in our management options for this disease. Importantly, there are situations in which topical calcineurin inhibitors may be advantageous over topical corticosteroids and may be useful as first line therapy. These would include treatment of patients who are poorly responsive to topical steroids or have steroid phobia, and treatment of face and neck dermatitis where ineffective, low-potency topical corticosteroids are usually used due to fears of steroid-induced skin atrophy. The potential use of topical calcineurin inhibitors as maintenance therapy is also intriguing for prevention of AD flares and progression of the atopic march.[79]

Topical tacrolimus and pimecrolimus have both been shown to be effective in vehicle-controlled studies. For 0.03 % and 0.1 % tacrolimus, the rate ratios for the proportion of patients who were clear or who had excellent improvement at 12 weeks were 4.50 (95% confidence interval, 2.91 to 6.96) and 5.62 (95% confidence interval, 3.67 to 8.61), respectively, in three vehicle-controlled trials involving 656 patients. [80] Short-term studies suggest that 0.1% topical tacrolimus may be similar in strength to potent topical corticosteroids, [80] whereas topical pimecrolimus is considerably weaker. [81, 82] Few long-term studies compare intermittent use of topical calcineurin inhibitors with intermittent use of topical corticosteroids. A 12-month vehicle-controlled study of children with atopic dermatitis showed that early use of pimecrolimus reduced the frequency of flares from 51 percent to 28 percent, [39] although early use of mild topical corticosteroids might have shown similar effects. Cyclosporine (Sandimmune) has been effective in patients with refractory atopic dermatitis. The condition returns after the cessation of therapy, although not always at the original level of severity. [83] Tacrolimus (Prograf), which is used in an oral form to prevent organ transplantation rejection, is also available in a topical

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form. This topical agent appears to be effective in the treatment of refractory atopic dermatitis, with few adverse effects. [31] Azathioprine (Imuran) may also be efficacious in patients with atopic dermatitis. Treatment with this immunosuppressant is more cost-effective than therapy with cyclosporine or tacrolimus. [88]

In the United Kingdom, the National Institute of Clinical Excellence approves the use of topical tacrolimus for children older than two years of age with moderateto-severe atopic dermatitis not controlled by topical corticosteroids, and of topical pimecrolimus as a second-line option for resistant dermatitis of the head and neck. In the United States, both of these topical calcineurin inhibitors are approved as second- line agents, and the site of application is not restricted for pimecrolimus.

Tacrolimus and pimecrolimus also have adverse effects, although they are different than those associated with topical corticosteroids. The most common local adverse effects are skin burning and irritation. Patients using topical calcineurin inhibitors should be counselled on appropriate sun protection, including sunscreen application. Whether these agents induce local or distant malignancy is unclear. In March 2005, the Food and Drug Administration issued an alert to health care professionals concerning a potential link between topical pimecrolimus and tacrolimus and cancer (mainly lymphoma and skin cancer) on the basis of studies in animals, case reports, and knowledge of how these drugs work. [85, 86] The alert emphasizes the importance of using these preparations only as labelled and when first-line treatment has failed or cannot be tolerated. Because of several case reports and additional animal data, the U.S. Food and Drug Administration has

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approved label revisions for these agents including a second-line indication, enhanced warnings, and a patient education guide. [87] The warning statement recommends avoiding long term use in all patient populations and limits use to children older than two years. [87]

The complete label information is available on the FDA website. [88]

2.2.2.3 Tar preparations

Coal tar, actually made by melting coal, has long been a treatment for a variety of skin conditions like atopic dermatitis. Coal tar preparations are available as creams, gels, bandages and bath additives. Shampoos and soaps containing coal tar can help with mild cases of atopic dermatitis. Coal tar tends to work better on thickened skin that is not scaly, or to ease very early symptoms of itching. However, coal tar can be very irritating to already inflamed skin and should be discontinued immediately if there is any increase in itching or redness of the rash. [89]

Tar preparations have anti-inflammatory and antipruritic effects on the lesions of atopic dermatitis. These preparations are effective when used alone or with topical corticosteroids but do not appear as effective as topical steroids. [90] Some tar preparations, especially gels, may contain alcohol and thus can be irritating to the inflamed skin. Shampoos, bath solutions and creams are less irritating. Tar preparations can be purchased over the counter (e.g., Cutar, Aquatar, Estar).The disadvantages of tars are their odour and dark staining colour they can be very smelly and messy. Using the products at night and covering the treated areas can decrease these problems. [90]

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2.2.2.4. Wet wraps

'Wet wraps' are wet bandages applied over emollients +/- topical corticosteroids thus allow for increase absorption of the corticosteroids. Tubular elastic bandages are convenient. Wet wraps are used in acute red, hot, weeping eczema and usually require admission to hospital. They can quite quickly gain control of eczema and appear to work by cooling and moisturizing the skin. They also protect the skin from damage due to scratching. They can be repeated for several days or longer, reapplied as they dry out.

Studies show that wet-wrap therapy can effectively re-hydrate and calm the skin. In one such study, children with severe atopic dermatitis who had not responded to other treatments were treated with wet-wrap therapy. All children experienced significant improvements. In just 1 week, there was a 74% average reduction in itch and the children had less sleep loss. Improvements to their skin lasted well beyond the 2 weeks of treatment. [91] However using wet-wrap therapy has one major drawback. It can be very time-consuming and treating a child requires a great deal of patience and supervision. Children tend to squirm when the bandages are being applied and may try to remove the bandages. Most parents and or patients agree that the results are worth the effort when other therapies are not effective.

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2.2.3. Systemic treatments for AD

Systemic therapy is indicated for severe, resistant disease. Systemic corticosteroids are effective at acutely controlling AD in adults, but their use should be restricted to the short term because of their long term side effects. Rebound flare-ups and diminishing effectiveness severely limit use. [92] Agents such as cyclosporine (Sandimmune) and IFN-g -1b (Actimmune) may be effective for severe AD. Data on the use of mycophenolate mofetil (Cellcept), azathioprine (Imuran), and intravenous immune globulin (human; Baygam) are conflicting, and there is no evidence to support the use of leukotriene inhibitors, methotrexate, desensitization injections, theophylline, or oral pimecrolimus. [50]

2.2.3.1. Antibiotics

AD reduces the skin's natural defences, making it easier for skin to become infected with microbes. If a person's AD is not improving as expected, this may be because the skin is infected. Secondary infection with *S. aureus* is common in patients with AD and usually is treated with short courses of antibiotics such as flucloxacillin, cephalexin, or amoxicillin– clavulanate. [67] Treatment of patients with chronic AD with antistaphylococcal antibiotics can markedly reduce the severity of their skin disease. [97] This clinical effect may be due to the capacity of antibiotics to reduce toxin (superantigen) production by *S. aureus*. The relationship between *S. aureus* infection and AD flare-ups has been debated but remains unclear. Concerns about resistance limit the use of antibiotics to treating acute skin lesions, rather than decolonization when the skin has not been affected. [97] One

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randomized trial found no benefit to prescribing floxacillin continually for four weeks as compared with placebo, and methicillin-resistant strains were more common in those who were prescribed antibiotics. [93] Although combinations of topical corticosteroids and antibiotics are used for atopic dermatitis, no good evidence suggests that they offer additional benefits as compared with topical corticosteroids alone. [56]

2.2.3.2. Oral antihistamines

Oral antihistamines, such as Benadryl (diphenhydramine) or Aterax (hydroxyzine), can be used to treat symptoms like itch associated with atopic dermatitis. The older generation antihistamines have a sedative effect and are frequently used especially at bedtime to facilities peaceful sleep as itch intensity often increase at night. [94.] Interestingly, these studies done on AD provided an insight as to why some antihistamines may be helpful only to selective AD patients: One theory is that the improvements in the clinical condition and patient quality of life may be due primarily to the promotion of restful sleep, rather than to a direct reduction of symptoms. [95, 96] Consequently, sedation as an unintended side‑effect might be the reason why antihistamines are effective in the treatment of AD patients. Nonetheless, not all antihistamines are effective only because they are sedative. Promethazine, [96] chlorpheniramine [97] and clemastine, [94] for example, are sedative antihistamines but have been found ineffective in clinical trials. Therefore, the effectiveness of some sedative antihistamines has to be independent of a sedative side‑effect. [98]

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Day time use is however problematic with this older generation antihistamines because of the sedative side effects, but the new generation antihistamines for example, loratadine has been prescribed to provide day time relief devoid of the sedative side effects. It's important to note, however, that they may not help in all cases of atopic dermatitis. It is very important to note that antihistamine creams should not be used on atopic dermatitis rashes because of their sensitizing potential, they contain chemicals that can actually worsen the rash. Topical forms of doxepin (Zonalon), diphenhydramine (in cream, gel or spray forms) and benzocaine (Americaine) are available. However, these forms may be systemically absorbed, and they can cause allergic contact dermatitis. [99, 100] Evidence is lacking to support the use of antihistamines for the treatment of atopic dermatitis [97], although they are sometimes recommended for their sedative effects [94]. Reports on non-sedative antihistamines are conflicting. [56, 94, 101] The largest study failed to demonstrate any overall benefit from prolonged use of cetirizine in children with atopic dermatitis. [102]

The value of non- sedating second‐generation antihistamines in the treatment of AD is not necessarily seen in their anti‐pruritic effect but in their anti‐inflammatory properties and it appears to be acknowledged that these anti‐inflammatory properties are not strictly histamine receptor related, [103] For example, cetirizine, a metabolite of hydroxyzine, is successfully deployed in the treatment of AD due to its anti‐inflammatory impact: cetirizine inhibits eosinophil chemotaxis, lowers eosinophilic chemokine release and reduces the expression of endothelial adhesion molecules. [104, 105]

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2.2.3.3. Oral Corticosteroids

Systemic corticosteroids should be reserved for use in patients with severe treatment-resistant atopic dermatitis. Oral corticosteroids improve the lesions of atopic dermatitis, but a disease flare up may occur when these medications are withdrawn. If a systemic corticosteroid is used to treat a severe flare of atopic dermatitis, the potential for a rebound effect can be decreased by tapering the drug while increasing topical corticosteroid treatment and aggressively hydrating the skin. [106] Oral corticosteroids, such as prednisone and prednisolone, may be used for more severe flares of atopic dermatitis. They are used if the rash covers a large part of the body and face. Oral steroids used long term have numerous side effects, including water retention, decrease in bone density, and suppression of the immune system. Though they may be effective in clearing atopic dermatitis, the long term side-effects are too risky to warrant using them as a first-line treatment. [50, 56] To avoid these side effects, but still benefit from the medication, oral steroids are often prescribed for a short course (5 days) to calm the rash. Topical steroids can then be used on the remaining rash.

2.2.4. Alternative treatments for AD

2.2.4.1. Light (UV) therapy

Phototherapy is effective in treating refractory atopic dermatitis and may be considered as a treatment option depending on the patient's age. [67] This treatment may be administered as ultraviolet A (UVA), ultraviolet B (UVB) or combined UVA and UVB. Psoralen plus UVA (PUVA) is known as photochemotherapy and

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may be a treatment option in patients with extensive refractory atopic dermatitis. [107]

Randomized clinical trials have shown that ultraviolet light (ultraviolet B, narrow-band ultraviolet B, and high-intensity ultraviolet A) is beneficial for atopic dermatitis in the short term. [56] Burning and itching may occur, and carcinogenicity is a long term concern. Phototherapy is usually used as a second- or third-line treatment. [57] Ultraviolet light (UVB or PUVA) therapy may be a useful adjunctive modality in the treatment of chronic recalcitrant AD. Under professional supervision UVB can be effective and has been found to have anti-inflammatory effects resulting in part from its ability to inhibit lymphocyte trafficking and antigen-processing. [108] The photo-immunologic effects target key cells in atopic inflammation, such as lymphocytes (LC) and keratinocytes, interfering with cytokine production and decreasing the expression of activation markers such as HLA-DR and IL-2 receptor on CLA+ T cells. Unlike traditional UVA-UVB phototherapy, which appears less effective for acute exacerbations and acts primarily in the epidermis, high-dose UVA1 therapy has been shown to significantly decrease dermal IgE-binding cells, including mast cells, LCs, and dendritic cells (DCs). UVA1 also inhibits LC migration out of the epidermis. [109] Photochemotherapy with oral psoralen therapy, followed by UVA (PUVA), may also be helpful for patients with severe disease. However, PUVA is reserved for patients with more recalcitrant disease because of the expense and the potential increased risk of skin cancer.

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2.2.4.2. Leukotriene Inhibitors

The U.S. Food and Drug Administration have labelled leukotriene inhibitors such as Zileuton (Zyflo), Zafirlukast (Accolate) and Montelukast for the treatment of asthma. They also have been approved for the treatment of allergic rhinitis. Experimental data have suggested that leukotriene's may be involved in the pathogenesis of atopic dermatitis, because the majority of children with atopic dermatitis later develop allergic rhinitis and asthma, also perhaps due to the fact that asthma, allergic rhinitis and atopic dermatitis have a similar pathogenesis. Leukotriene's are synthesized in the cell from arachidonic acid by 5-lipoxygenase. The catalytic mechanism involves the insertion of an oxygen moiety at a specific position in the arachidonic acid backbone their over production is n major cause of inflammation. Leukotriene's are synthesized in response to many triggers, including receptor activation, antigen-antibody interaction, physical stimuli such as cold, and any stimulation that increases intercellular calcium. [110] These potent inflammatory mediators promote neutrophil-endothelial interactions, inducing bronchoconstriction and enhancing airway hyperresponsiveness. They also stimulate smooth muscle hypertrophy, mucus hypersecretion, and the influx of eosinophils into airway tissues. [111]

It is conceivable that early leukotriene inhibitor use could not only treat atopic dermatitis but also modify the disease course of allergic rhinitis and asthma in children. However, there are only a few small studies of the use of leukotriene inhibitors in the treatment of atopic dermatitis, most of which are either case reports or small randomized crossover trials. Two studies showed small but

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significant improvements in atopic dermatitis with the use of these agents. [112, 113] Another study on the use of either Montelukast or Zafirlukast in seven patients in a nonrandomized, no-control, add-on usage trial showed that leukotriene inhibitors did not lead to a sustained benefit for extensive atopic dermatitis. [114] The role of leukotriene inhibitors in atopic dermatitis has yet to be defined.

A study done by Carucci JA *et. al* described four patients with atopic dermatitis who responded to Zafirlukast therapy. [112] Prescriptions of leukotriene inhibitors have outpaced the evidence supporting their use, perhaps because of perceived ease of use compared with other asthma medications.

2.2.4.3. Immunosuppressants and Antineoplastics

Because patients with AD manifest abnormalities in immune regulation, therapy directed toward correction of their immune dysfunction represents an alternative approach. In this regard, therapeutic trials with several experimental immunomodulators have been reported. Immunosuppresent agents are drugs that prevent or reduce the immune response. They are used in the treatment of a variety of severe inflammatory conditions such as uveitis, scleritis, keratoconjunctivitis, and is also use to prevent graft rejection. [7] Mainly antineoplastic antimetabolites inhibit the conversion of folic acid to tetrahydrofolic acid by inhibiting the conversion of dihydrofolate to tetrahydrofolate by binding the enzyme dihydrofolate reductase, thereby interfering with DNA synthesis; it is specific for the S phase of the cell cycle. Antineoplastic agents are used in the treatment of leukemias, lymphomas, sarcomas, and cancer of the head and neck, as well as some organs like for example the colon. [115]

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At length, a case series that included 24 patients with atopic dermatitis who were treated with an antineoplastic, a human interferon gamma, for two years demonstrated both safety and efficacy for this therapy [116.] In a later case study Fifty-one patients with severe refractory AD were treated with recombinant interferon-gamm (rIFN-gamma). Twenty patients were treated with a low-dose rIFN-gamma; 21 patients received a high-dose and 10 patients received placebo. The patients were injected subcutaneously 3 times a week for 12 weeks. The disease severity of the 2 groups treated with rIFN-gamma was reduced significantly at the end of treatment period compared with that of the placebo group, also more rapid clinical improvement and more effective treatment outcome were seen in the high-dose group than in the low-dose group for the initial 6-week treatment period; however, the clinical improvement in both of the treated groups was stable and maintained after week 8 of treatment. [117] Thus a higher dosage of rIFN-gamma is more effective but for the maintenance of clinical improvement, a lower dosage of rIFN-gamma is recommended. A similar more recent study also showed that IFN-gamma can cause marked clinical improvement in AD patients, and interleukin (IL)-4 and IL-6 are two therapeutic targets in mechanism of action of IFN-gamma. [118] Although the exact mechanism of action of IFN-gamma therapy in AD is not clear, the beneficial effects of IFN-gamma have been attributed mainly to an immunomodulating effect on the expression of certain immunologic markers. [123] To date, however, large placebo-controlled studies are lacking.

Thymopentin, a synthetic pentapeptide, which promotes differentiation of thymocytes and suppressor/cytotoxic T-cell function, has been found to provide significant relief of pruritus and erythema caused by AD. [119] IFN-γ, a cytokine that downregulates Th-2 cell function, has also been found in placebo-controlled trials to reduce clinical severity associated with AD and decrease total circulating

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eosinophil counts. [3, 126] Tacrolimus (Prograf), which is used in an oral form to prevent organ transplantation rejection, is also available in a topical form. This topical agent also appears to be effective in the treatment of refractory atopic dermatitis, with few adverse effects. [120] Azathioprine (Imuran) may also be efficacious in patients with atopic dermatitis. Treatment with this immunosuppressant is more cost-effective than therapy with cyclosporine or tacrolimus. [127]

Finally, Cyclosporine, an immunosuppressant drug that down regulates cytokine production, has also been reported in double-blind, placebo-controlled trials to cause a significant improvement in AD. [121] Cyclosporine (Sandimmune) has been effective in patients with refractory AD. The condition returns after the cessation of therapy, although not always at the original level of severity. [83] Cyclosporine therapy did, however, lead to mild renal and liver toxicity. Thus the side effects associated with prolonged systemic cyclosporine therapy make it an unlikely candidate for long-term treatment of AD. Nevertheless, these observations suggest that modulation of the immune system with approaches that inhibit IgE responses or interfere with the action of IL-4 and IL-5 may represent exciting future alternatives to be used in patients with AD that is resistant to currently available therapy. In addition, the new high-potency phosphodiesterase inhibitors may be useful in targeting the increased phosphodiesterase activity in atopic monocytes and have demonstrated promising preliminary clinical results. [122]

New immunologic therapies for chronic allergic diseases in the future are likely to include the modulation of IgE secreting cells as well as modulation of T-h2 cells and their cytokines targeting specific organ. This is stated because of the observation that IgE has a multifunctional role in the pathogenesis of allergic

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inflammation. Aside from its involvement in IgE-mediated degranulation of mast cells and basophils, it is also involved in the activation of macrophage/monocytes and the stimulation of Th-2 cells. Recent studies with recombinant IFN-γ and cyclosporine are promising and serve as a proof of the concept that immunomodulatory therapies that down regulate T-cell function and cytokine secretion are effective in reducing the clinical severity of AD. [123] As our understanding of the immunopathogenesis of allergic responses continues to grow, manipulation of the immune response in AD and other allergic diseases is likely to take an exciting new direction in the treatment of this common group of illnesses. Table 6 summaries the development for new evolving therapeutic targets for AD.

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Table 6 : Evolving therapeutic targets in AD
Anti-allergic approaches
Anti-IgE
Allergen-selective immunotherapy
Immunization with CpG motifs
T cell targets
Probiotics (e.g., Lactobacillus rhamnosus strain GG)
Reduction of Th2 cytokine responses (anti–IL-4, soluble IL-4
receptor, anti–IL-13,
antisense oligonucleotide approaches)
Mycobacterium vaccae vaccination
Inhibition of T cell activation; e.g., alefacept (blocks T cell
CD2–leukocyte functional antigen-1 [LFA-1] and –LFA-3 APC
interactions), efalizumab (blocks LFA-1–ICAM-1 and –ICAM-2
APC interactions)
Anti-inflammatory agents
Oral pimecrolimus
TNF inhibitors
Antimicrobial approaches augmenting T cell responses
Antimicrobial peptides
Blockade of inflammatory cell recruitment
Chemokine antagonists (C-C chemokine receptor-4, cutaneous
lymphocyte–associated antigen)
CLA inhibitors

Adapted from Donald YM Leung.... New insight into AD [109]

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2.3. Animal models for AD

Our understanding of atopic dermatitis in humans has been enormously expanded by the use of animal models, because they allow in-depth investigation of pathogenesis of the disease and provide invaluable tools for diagnostic and pharmaceutical purposes. Appropriate animal models are essential for the identification of potential drugs. Because AD is a common disease for which there is no satisfactory therapy, understanding AD through the study of animal models is a pressing need. Although species other than mice, for example, dogs and guinea pigs, can develop AD-like lesions, mouse models are primarily used because of the ease of manipulation, low cost, repeatability and most importantly the availability of genetically manipulated strains. Since the description of the Nc/Nga mouse as the first spontaneously occurring model of AD in 1997 a number of mouse models have been developed. [124]

These models can be categorized into three groups:

1. Mice that spontaneously develop AD-like skin lesions for example NC/Nga mice. (Fig. 4)
2. Models induced by Epicutaneous (EC), application of sensitizers for example Balb/c mice painted repeatedly with a hapten. (Fig 5)
3. Genetically engineered mouse models; Transgenic mice that either over express or lack selective molecules [125]. (Fig. 6)

These mousse models display many features of human AD, and their study has resulted in a better understanding of the pathogenesis

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2.3.1. Mice that spontaneously develop AD-like skin lesions

The NC/Nga strain of mice (Fig. 4) was established as an inbred strain by K. Kondo in 1957 [126] and originated from Japanese fancy mice. The mouse has been reported to have certain characteristics such as high susceptibility to irradiation and to anaphylactic shock induced by ovalbumin. [126, 127] The NC/Nga mice have also been reported to develop an eczematous condition when kept in conventional surroundings but not when kept under specific pathogen–free (SPF) conditions. [124] The eczema develops at the age of 8 weeks, with maximum activity at around 17 weeks with lesions characterized by edema, hemorrhage, erosion, dryness, and alopecia typically localized on the ears, back, and neck and in the facial region. Serologically, the IgE level is markedly elevated from the age of 8 weeks**.** Although these animal models spontaneously developing AD-like lesions are useful for research on atopic dermatitis, this strain is not widely available, thus limiting its usefulness. Thus there was a great need to develop a simple and reproducible animal model of atopic dermatitis by using laboratory animals that are widely available.

Figure 4: NC/Nga mice established as an inbred strain by K. Kondo in 1957

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2.3.2. Models induced by Epicutaneous (EC), application of sensitizers

The second simple and reproducible model for AD is induced by Epicutaneous (EC) application of sensitizers for example house dust mite allergen or a hapten. Haptens are low-molecular-weight organic chemicals that aren't allergenic on their own but binds to peptides or proteins, thereby altering its configuration and rendering it foreign and allergenic. Examples of haptens include antibiotics, some other drugs, as well as chemicals present in toiletries, processed foods, powdered milk, preservatives used in vaccines, and metal jewellery. Balb/c mice (Fig. 5) subjected to EC application of the recombinant mite allergen termed, Der p8, exhibited features of dermatitis with epidermal hyperplasia and spongiosis, skin infiltration with CD4þ and CD8þ cells, and a skewed Th2 response locally and systemically. [128] Noted in earlier findings it was found that an antigen specific IgE antibody is preferentially produced in mice repeatedly painted with a hapten. [129] In 1995 Kitagaki H *et. al.* reported that repeated application of 2,4,6-trinitrochlorobenzen (TNCB) at 2-day intervals for 24 days to the same skin site results in a site-restricted shift in the time course of antigen-specific hypersensitivity responses from a typical delayed-type to an ITH reaction followed by a late reaction, [137] a finding often seen in skin lesions of AD patients. Soon-Hee Lee *et al.* induced atopic dermatitis-like immunologic skin disorder by repeatedly applying DNCB onto mice skin, after complete removal of dorsal hair. [138] This model had originally been developed using Balb/c mice. [137] This simple and reproducible model is of enormous value in the assessment of potentially therapeutic agents for the treatment of atopic dermatitis. The major advantage of using this model in trials of possible therapies over other models is its

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reproducibility as well as the ease of quantitative assessment by measuring for example ear thickness.

Super antigen-induced mouse models of AD is another way of producing mouse models for AD. It has been shown that application of super antigens such as exotoxins with superantigenic properties produced by *Staphylococcus aureus* strains to tape-stripped skin of Balb/c mice was able to elicit Th2-dominated allergic skin inflammation accompanied by systemic Th2 response to the super antigen. [130]

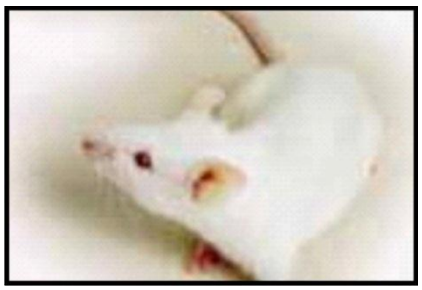

Figure 5: Healthy Balb/c mouse

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2.3.3. Genetically engineered mouse models

The third means of producing a mouse model for AD is by genetically engineering mouse models, these are transgenic mice that either over-express or lack selective molecules. IL-4 transgenic mice,[131] TSLP transgenic mice, [132] Caspase-1 transgenic mice [133] and also Apolipoprotein C1 (APOC1) transgenic mice (Fig.6).[134] These are only a few examples of genetically engineered mouse models for AD. APOC1 is an Apolipoprotein involved in lipoprotein metabolism.[134] Apolipoprotein C-I is a protein component of lipoproteins that in humans is encoded by the APOC1 gene. [135, 136] In healthy individuals, the protein is predominantly expressed in liver, skin, and brain tissue with macrophages and keratinocytes as major cell types. The protein is highly conserved and a high degree of homology exists between APOC1 in mice and humans. Mice transgenic for human Apolipoprotein C1 (APOC1Tg mice) in liver and skin have increased levels of free fatty acids, cholesterol, and triglycerides, but show a complete absence of subcutaneous fat and atrophic sebaceous glands. [125] APOC1Trans genic mice not only have disturbed serum levels of lipids but they also spontaneously develop with age severe dermatitis. Various features are suggestive for a Th2-mediated mechanism, i.e. the involvement of eosinophils, mast cells and IgE. Symptoms of atopic dermatitis develop gradually and are associated with moderate epidermal hyperplasia, and hyperkeratosis and parakeratosis, scaling, lichenification, excoriations, and pruritus. At an age of 6 weeks already an increased in transepidermal water loss can be detected in APOC1tg mice have, possibly, this enables the activation of the immune system by a trigger a Th2-mediated mechanism. [137]

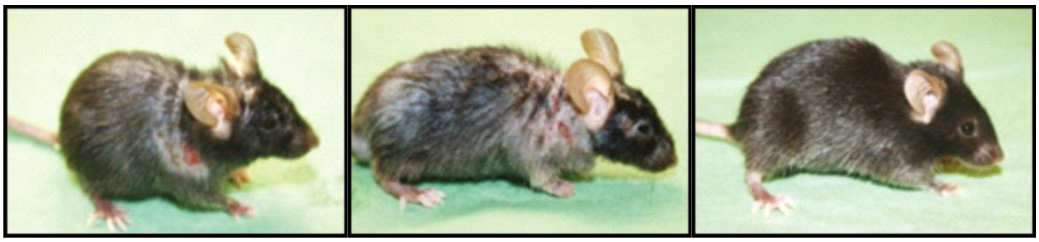

Figure 6: Appearance of atopic dermatitis in APOC1(+/+) mice. AD in APOC1(+/+) mice is associated with scaling, papules, lichenification, and excoriations. Left, 12-week-old APOC1(+/+) mouse; middle panel, 18-week-old APOC1(+/+) mouse; right, 12-week-old APOC1(+/-) control mouse.Homozygous APOC1+/+ mice gradually develop symptoms of dermatitis evident from increased scaling, papules, lichenification and excoriations

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2.4. Overview of *Aloe*

Genus Aloe plant has been used in medicinal practice over thousands of years in many cultures of the world. The historical use of various *Aloe* species by humans is well documented although documentation of the clinical effectiveness is relatively limited. [138] Of the 500+ species of *Aloe*, [139] only a few are used as a herbal medicine, *Aloe vera* again being the most commonly used version of *Aloe* in herbal medicine. Also included are *Aloe perryi* (found in north-eastern Africa) and *Aloe ferox* (found in South Africa).

The use of *Aloe* is being promoted for a large variety of conditions. Often general practitioners (GPs) seem to know less than their patients about its alleged benefits. *Aloe* products, which include the latex, gel, and whole leaf, are used, among other reasons, as laxatives, in creams for skin ailments, and as a treatment for a wide range of diseases, respectively. [149] The heterogeneous nature of *Aloe* products may contribute to the diverse biological and therapeutic activities that have been observed. Variations in the composition of *Aloe* can result in products with different chemical and physical properties, making the comparison of products difficult. The Department of Complementary Medicine at the University of Exeter receives more enquiries from colleagues related to *Aloe vera* than for any other herbal remedy. Considering this high level of interest, it is relevant to review systematically the evidence for or against its clinical effectiveness.

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2.4.1. *Aloe vera*

Aloe arborescens Miller also known as *Aloe vera* (Fig.7 & Fig. 8) is a stem-less, drought-resistant succulent plant and part of the family Liliaceae. [140] The *Aloe* leaf can be divided into two major parts, namely the outer green rind, including the vascular bundles, and the inner colourless parenchyma containing the aloe gel. The green skin of the *Aloe* leaf has a hard, waxy surface and is about 15 cell layers thick. Vascular bundles just below the rind contain tubes of xylem or phloem that transport water and nutrients. The long, narrow tubes are the source of "*Aloe* juice." A bitter, sticky yellow or orange sap will drain from the larger tubules when a leaf is cut. Description of the inner central part of the aloe leaf may sometimes be confusing, due to the different terms that are used interchangeably such as inner pulp, mucilage tissue, mucilaginous gel, mucilaginous jelly, inner gel and leaf parenchyma tissue. Technically, the term 'pulp' or 'parenchyma tissue' refers to the intact fleshy inner part of the leaf including the cell walls and organelles, while 'gel' or 'mucilage' refers to the viscous clear liquid within the parenchyma cells.[141] The three structural components of the *Aloe vera (A.vera)* pulp are the cell walls, the degenerated organelles and the viscous liquid contained within the cells. These three components of the inner leaf pulp have been shown to be distinctive from each other both in terms of morphology and sugar composition. Many compounds with diverse structures have been isolated from both the central parenchyma tissue of *A. vera* leaves and the exudate arising from the cells adjacent to the vascular bundles. The bitter yellow exudate contains 1,8 dihydroxyanthraquinone derivatives and their glycosides, which are mainly used for their cathartic effects.[142] The *Aloe* parenchyma tissue or pulp has been shown to contain proteins, lipids, amino acids, vitamins, enzymes, inorganic compounds and small organic

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compounds in addition to the different carbohydrates. Some evidence of chemotaxonomic variation in the polysaccharide composition of aloes exists. [141]

A. vera is found in north Africa as well as certain parts of Australia, Europe and the East also South America, Turkey and the USA. *A vera* is used as a herbal medicine and has been shown to have anti-inflammatory [140, 143, 144, 145], anti-oxidant, anti-cancer, wound healing, anti-aging and anti-diabetic properties [140]. It contains more than 70 biological active compounds. [140] These include alkaloids, saponins, fatty acid materials, glycoproteins, resins, sterols, gelonins, minerals, vitamins (A, C and E) amino acids, enzymes, [146] glucosyl chromone, [147] carbohydrate polymers, glycomannans (peptic acid) and acemannan. [148]

The gel of *A. vera* consists of the following components with anti-inlammatory activity: salicylates, magnesium lactase, bradykinin, thromboxane inhibitors, sterols and beta linked acetyl mannan. [149] The inner gel of the *A. vera* leaf has been proven to decrease levels of tumour necrosis factor a (TNF-a) [145] and interleukin 1β [149] in monocyte cultures, which are associated with inflammation due to bacterial infections. [149, 150] In contrast to what is mentioned above, an aqueous extract of *A. vera* did not decrease TNF-a and interleukin 1β which are the main inflammatory mediators in *Propionibacterium acnes.* [151] An aqueous extract of *A. vera* did however inhibit the in vitro conversion of arachidonic acid to Prostaglandin E2 [152, 153] and reduces the production of thromboxane A2 synthase [153] that is responsible for platelet aggregation.

In vivo animal studies done on *A. vera* has shown to be effective in treating inflammatory bowel disease. It has an inhibitory effect on the production of reactive oxygen metabolites and prostaglandin E2 release by $CaCo_2$ epithelial

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colon cancer cells but had no effect on thromboxane A2. Furthermore it reduces the interleukin-8 levels which are pathogenic in inflammatory bowel disease. [140] However a study done on anti-inflammatory effects of A. *vera* showed that it had no inhibitory effect on the reactive oxygen metabolites, [151] which is contrary to the findings of other researchers. [140, 154, 152]

The lipooxygenase pathway which leads to the synthesis of leukotrienes or lipoxins [150] has been shown to be blocked by *A.vera* during the acute inflammation process, especially when applied topically to minor burns and skin ulcers. [155] In an *in vivo* study it has been shown that *A. vera* possesses gastro-protective properties at lower concentrations by inhibiting gastric acid secretion, indicating a possible use in the treatment of peptic ulcers. [146]

In addition *A. vera* has been shown to reduce carrageenan induced edema in rats at the site of injection by decreasing neutrophil migration. [152] *Aloe vera* contains the enzymes carboxypeptidase and bradykinase, both of which have been shown to relieve pain and decrease inflammation and swelling. [24] Salicylic acid has also been detected in *A. vera*, as has a relatively high concentration of mannose 6-phosphate. [156] The potent anti-oxidative compound 8-C-β-D-glucopyranosyl-2-propyl-7-methoxy-5- methylchromone, has been isolated from a methanol extract of *A. vera* [157] and iso-aloesin (2- acetonyl-6-C-β-D-glucopyranosyl-7-hydroxy-5-methylchromone) from the leaves this plant. [158, 159]

Aloe arborescens Miller is widely cultivated and used in Japan as well as all over the world for its therapeutic and cosmetic purpose. However, the preparation of this *Aloe* species is more preferable to use the whole-leaf rather than the isolated gel. [170] In regards to the healing properties, many researchers have demonstrated

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that the mucilaginous polysaccharides contained in the clear pulp of *Aloe* leaf are the major ingredient responsible for the healing. However, new evidence has shown that emodin, one of the derivatives of anthraquinones produced by superficial pericyclic cells, is also capable of promoting the repair of rats' excisional wounds via stimulating tissue regeneration. [160, 161] This is supporting evidence to the claim that the healing function of *Aloe* plant is essentially a result of the synergistic mode of action of many bioactive compounds, rather than one single "magic bullet". [162]

Long-term ingestion of moderate levels of *A. vera* has no apparent adverse effects on body weight, food intake gastrointestinal transit time and gross pathology in rats. However, at high doses, diarrhoea and decrease in body weight occurred [163].

Also long term ingestion at high doses has been associated with dermatitis, hepatitis, and thyroid dysfunction in humans.[164, 165, 166.]

Figure 7: *Aloe vera* plant with yellow flowers

Figure 8: *Aloe arborescens Miller* also known as *Aloe vera*

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2.4.2. *Aloe ferox*

Aloe ferox and *Aloe vera* both belong to the family Liliaceae and the tribe Aloineae. *Aloe ferox* (Fig. 9) are characterized by stem less large, thick, fleshy leaves that are lance shaped and have a sharp apex and a spiny margin. *Aloe ferox* (*A. ferox*) produces a single candelabrum-like inflorescence with dense erect spikes of scarlet flowers from May to October. Individual specimens have an estimated life span of 150 years. [167] *A. ferox* is indigenous to South Africa and Lesotho, and is the only species used in South Africa for the extraction of bitters and aloe gels.[167] It is a robust plant with persistent dry leaves on the lower portion of the single stem plant. *A. ferox* occurs in habitats ranging from easily accessible valleys to inaccessible mountain ridges, which ensures the survival of the species in the wild even if agricultural development has decreased its range on arable lands. [167] *Aloe* leaves have yellow latex, which is referred to as *Aloe* juice or sap and has a bitter taste. The dried product derived from *A. ferox* leaves is commonly known as Cape aloe. Cape aloe extract possesses the following pharmacological effects: anti-inflammatory, anti-bacterial, anti-fungal and protections against liver injury. [168]

A. ferox gel is an aqueous mixture of peptic substances, polysaccharides, amino acids, minerals, trace element, organic acids and various minor compounds. *Aloe* gel is prepared from the inner part of the leaf. The leaf pulp, is the inner most portion of the leaf and is composed of the parenchyma cells that contain the gel. [169] Other *A. ferox* components include antioxidant polyphenols, indoles, alkaloids, vitamins, enzymes, minerals polysaccharides, athraquinones, amino acids, saponins [168] and the enzymes carboxypeptidase and bradykinase, for the relieve pain and decrease inflammation and swelling. [24]

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A. ferox is widely used in traditional medicine in South Africa especially by traditional healers in African cultures. According to many authors, the species is used as an anti-inflammatory and anticancer agent such as in the treatment of leukaemia and against neuroectodermal tumour. [12, 170] The antimicrobial properties have been widely reported in literature. [171, 172] However, the reports have focused mainly on their activities against commensal microbial flora while information on their activity against human pathogens is scanty. Methanol and water extracts of *A. ferox* have shown to be effective as a treatment for *Neisseria gonorrhoea* infections, whereas the pure aloin isolated from *A. ferox* inhibits the growth of *Candida albicans* and *N. gonorrhoea* in vitro. [173] The anti-inflammatory compound aloeresin I was recently isolated from *A. ferox* and the presence of two dihydrocoumarins with immunomodulatory and antioxidative properties have also been reported. [26]

Unfortunately no literature on any possible clinical trials on the safety and efficacy of *Aloe* are available but the FDA has approved the internal use of the *Aloe* gel only as a dietary supplement and its external use only as a cosmetic ingredient, even though the clinical effectiveness of oral or topical *Aloe* is not sufficiently defined at present.

Figure 9: *Aloe ferox* plant

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Chapter 3

Experimental procedures

3.1. Study design

This was a proof of concept, double blind, randomized, parallel-group comparative study over a 15 day treatment period. This study had 2 study arms namely a Pilot study and a Main study consisting of 3 treatment groups each; two active drugs were compared to each other as well as to a placebo. *Aloe ferox* and *Aloe vera* were tested for its therapeutic efficacy in treating atopic dermatitis induced in BALB/c mice. In the event of any systemic adverse effects or if an animal causes severe damage to itself, for example by scratching itself severely, the experiment will be terminated.

3.2. Animals

3.2.1. Balb/c mice

66 Male 5-week-old Balb/c mice were used in this study; 6 for the Pilot study and 60 for the Main study. Ethical clearance was obtained from the AUCC (Animal Use and Care Committee) of the University of Pretoria (reference no: H017-10) for the use of the animals. Mice where bred and raised in the Centre for Animal services at the University of the Witwatersrand in Johannesburg until 4 weeks of age where after the mice were transported to the University of Pretoria Biomedical Research Centre (UPBRC) at Onderstepoort.

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For the Pilot study the animals were randomly divided into 3 groups/arms, namely the *Aloe ferox* arm, consisting of 2 mice, the *Aloe vera* arm consisting of 2 mice, and a negative control group (placebo gel) arm also consisting of 2 mice. For the Main study 60 mice where randomly and equally divided into the same study arms, the *Aloe ferox* arm consisting of 20 mice, the *Aloe vera* arm consisting of 20 mice, and the negative control group (placebo gel) arm also containing 20 mice.

3.2.2. Housing and feeding conditions

Male mice weighing between 20 and 40 grams were housed 5 per cage in a controlled environment. The mice were maintained in a room with a 12-h light / 12-h dark cycle, with the temperature and humidity set at 23 ± 2C and 55 ± 5%, respectively. The animals were provided food and tap water ad libitum. They were fed a conventional rodent diet obtained from Epol (Pty) Ltd (Randburg, South Africa) and had an unlimited supply of drinking water. Environmental enrichment, for example, bedding (wood wool), was provided to keep the mice occupied. Previous work suggests that the provision of enrichment items, such as wood wool, gives laboratory mice the opportunity to perform exploratory and gnawing activities. This can be used to improve their well-being and to distract them from scratching and fighting. [174]

3.2.3. Preparation of animals

The cages of the rats were labelled from 1 to 14 for identification purposes. The mice were also numbered by clipping the ears a standard method of identification used by the UPBRC at Onderstepoort. The mice were kept in their cages for at least five days prior to commencement of the experiment to allow for

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acclimatisation to the laboratory conditions 175 and they were handled daily during this period. They were not immunosuppressed.

3.3. Pilot Study

The Pilot study was done to test logistics and in order to improve the design of the main study; this included aspects such as better quality, efficiency and analyses of information to be gathered. The pilot study was conducted using 6 Balb/c mice, two mice per group a *Aloe ferox* arm, a *Aloe vera* arm and a negative control group (placebo gel) arm. The method of the Pilot study was adapted from previous research done by Kitagaki H et al. [186] The Pilot study, was planned over 36 days. For the development of AD, mice where sensitized by a single topical application of 20 µl of 0.25% DNFB solution to the right ear 7 days before initiation of the study on day -6. The first DNFB challenge took place on day 0, mice were challenged every second day. As control the vehicle was applied to the left ears in the same manner to insure that the application of DNFB cause the development of AD.

The intention of the Pilot study was to gather information regarding the following:

1. Verification that the instructions given to investigators (e.g. randomization procedures) were comprehensible.
2. Verification that investigators and technicians were sufficiently skilled in the procedures and handling of the animals (BALB/c mice).
3. Verification on the correct operation of equipment
4. Assessment on the level of intervention (e.g. the dose of a drug).

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5. Lastly identification of any adverse effects (pain, suffering, distress or lasting harm) caused by the procedure, and the effectiveness of actions to reduce them.

3.3.1. Materials

3.3.1.1. Drugs

Table 7: List of Drugs used during study		
Drug	**Properties**	**Source**
***Aloe ferox* gel**	• 97%- *Aloe ferox* • Whole leave extract • Applied topically	Albertinia South Africa
***Aloe vera* gel**	• 97%- *Aloe vera* • Whole leave extract • Applied topically	PharmaNatura Wynberg, Sandton
Placebo gel	• No active ingredient • Applied topically	Albertinia South Africa

3.3.1.2. Chemicals

Table 8: List of Chemicals used during study		
Chemical	**Properties**	**Source**
Chemicals used for induction of Atopic Dermatitis		
Acetone	• Formula: $(CH_3)_2CO$. • Molar mass: 58.08g/mol • Appearance: Colourless liquid • Odour: Pungent, irritating, floral	Merck (Darmstadt, Germany)

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Dinitrofluorobenzene (DNFB)	• 1-fluoro-2,4-dinitrobenzene, or Sanger's reagent, is a chemical used for polypeptide sequencing • Density: 1.47 g/cm³ • Boiling point: 296° C	Sigma-Aldrich (St Louis, Missouri USA)
DMSO	• Dimethyl sulfoxide (DMSO) is an organosulfur compound • Formula: $(CH_3)_2SO$. • Appearance: Colourless liquid • An important polar aprotic solvent that dissolves both polar and nonpolar compounds • Boiling point: 189° C • Density: 1.10 g/cm³ • Molar mass: 78.13 g/mol	Merck (Darmstadt, Germany)
Sesame Oil	• 100% pure sesame seed oil	Merck Chemicals (Gauteng)

3.3.1.2. Equipment

- Adjustable pipettes to measure volumes ranging from 2 µl to 1 ml
- Tubes to prepare standard dilutions
- Calliper to measure ear thickness of mice
- Electronic metric scale

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3.3.2. Method for Pilot Study

3.3.2.1. Preparation of reagents for AD sensitization

2,4 Dinitrofluorobenzene (DNCB) as sensitisizing agent, was dissolved in acetone and made up to a concentration of 0.25% with sesame oil. Pure sesame oil was used as the placebo control and contained no active ingredient

3.3.2.2. Sensitization and challenging procedures for Pilot study

Mice where sensitized and repeatedly challenged at the same skin site (dorsal part of ears) with DNFB solution. Briefly, mice where sensitized by a single topical application of 20 µl of 0.25% DNFB solution to the right ear 7 days before the first challenge (day 0), as control the vehicle was applied to the left ears in the same manner. 10 µl of 0.25% DNFB solution was repeatedly applied to the sensitized right ears as well as 10 µl placebo solution to the left ears at 2-day intervals.

3.3.2.3. Experimental procedure for Pilot study

For assessment of curative effects, the application of the investigational medicinal product, *Aloe ferox, Aloe vera* and a placebo gel, began from day 16 up to day 35. Test compounds where applied 2 hours after the DNFB challenge. For estimation of the efficacy of the test compounds against chronic contact hypersensitivity reaction, changes in the ear thickness were measured at 2-day intervals with an electronic calibre just before DNFB application, animals were also weighed every

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second day. At the end of the experimental period (day 36), all the animals were terminated by isoflurane

overdose and blood was collected by cardiac puncture for measurement of serum IgE. Experimental procedure is detailed in Table 9.

Table 9: Experimental procedure for pilot study.

	***Aloe ferox* group 20 mice**	***Aloe vera* group 20 mice**	**Placebo group 20 mice**
Day -6	Single topical application of 20 µl of 0.25% DNFB solution once daily to the right ear of each mouse, as control the vehicle (extra virgin olive oil) was applied to the left ears in the same manner.	Single topical application of 20 µl of 0.25% DNFB solution once daily to the right ear of each mouse, as control the vehicle (extra virgin olive oil) was applied to the left ears in the same manner.	Single topical application of 20 µl of 0.25% DNFB solution once daily to the right ear of each mouse, as control the vehicle (extra virgin olive oil) was applied to the left ears in the same manner.
Day -5	No treatment	No treatment	No treatment
Day -4	No treatment	No treatment	No treatment
Day -3	No treatment	No treatment	No treatment
Day-2	No treatment	No treatment	No treatment
Day -1	No treatment	No treatment	No treatment
Day 0	Weigh all mice measure ear thickness of both ears.	Weigh all mice measure ear thickness of both ears.	Weighe all mice measure ear thickness of both ears.
Day 1	Apply10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.
Day2	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.
Day3	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.

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Day4	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.
Day5	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.
Day6	Weig all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.
Day7	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.
Day8	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.
Day9	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.
Day10	Weigh all mice measured ear thickness of both ears	Weigh all mice measured ear thickness of both ears	Weigh all mice measured ear thickness of both ears
Day11	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.
Day12	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.
Day13	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.
Day14	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.
Day15	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.
Day16 **Treat-**	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.	Weigh all mice measured ear thickness of both ears.

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ment day 1	Apply 10 µl of *Aloe ferox* test compound to both ears.	Apply 10 µl of *Aloe vera* test compound to both ears.	Apply 10 µl placebo gel to both ears.
Day17 **Treat-ment day 2**	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe ferox* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe vera* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl placebo gel to both ears 2 hours after the DNFB challenge.
Day18 **Treat-ment day 3**	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe ferox* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe vera* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl placebo gel to both ears.
Day19 **Treat-ment day 4**	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe ferox* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe vera* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl placebo gel to both ears 2 hours after the DNFB challenge.
Day20 **Treat-ment day 5**	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe ferox* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe vera* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl placebo gel to both ears.
Day21 **Treat-ment day 6**	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe ferox* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe vera* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl vehicle placebo to the left ears. Apply 10 µl placebo gel to both ears 2 hours after the DNFB challenge.

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Day22 **Treat-ment day 7**	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe ferox* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe vera* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl placebo gel to both ears.
Day23 **Treat-ment day 8**	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe ferox* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe vera* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl placebo gel to both ears 2 hours after the DNFB challenge.
Day24 **Treat-ment day 9**	Weigh all mice measure ear thickness of both ears. Apply 10 µl of *Aloe ferox* test compound to both ears.	Weigh all mice measure ear thickness of both ears. Apply 10 µl of *Aloe vera* test compound to both ears.	Weigh all mice measure ear thickness of both ears. Apply 10 µl placebo gel to both ears.
Day25 **Treat-ment day 10**	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe ferox* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe vera* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl placebo gel to both ears 2 hours after the DNFB challenge.
Day26 **Treat-ment day 11**	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe ferox* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe vera* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl placebo gel to both ears.
Day27 **Treat-ment day 12**	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe ferox* test compound to both ears	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe vera* test compound to both ears 2	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl placebo gel to both ears 2 hours after the

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	2 hours after the DNFB challenge.	hours after the DNFB challenge.	DNFB challenge.
Day28 **Treat-ment day 13**	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe ferox* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe vera* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl placebo gel to both ears.
Day29 **Treat-ment day 14**	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe ferox* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe vera* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl placebo gel to both ears 2 hours after the DNFB challenge.
Day30 **Treat-ment day 15**	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe ferox* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe vera* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl placebo gel to both ears.
Day31 **Treat-ment day 16**	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe ferox* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe vera* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl placebo gel to both ears 2 hours after the DNFB challenge.
Day32 **Treat-ment day 17**	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe ferox* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe vera* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl placebo gel to both ears.
Day 33 **Treat-ment day**	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears.

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18	Apply 10 µl of *Aloe ferox* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of *Aloe vera* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl placebo gel to both ears 2 hours after the DNFB challenge.
Day 34 **Treat-ment day 19**	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe ferox* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl of *Aloe vera* test compound to both ears.	Weigh all mice measured ear thickness of both ears. Apply 10 µl placebo gel to both ears.
Day 35 **Treat-ment day 20**	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe ferox* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl of *Aloe vera* test compound to both ears 2 hours after the DNFB challenge.	Apply 10 µl of 0.25% DNFB solution to right ears as well as 10 µl placebo solution to the left ears. Apply 10 µl placebo gel to both ears 2 hours after the DNFB challenge.
Day 36	Terminate animals by isofluorane overdose without DNFB challenge or test ointment application. Collect blood by cardiac puncture under isoflurane anaesthesia.	Terminate animals by isofluorane overdose without DNFB challenge or test ointment application. Collect blood by cardiac puncture under isoflurane anaesthesia.	Terminate animals by isofluorane overdose without DNFB challenge or test ointment application. Collect blood by cardiac puncture under isoflurane anaesthesia.

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3.4. Main Study

As a result of unexpected complications in the Pilot study the Main study followed a different method for the induction of AD.

The following issues complicated the pilot study:

- Clipping of the mice ears for identification purposes interfered with measuring of ear thickness.
- After application of the DNFB to the dorsal part of the ears, the Balb/c mice did not develop AD, this could have been due to the fact that it was easier for the mice to brush off the applied DNFB with their paws.
- The area of application was too small and resulted in too little DNFB being absorbed through the dorsal part of the mouse ear.

A new method adopted by (Lee SH 2006) [186] was followed. This method involved the 2, 4-dinitrochlorobenzene (DNCB) dorsal skin application model to induce atopic dermatitis-like phenomena in mice. This modal has been reported as a reliable model demonstrating similar immunologic and skin alteration as human atopic dermatitis.[186]

The following changes were made to the Main study:

- For induction of AD, DNCB was applied onto mice dorsal skin, after complete removal of dorsal hair. This method differs to the pilot study as in the pilot study the DNFB was applied to the dorsal part of the ear.

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- Another change that was made is that a higher concentration of DNFB was used as well as a minute amount of DMSO was added to the DNFB solution to increase the absorption of DNFB through the skin.

3.4.1. Materials

3.4.1.1. Drugs

Table 10: List of Drugs used during main study		
Drug	**Properties**	**Source**
***Aloe ferox* gel**	• 97%- *Aloe ferox* • Whole leave extract • Applied topically	Albertinia South Africa
***Aloe vera* gel**	• 97%- *Aloe vera* • Whole leave extract • Applied topically	PharmaNatura Wynberg, Sandton
Placebo gel	• No active ingredient • Applied topically	Albertinia South Africa

3.4.1.2. Chemicals

Table 11: List of Chemicals used during main study		
Chemical	**Properties**	**Source**
Chemicals used for induction of Atopic Dermatitis		
Acetone	• Molecular Formula: $(CH_3)_2CO$. • Molar mass: 58.08g/mol • Appearance: Colourless liquid • Odour: Pungent, irritating, floral	Merck (Darmstadt, Germany)
Dinitrofluorobenzene (DNFB)	• 1-fluoro-2,4-dinitrobenzene, or Sanger's reagent, is a chemical used for polypeptide sequencing • Density: 1.47 g/cm^3	Sigma-Aldrich (St Louis, Missouri USA)

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	• Boiling point: 296° C	
DMSO	• Dimethyl sulfoxide (DMSO) is an organosulfur compound • Appearance: Colourless liquid • An important polar aprotic solvent that dissolves both polar and nonpolar compounds • Formula: $(CH_3)_2SO$. • Boiling point: 189° C • Density: 1.10 g/cm³ • Molar mass: 78.13 g/mol	Sigma-Aldrich (St Louis USA)
Sesame Oil	• 100% pure sesame seed oil	Merck Chemicals (Guateng)
Chemicals used for Preparation of mouse skin samples		
Isoflurane	• Isoflurane (2-chloro-2-(difluoromethoxy)-1,1,1-trifluoro-ethane) is a halogenated ether used for inhalational anesthesia • Molecular Formula: $C_3H_2ClF_5O$ • Molar mass:184.5 g/mol	Sigma-Aldrich (St Louis USA)
Formaldehyde	• Formaldehyde is an organic compound. • A gas at room temperature, • Formaldehyde is colourless and has a characteristic pungent, irritating odour. • Molecular Formula**:** CH_2O • Density**:** 815.30 kg/m³ • Boiling point**:** -19° C • Molar mass**:** 30.031 g/mol	Sigma-Aldrich (St Louis USA)
Xylene	• Molecular Formula: C_8H_{10}, $C_6H_4(CH_3)_2$ or $C_6H_4C_2H_6$ • Molar Mass:106.16 g/mol • Appearance: clear, colourless liquid	Merck (Darmstadt, Germany)
Absolute ETOH	• Molecular Formula: C_2H_6O • Molar Mass: 46.07 g/mol • Appearance: Colourless liquid • Density: 0.789 g/cm^3	Illovo Sugar Limited (Durban, RSA)
Wax		

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Chemicals used for ELISA supplied together with BioLegend's ELISA MAX™ Deluxe Sets		
Mouse IgE ELISA MAXTM Detection Antibody (200X)		
Mouse IgE ELISA MAXTM Capture Antibody (200X)		
Mouse IgE Standard		
Avidin-HRP (1000X)		
Substrate Solution A		
Substrate Solution B		
Coating Buffer A (5X)		
Assay Diluent A (5X		
Chemicals used for ELISA that was not supplied with BioLegend's ELISA MAX™ Deluxe Sets		
PBS (Phosphate-Buffered Saline	• Consist of 8.0 g NaCl, 1.16 g Na2HPO4, 0.2 g KH2PO4, 0.2 g KCl, (FTA Hemagglutination buffer) • Add deionized water to 1 L • pH to 7.4 • 0.2 µm filtered	BD Bioscience (Sparks, USA)
Wash Buffer is recommended.	• (Phosphate-Buffered Saline (PBS) + 0.05% Tween-20, pH7.4).	BioLegend Cat. No. 421601
Sulphuric acid	• Stop Solution (2N H_2SO_4) • Molecular formula: H2SO4. • Appearance: colourless to slightly yellow viscous liquid which is soluble in water at all concentrations • Density**:** 1.84 g/cm³ • Molar mass**:** 98.079 g/mol • Boiling point**:** 337° C	Merck (Darmstadt, Germany)
Chemicals used for H&E staining		
Shandon Instant Haematoxylin	• No mucoid staining. • Stains from blue-black to light blue depending upon preparation	Shandon 6765015, Thermo Fisher Scientific, Manor Park, Runcorn UK)
Eosin	• Fluorescent red dye resulting from the action of bromine on fluorescein • Used as a counterstain to haematoxylin in H&E (haematoxylin and eosin) staining • Molecular Formula:	(Certistain CI 45380, Merck 1.15935, Darmstadt, Germany)

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	$C_20H_6Br_4Na_2O_5$. • Molar mass, 691.85 g/mol	
DPX mountant	• Liquid mixture/solution: -Polystyrene solution in Xylene • Melting Point: 80-88ºC • Density: 0.94g/ml at 25ºC • Flammable	(Saarchem 1935000KF, Merck (pty) Ltd, Wadeville, Gauteng).
Ethanol	• Molecular formula CH_3CH_2OH • Molar mass: 46.07g/mol • Appearance Colorless liquid • Density: 0.789 g/cm^3	Illovo Sugar Limited (Durban, RSA)
Hydrochloric acid	• Hydrochloric acid is a clear, colourless solution of hydrogen chloride in water. • Formula: HCL • Molar mass: 36.406g/mol	Merck (Darmstadt, Germany)
Acetone	• Formula: $(CH_3)_2CO$. • Molar mass: 58.08g/mol • Appearance: Colorless liquid • Odor: Pungent, irritating, floral	Merck (Darmstadt, Germany)
Acetic acid	• Acetic acid is an organic compound • Acetic acid is the main component of vinegar, and has a distinctive sour taste and pungent smell. • Formula**:** $C_2H_4O_2$ • Density**:** 1.05 g/cm^3 • Molar mass**:** 60.05 g/mol • Boiling point**:** 118° C	Merck (Darmstadt, Germany)
Xylene	• Formula: C_8H_{10} • Density: 0.864g/ml, liquid phase • Melting point: -47.4ºC • Boiling point: 138.6 ºC	Merck (Darmstadt, Germany)

3.4.1.3. Equipment

- NUNC MaxisorpTM 96 MicroWell Plates
- A micro plate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 µl to 1 ml

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- Wash bottle or automated micro plate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Sealer
- Absorbent paper

3.4.2. Method

3.4.2.1. Preparation of reagents for AD sensitization

DNCB was dissolved in acetone and made up to a concentration of 1% DNFB with sesame oil. 0.2ml DMSO was then added to 2ml of the 1% DNCB solution to make up a final concentration of 0.9% DNFB. DMSO was added to improve the absorption of the DNCB through the skin.

Study groups (Placebo, *Aloe ferox*, *Aloe vera*) were compared with respect to IgE concentration using an one-way analysis of variance (ANOVA). No baseline blood for analysis of IgE concentration was collected as mice has very small blood volume and would have resulted in death of the mice.

3.4.2.2. Experimental procedure for AD sensitization

For induction of atopic dermatitis-like immunologic skin disorder, 10 μL of 0.9% DNCB was applied on their dorsal skin once every day for 10 consecutive days, after complete removal of the hair on an area of approximately 4 cm^2. The mice were then left for 14 days to rest and the sensitized again for the next 5 days. On day 16 the animals were randomly divided by an independent individual using

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'Research Randomizor' software into 3 groups, namely *Aloe ferox* (20 mice), *Aloe vera* (20 mice), and a negative control group, placebo gel (20 mice). For assessment of curative effects, the investigational medicinal product's application was begun from day 16 up to day 26. An amount of 0.5 ml of each investigational product was topically applied to the dorsal side each respective mouse daily. For estimation of the efficacy of the investigational medicinal products against atopic dermatitis, blood was collected on day 27 by isoflurane overdose for measurement of serum IgE. The dorsal skin of each mouse were dissected, carefully removed and placed in 10% buffered formalin until further histopathological analysis.

3.4.2.3. Measurement of serum IgE

Blood was collected by cardiac puncture under isoflurane anaesthesia; serum harvested from each blood sample will be used for IgE quantification. Serum IgE was determined by a sandwich enzyme-linked immunosorbent assay (ELISA) (BioLegend's ELISA MAX™ Deluxe SetBioLegend's ELISA MAX™ Deluxe Set).

BioLegend's ELISA MAX™ Deluxe Sets contain the components necessary for the accurate quantification of natural and recombinant mouse IgE. These sets are designed for cost-effective and accurate quantification of mouse IgE in cell culture supernatant, serum, plasma or other biological fluids. They are sensitive, accurate, and robust.

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3.4.2.3.1. Principle of the ELISA Test

BioLegend's ELISA MAX™ Deluxe Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A mouse IgE specific monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and IgE binds to the immobilized capture antibody. Next, a biotinylated anti-mouse IgE detection antibody is added, producing an antibody-antigen-antibody "sandwich". Avidin-horseradish peroxidase is subsequently added, followed by TMB Substrate Solution, producing a blue color in proportion to the concentration of IgE present in the sample. Finally, the Stop Solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm with a microplate reader.[176]

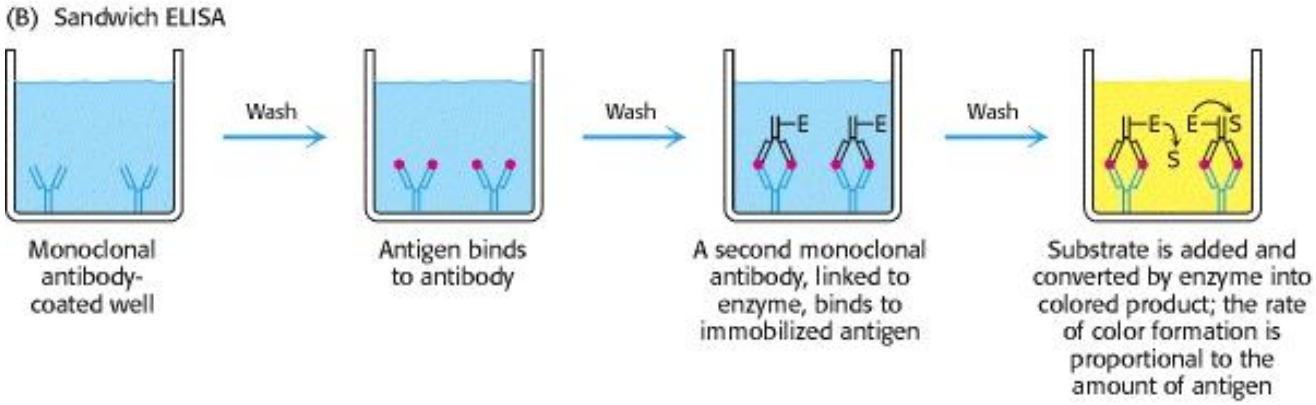

Figure 10: Principal of ELISA test

Adapted from; http://exploreable.wordpress.com/2011/05/25/elisa-enzyme-linked-immunosorbent-assay/

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3.4.2.3.2. Reagent Preparation – ELISA

1. 5X Coating Buffer was diluted to 1X with deionized water. For one plate 2.4 mL 5X Coating Buffer in 9.6 mL deionized water.

2. Pre-titrated Capture Antibody was diluted 1:200 in 1X Coating Buffer. For one plate 60 µL Capture Antibody was diluted in 11.94 mL 1X Coating Buffer.

3. 5X Assay Diluent was diluted to 1X with PBS (pH 7.4). For 50 mL, 10 mL 5X Assay Diluent was diluted in 40 mL PBS.

4. Lyophilized vials were under vacuum pressure. Lyophilized standard were reconstitute with 0.2 mL of 1X Assay Diluent.The reconstituted standard was allowed to sit for 15 minutes at room temperature, then mixed gently prior to making dilutions.

5. Prior to use, a 1,000 µL of the top standard was prepared at a concentration of 10 ng/mL from the stock solution in Assay Diluent (referd to Lot-Specific Instruction/Analysis Certificate).

6. The pre-titrated Biotinylated Detection Antibody was diluted 1:200 in 1X Assay Diluent. For one plate 60 µL Detection Antibody was diluted in 11.94 mL Assay Diluent.

7. Avidin-HRP 1:1000 was diluted in 1X Assay Diluent. For one plate 12 µL Avidin-HRP was diluted in 11.99 mL Assay Diluent.

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8. TMB Substrate Solution is a mixture of equal volumes of Substrate Solution A with Substrate Solution B. The two components were mixed immediately prior to use. For one plate 6 mL Substrate Solution A was mixed with 6 mL of Substrate Solution B in a clean container (the solution was clear and colourless).

3.4.2.3.3. Assay Procedure for ELISA

One day prior to running the ELISA, Capture Antibody was diluted in 1X Coating Buffer as described in Reagent Preparation. 100 µL of this Capture Antibody solution was added to all wells of a 96-well plate provided in this set. Plates were sealed and incubated overnight (16-18 hrs) at 4°C.

All reagents were brought to room temperature (RT) prior to use. All standards and samples were run in duplicate. A standard curve was required for each assay.
Plates were washed 4 times with 300 µL wash Buffer per well and residual buffer was blotted by firmly tapping plates upside down on absorbent paper. All subsequent washes were performed similarly.

To block the non-specific binding and reduce the background, 200 µL 1X Assay Diluent per well were added. Plates were sealed and incubated at room temperature for 1 hour with shaking at 200 rpm on a plate shaker.

While plates were being blocked, the appropriate sample dilutions and standard were prepared. 1,000 µL of top standard was prepared at 10 ng/mL in 1X Assay Diluent (refer to “Reagent Preparation” section). Six two-fold serial dilutions of the 10 ng/mL top standard with Assay Diluent were performed in separate tubes.

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After diluting, the mouse IgE standard concentrations were 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.625 ng/mL, 0.313 ng/mL and 0.156 ng/mL, respectively. The Assay Diluent served as the zero standard (0 ng/mL).After incubation plates were washed 4 times with Wash Buffer.

100 µL per well of standards or samples were added to the appropriate wells. Plates were sealed and incubate at room temperature for 2 hours with shaking. After incubation time of 2 hours plates were washed 4 times with wash buffer.

100 µL of diluted Detection Antibody solution was added to each well, plates were sealed and incubated at room temperature for 1 hour with shaking. Plates were then washed 4 times with a Wash Buffer.

100 µL of diluted Avidin-HRP solution was added to each well. Plates were sealed and incubate at room temperature for 30 minutes with shaking.

Plates were then washed 5 times with a Wash Buffer. For the final wash, wells were soaked in Wash Buffer for 30 seconds to 1 minute for each wash. This helped minimizing background. 100 µL of freshly mixed TMB Substrate Solution were then added and incubate in the dark for 20 minutes*. The positive wells turned blue in colour. It wasn't necessary to seal the plate during this step.

Reaction was stopped by adding 100 µL of Stop Solution (2N $H_2SO_{4)}$ to each well. The positive wells turned from blue to yellow.

Absorbance was read at 450 nm and 570 nm within 30 minutes. The absorbance at 570 nm was subtracted from the absorbance at 450 nm.

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3.4.2.4. Hematoxylin and Eosin staining

Hematoxylin and Eosin are used for general tissue morphology. The staining method involves application of hemalum, which is a complex formed from aluminium ions and oxidized haematoxylin. The nuclear staining is followed by counterstaining with an aqueous or alcoholic solution of eosin Y, which colours other, eosinophilic structures in various shades of red, pink and orange.

Following termination, the dorsal skin of each mouse were dissected, carefully removed and placed in 10% buffered formalin until further analysis. One week later the dorsal skin samples were dehydrated in ascending grades of ethanol, cleared and processed through to paraffin wax by standard protocols, where after thin 3µm sections was made.

The paraffin wax was removed and the skin sections stained with hematoxylin and eosin for general tissue morphology. The staining method involves application of hemalum, which is a complex, formed from aluminium ions and oxidized haematoxylin. The nuclear staining is followed by counterstaining with an aqueous or alcoholic solution of eosin Y, which colours other, eosinophilic structures in various shades of red, pink and orange. [177, 178]

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3.4.2.4.1. Specimens

Standard paraffin sections fixed in neutral buffered formalin.

3.4.2.4.2. Procedure for Hematoxylin and Eosin staining

Three micron sections of the formalin fixed paraffin embedded (FFPE) tissue blocks were cut, mounted onto glass slides and baked at 60°C for twenty minutes. The sections were stained in a Leica Autostainer XL (Leica Microsystems, Nussloch, Germany) of which the protocol was as follows. The sections were dewaxed in two changes of xylene for five minutes each, hydrated in two changes of absolute ethanol followed by 70% ethanol. The sections were washed in tap water for four minutes and rinsed in distilled water. Thereafter the sections were stained in Shandon Instant Haematoxylin for 5 minutes at room temperature, washed in tap water for three minutes and rinsed in distilled water. The cytoplasm was stained by immersing the slides in a 0.2% Eosin Y, absolute ethanol solution for seven minutes at room temperature. Finally the sections were dehydrated through 95% and absolute ethanol, cleared in xylene and mounted onto a coverslip with DPX mountant. [177, 178]

3.4.3 Statistical Analysis

Statistical planning and analysis was done in association with Prof. P Bekker from the Medicinal Research Council associated with the University of Pretoria.

Resources limited the respective arms of the study to 20 mice per group. The expected standard mean of the IgE concentration in a normal population is 0.5µg/ml. The active arms were respectively compared to the placebo arm in order to show proof of concept. Data summary or employed summary statistics mean and

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standard deviation of Study groups (Placebo, *Aloe ferox and Aloe vera*) were compared with respect to concentration using an one-way analysis of variance (ANOVA). Specific differences between study groups, following significance in the ANOVA, was done using two-group t-tests employing the pooled standard deviation determined from the within group mean squares. These pair-wise comparisons were done at the Bonferroni adjusted 0.017(=0.05/3) level of significance.

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Chapter 4

Results

4.1. Pilot Study Results

The Pilot study presented a variety of problematic issues. This led to no results being obtained from the Pilot study, the following issues complicated the pilot study:

- Clipping of the mice ears for identification purposes interfered with measuring of ear thickness.
- After application of the DNFB to the dorsal part of the ears, the Balb/c mice did not develop AD, this could have been due to the fact that it was easier for the mice to brush off the applied DNFB with their paws.
- The area of application was too small and resulted in too little DNFB being absorbed through the dorsal part of the mouse ear.

In light of the above the main study was slightly modified.

4.2. Main Study Results

Of the 20 mice per study arm, results were only obtained from 18 mice in the placebo group, as a result of 2 mice which had a severe reaction to the DNFB applied to dorsal skin and therefore died from dehydration. In the *Aloe ferox* group results were also obtained from only 18 mice. Two of the Balb/c mice had to be euthanized due to severe injuries from fighting.

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4.2.1. Macroscopic Results

The following macroscopic photographs were obtained with a Canon Cu250 camera and presented in figure 11.Clinical skin features and grade of dermatitis is presented after a 10 day treatment period with the two different medicinal investigational products, *(Aloe ferox* and *Aloe vera*) and the negative control (placebo gel).

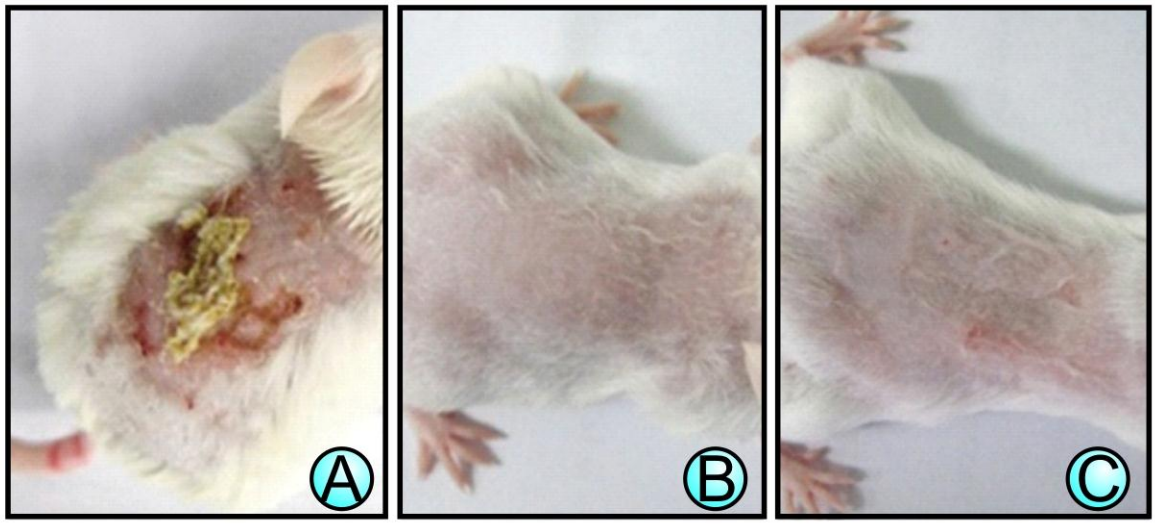

Figure 11: Macroscopic photograph of 2,4-dinitrochlorobenzene (DNCB) induced atopic dermatitis mouse skin.

(A) Placebo gel application did not result in any improvement after atopic dermatitis induction, as this was the control group no improvement was expected.

(B) With the *Aloe ferox* application for 10 consecutive days, after atopic dermatitis induction no AD could be observed after treatment and was completely cleared up by the end of the study.

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(C) *Aloe vera* application also applied for 10 consecutive days, after atopic dermatitis induction by DNCB, AD was virtually cleared up compared to the placebo group.

4.2.2. Histological Findings

Figure 12 shows the histological features and general tissue morphology of skin lesions of Balb/c mice when stained with Hematoxylin and Eosin (H&E). H&E stain, ×100. Marked hyperplasia of the epidermis, a dermal infiltrate, and mild spongiosis was seen. The cellular infiltrate consists of neutrophils, eosinophils, and lymphocytes.

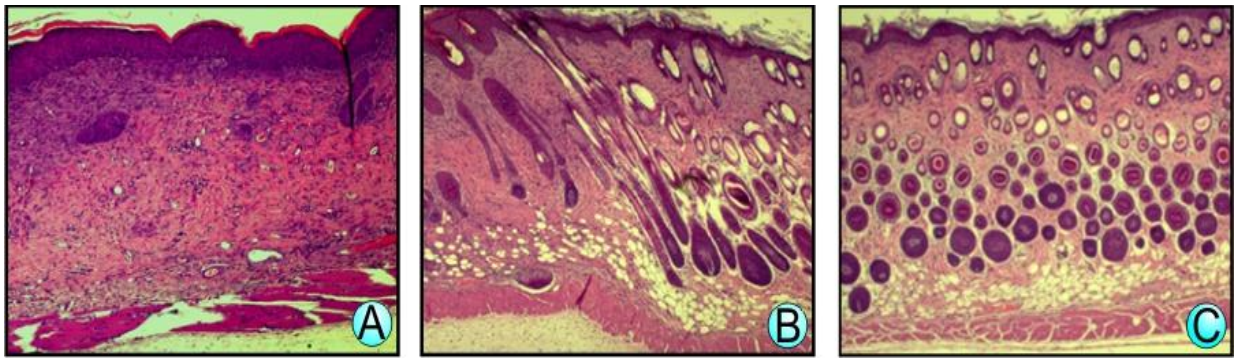

Figure 12: Hematoxylin & Eosin-stained dorsal skin sections.

(A) Placebo gel application as control mice, showing an absence of skin structures for example, hair follicles and sweat glands as well as ulceration.

(B) *Aloe ferox* arm resulted in the closest to normal results in epidermal skin with an an increase of sub-epithelial stromal cells.

(C) *Aloe vera* arm also resulted in an increase of sub epithelial stromal cells.

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4.2.3. Ig E Results

Serum IgE levels were measured at the end of the study after the 10 day treatment period. The results presented in Table 12 are expressed as means ± Standard deviation.

Table 12: Summary statistics by Treatment Group

(IgE concentration measured in ng/ml)

	N	Mean [IgE]	Sd [IgE]
Placebo	18	4356.183	361.186
Aloe ferox	18	4035.031	292.340
Aloe vera	20	4105.793	294.297

Table 12 shows the summary statistics of the three different treatment groups.

The results show that the IgE concentrations in both the treatment arms were significantly lower than in the placebo-arm with the *A.ferox* arm being the lowest.

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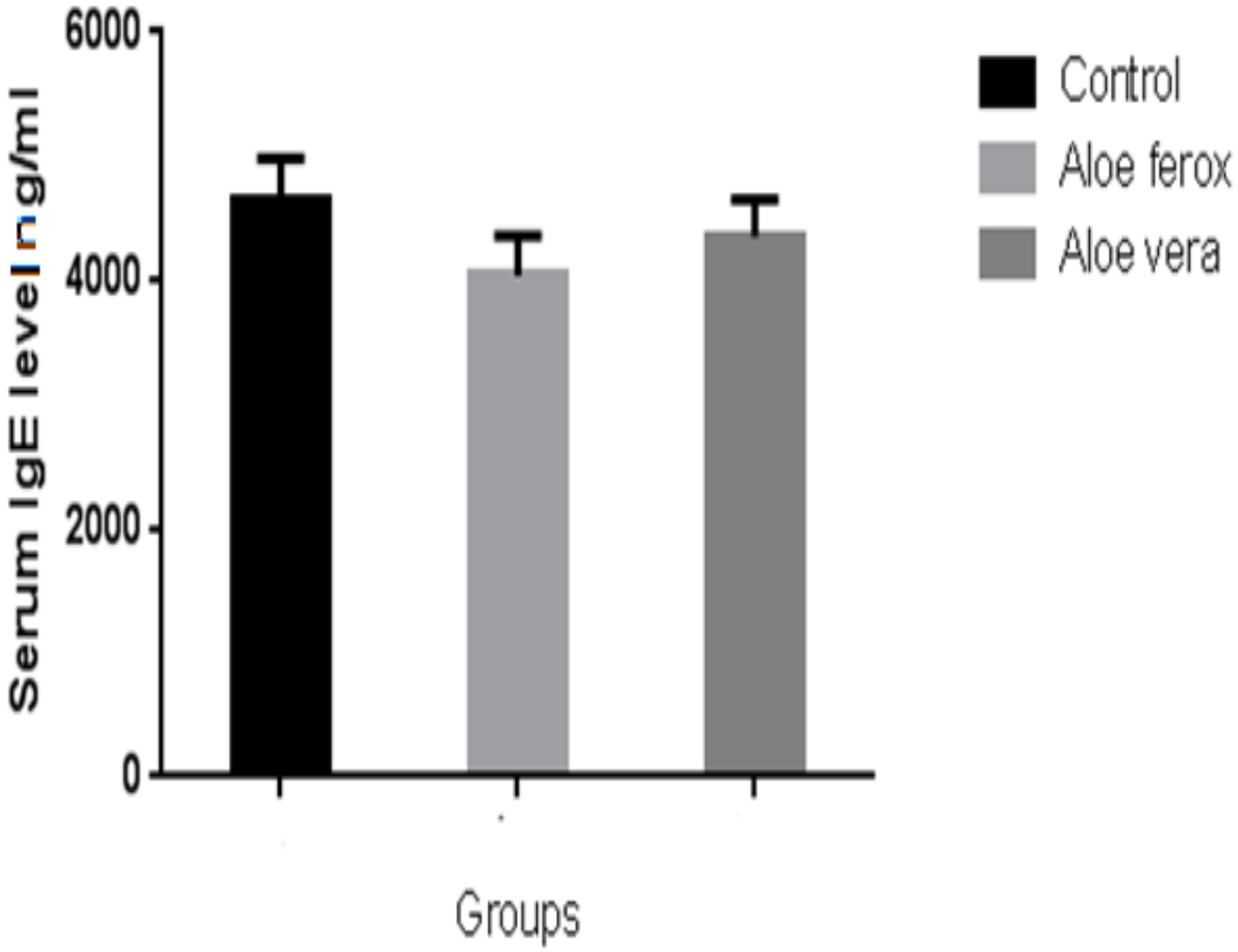

Figure 13:*Aloe ferox* and *Aloe vera*-mediated suppression of IgE hyperproduction.

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Table 13: 0ne Way ANOVA, Analysis of Variance					
Source	**SS**	**Degrees of Freedom (df)**	**Mean square (MS)**	**F**	**Prob > F**
Between groups	0.112529736	2	0.056264868	22.92	<0.0001
Within groups	0.130131878	53	0.056264868		
Total	0.242661614	55			

In Table 13, one-way ANOVA demonstrated a significant difference between groups ($P < 0.0001$).

Table 14: Comparison of IgE by group (Bonferroni)		
Row Mean Column Mean	**Placebo**	***Aloe ferox***
Aloe ferox	-0.11175 <0.001	
Aloe vera	-0.052608 0.006	0.059142 0.002

Table 14 above, furthermore demonstrates that at the Bonferroni adjusted p=0.017, all groups differed significantly in terms of IgE levels.

- The Placebo arm yielded higher IgE levels than the *Aloe ferox* arm. ($p < 0.001$)
- The Placebo arm yielded higher IgE levels that the *Aloe vera* arm. ($p = 0.006$)
- The *Aloe ferox* arm yielded a lower IgE level than the *Aloe vera* arm. ($p = 0.002$)

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Table 15: Two-sample T test with equal variances (T test IgE if groep ~=1, by(groep))						
Group	**Obs**	**Mean**	**Standard Error**	**Standard Deviation**	**[95% Confidence Interval]**	
Aloe ferox	18	4035.031	68.905	292.340	3889.640	4180.420
Aloe vera	20	4356.183	65.806	294.297	4218.647	4493.719
Combined	38	4195.607	49.770	306.803		
Difference		-.328.152			*(-.0898755	*-.0284079)

Superiority of *Aloe ferox* vs. *Aloe vera* was demonstrated following application of the appropriate pair-wise t-test (p=0.002) as well as from the fact that the upper limit of the 95% confidence interval *(-.090; -.028) for the difference between *Aloe ferox* and *Aloe vera* was less than zero.

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Chapter 5

5.1. Discussion

Atopic dermatitis is a chronic disease that is difficult to define because of its variable morphology and distribution and its intermittent nature. Within the last several decades, there has been much evidence to support the concept that AD has an immunologic basis; this is also supported by the observation that patients with primary T-cell immunodeficiency disorders frequently have elevated serum IgE levels and eczematous skin lesions that are indistinguishable from AD. [179]

Although the majority of AD patient are still being treated with topical corticosteroids as the first line treatment for AD, there is still an pressing requirement for other anti-inflammatory drugs that can be used as an alternative to, or as an intermittent or alternating therapy during, long-term treatment with topical corticosteroids because of the concern regarding long term side-effects of corticosteroid therapy.

The present study was undertaken to assess the efficacy of *Aloe ferox* and *Aloe vera* in alleviating atopic dermatitis-like immune alterations in Balb/c mice. Using animal models to represent AD in patients has been of great use to scientist. This gave them great insight into the pathology, underlying causes and treatment of AD. Looking at Figure 10, the macroscopic photograph of 2,4-dinitrochlorobenzene (DNCB) induced AD mouse skin after a 10 day treatment period, it is evident that both active arms namely *Aloe ferox* and *Aloe vera* responded well to the treatment however the best result was obtained from the *Aloe ferox* arm, followed by the

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Aloe vera arm and the placebo arm. The ulceration caused by AD in the *Aloe ferox* arm was completely healed after the 10 day treatment and virtually healed in the *Aloe vera* treatment group in comparison to the placebo group where the ulceration was still prominent after the 10 day treatment period.

With the H&E histological analysis as seen in Figure 12, there are some similarities as well as differences that can be observed between the different treatment groups. A number of observations were made from the histological analysis. These observed results, associated with AD were caused by the chronic state of inflammation due to the irritating chemical (DNFB) applied to the dorsal mice skin.

In the control/placebo group, ulceration as well as hyperkeratosis of the skin was observed. Hyperkeratosis is a thickening of the stratum corneum, the outer layer of the skin, often associated with a qualitative abnormality of the keratin, [180] and also usually accompanied by an increase in the granular layer. This was also observed in the *Aloe ferox* treatment group as well as the *Aloe vera* treatment group but to a lesser extent. This process is often part of the skin's normal protection against rubbing, pressure and other forms of local irritation, for example in this study it was caused by chronic inflammation, infection and irritating chemicals.

Other similarities are the presence of Pseudoepitheliomatous (pseudocarcinomatous) hyperplasia in all 3 treatment groups. This is a histopathological reaction pattern rather than a disease. It is characterized by irregular hyperplasia of the epidermis which also involves follicular infundibula and acrosyringia. [181, 182]

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Looking at some of the differences; only in the control group an absence of skin structures for example hair follicles and sweat glands was observed as well of some early signs of the formation of cancerous cells. This is a clear indication of the effectiveness of *Aloe* in treating AD. In the *Aloe ferox* and *Aloe vera* groups an increase of sub-epithelial stromal cells was found, with the *Aloe ferox* group showing the closest to normal results; this is suggestive of superiority of *Aloe ferox* over *A.vera*.

The use of *Aloe vera* as an herbal medicine has long been documented and has been publicized to have anti-inflammatory, [140, 183, 144, 145] anti-oxidant, anti-cancer, wound healing, anti-aging and anti-diabetic properties. [140] In preceding studies it was acknowledged that *Cape Aloe* extract possesses the following pharmacological effects: anti-inflammatory, anti-bacterial, anti-fungal and protection against liver injury. [194]

To date there has been no scientific investigations into *Aloe vera* and *Aloe ferox* for its effectiveness in the treatment of AD. This study demonstrates *Aloe's* immunoregulatory potential for alleviating atopic dermatitis through influencing of Ig E expression and Th_2 cell activation. IgE molecules contribute to the induction or stimulation of a mononuclear cell infiltrate by several mechanisms. In this regard, clinically significant allergen-induced reactions namely DNFB-induced-AD is associated with an IgE-dependent biphasic response. [184] In explanation of the above mentioned reaction; after exposure to an allergen, mast cells bearing IgE directed to the relevant allergen, releases various mediators, cytokines, and leukocyte chemotactic factors into local tissue within 15 to 60 minutes of allergen challenge. This immediate reaction is likely to contribute to the acute pruritus and erythema observed after exposure of patients with AD to relevant food and

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inhalant or contact allergens. Repeated application of an allergen like DNFB results in a shift in the time course from a typical delayed-type hypersensitivity reaction to an immediate-type response (ITR) followed by a late-type reaction (LTR). [185] The development of these early type responses (ITR, LTR) in a site-restricted fashion is antigen specific and is associated with elevated serum levels of antigen-specific IgE. The above mention reaction results in a shift in the local cytokine pattern from a T-helper (Th) 1-type to a Th2- type profile. This causes an imbalance between Th-1 and Th-2 cells a condition closely associated with AD. Thus skewedness toward type-2 responses is a background mechanism for the occurrence of atopic dermatitis. [186, 187]

Table12 shows the summary statistics by treatment group the placebo group had the highest IgE concentration, 4356.183ng/ml and *Aloe ferox* the lowest IgE concentration, 4035.031 ± 292.340 ng/ml. Study groups (Placebo, *Aloe ferox, Aloe vera*) were compared with respect to IgE concentration using a one-way analysis of variance (ANOVA). Following significance in the ANOVA, specific differences between study groups, was determined using two-group t-tests employing the pooled standard deviation determined from the within group mean squares (Table13 : Analysis of Variance). These pair-wise comparisons were done at the Bonferroni adjusted 0.017(=0.05/3) level of significance (Table 14). Superiority of *Aloe ferox* versus *Aloe vera* followed from the appropriate pair-wise t-test as well as from the fact that the upper limit of the 95% confidence interval for the difference between *Aloe ferox* and *Aloe vera* was less than zero (Table 15: . Two-sample T test with equal variances)

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Overall, it was found that not only did *Aloe ferox* demonstrate efficacy in treating AD, but also it was showed that it is superior to *Aloe vera* and possess an ability to influence Th2 cell activation involved with the onset or progression of AD, thus resulted in the suppression of IgE over-production. In addition, more rapidly healing of the AD skin lesions and ulceration, Fig 10, was observed in the *Aloe ferox* group compared to the *Aloe vera* group.

Considering this study's experimental results, it could be concluded that the application of *Aloe vera* and *Aloe ferox*, the latter being the more effective of the two, on the dorsal skin of AD induced Balb/c mice could correct the immune homeostasis skewed in favour of Th2 through a series of immunoregulatory processes to alleviate the occurrence or progression of AD.

Further investigations are necessary and will be directed towards identifying target immune cells for *Aloe*-mediated alleviation of atopic dermatitis-like immunologic and skin alterations and whether *Aloe* exerts its immune regulatory effects independently, sequentially, or concomitantly on each of the immune cells involved in the pathogenesis of AD.

5.2. Short comings

After completion of the study and analysis of results the following short comings was noted:

- More insight research into the group behaviour of male Balb/c mice should have been done, as it seemed 5 mice per cage resulted in territorial behaviour and fighting.

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- An AD model using larger animal models for example guineapigs will be more useful as no baseline IgE concentration before the 10 day treatment period could be obtained, drawing blood from mice to calculate base line IgE concentration would have resulted in demise of the mice.

5.3. Conclusion

These mouse models of AD that closely mimic AD in humans have shed important light into the pathogenesis of allergic skin inflammation caused by induced atopic dermatitis. These models have highlighted the role of allergic sensitization to epicutaneously introduced allergens and taken together, the present data indicated that topical application of *Aloe ferox* and *Aloe vera* can suppress chronic AD by a comparatively selective reduction of Th2 responses. Furthermore, is was shown that not only is *Aloe ferox* effective in treating AD but is also superior to *Aloe vera* for its effectiveness in treating AD and achieved a good cosmetic result without any side-effects such as alopecia, telangiectasia and skin atrophy. Therefore, *Aloe* may be useful as an alternative or intermittent treatment for the management of patients with recurring or chronic AD who require long-term therapy.

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