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Effect of Polyherbal Formulation (PHF) and its comparative study with other treatment in different types of dermatitis (Eczema) in experimental animals

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Abstract

Atopic dermatitis (AD) and allergic contact dermatitis are skin disorders that are a type of eczema. AD is a childhood disorder & allergic contact dermatitis. When the skin touches irritating bacteria, the skin inflammation causes itching and redness. The Poly Herbal Formulation uses this content: chaulmoogra oil 10%, neem oil 8%, and Karanja oil 30%, then made into an ointment. They are using this ointment topically for mice's skin after 30 days of redness, itching, dryness, and inflammation necessary for the basic treatment. This paper provides a different study of eczema AD & contact allergic dermatitis by using a polyherbal formulation.

Keywords: Atopic dermatitis; Allergic contact dermatitis; Polyherbal formulation; Animals

1. Introduction

1.1. Definition

Atopic dermatitis, often known as eczema, is a chronic skin inflammation that is more irritable. The vast majority of cases affect children. Atopy is a genetic disorder in which immunoglobulin E antibodies are created in response to allergens in the environment, such as dust and food. Dermatitis and eczema are frequently used synonymously, even though the name eczema is presently reserved for the acute symptom of the disease. In addition, managing the condition requires the repeated relevance of emollients and topically applied medication as well as sleep disruption. A D also poses major cost-effect trouble.⁽¹⁾

1.2. Epidemiology

Worldwide, dermatitis affected around 230 million people as of 2010 (3.5% of the population). Dermatitis is most commonly seen in infancy, with a female predominance of eczema presentations taking place all through the reproductive period of 15–49 years. In the UK, as regards 20% of children hold the condition, as in the United States, as regards 10% are affected.

While small records on the rates of eczema exist from the 1940s to the present, the speed of eczema has been increased significantly in the latter half of the 20th century, with eczema in school-aged kids being established to rise between the late 1940s and 2000. In the developed world, there has been an increase in the rate of eczema more than time. The prevalence and life span incidence of eczema in England has been seen to increase in current times.

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Population-based studies on the epidemiology of AD in India have been unusual and mainly provide epidemiological information. A study from Bihar reported an attendance frequency of 0.38% of the whole number of outpatient attendees. Relatively recent hospital-based studies have also determined a low incidence in the Northern and Eastern parts of the country, with the reported prevalence amongst dermatology outpatient sector attendees being 0.42% and 0.55%, correspondingly. Still, AD is the most common dermatitis in children registered at a paediatric dermatology clinic, where it constituted 28.46% of all registered patients. In the Indian study: 4.2 months was used for not fully formed AD and 4.1 years for childhood AD, compared to 4.5 months for babyish AD and 4 years for childhood AD. In a different study, the overall mean age of onset was 4.58 years to inform the world map of eczema occurrence after 5 to 10 years (ISAAC Phase Three) and include added data from over 100 new centers. Cross-sectional surveys using the ISAAC survey on eczema symptoms were completed by puberty aged 13 to 14 years old and by parents of children aged 6 to 7 years old. ^(2,3,4,5)

1.3. Types of eczema⁽⁶⁾

1.3.1. Atopic dermatitis

Atopic dermatitis is a type of eczema that is chronic and inflammatory. Though the exact cause of AD is unknown, it happens when the immune system goes into overdrive. AD regularly begins in early childhood, usually in the first six months of life. While you or your child have AD, its capacity may improve at times or it may get inferior.

1.3.2. Contact dermatitis

Contact dermatitis happens when the skin touches irritating substances or allergens. These make the skin inflamed, causing it to burn, itch, and become red. There are two kinds of contact dermatitis: irritant and allergic. Contact dermatitis regularly appears on the hands, or parts of the body that touch the irritant or allergen.

1.3.3. Symptoms

Atopic dermatitis commonly manifests itself in infants with dry and scaly patches appearing on the skin. These patches are regularly extremely itchy. The symptoms of atopic dermatitis are capable of varying depending on the age of the person with the condition ⁽⁶⁾.

Skin rashes to bumpy rashes, including blisters. while each category of dermatitis has different symptoms, present are assured symbols that are general for all of them, together with redness of the skin, swelling, itching and skin lesions with occasionally emission and scarring.⁽⁶⁾

Even though the symptoms of atopic dermatitis fluctuate from person to person, the main regular symptoms are dry, itchy, red skin. Usual affected skin areas embrace the folds of the arms, the back of the face, knees, wrists, and hands.⁽⁷⁾

2. Material and methods

2.1. Material

2.1.1. Animals

Mice (25-30 gm) of either sex were procured from the Rajarshi Shahu College of Pharmacy, Buldana, Dist-Buldana, and used in the experimental design. It was performed according to the committee for the purpose of control and supervision of experiments on animals (CPCSEA) guidelines for the use and care of animals. All the mice were divided into 7 groups, each containing 6 mice. The mice were individually identified by different markings done on their body parts. All the mice were fed on the standard mouse pellet diet and were allowed to drink water ad libitum by means of bottles. ^(7,8)

2.1.2. Drugs

Collection of standard drugs

Tacrolimus ointment 0.3% (perrigo pharma), cortisone cream (CVS pharmacy)

Collection and Authentication of the herbal drug

- Karanj oil (30%w/w) Vyas Pharmaceutical Indore

- Chaulmoogra oil (10% w/w) Vyas Pharmaceutical Indore
- Neem oil (8%w/w) and Vyas Pharmaceutical Indore were purchased from Shree Medical Buldhana and Authentication from the Botanical Department.

Chemicals and Reagents

- White soft paraffin, cetosteryl alcohol, liquid paraffin
- Dinitrocholobenzene (1%) 10 gm, oxazolone (2%), acetone (2%), 10% formaldehyde.
- Dimethylgloxime.

2.1.3. Appartus & Instrument

Separating funnel, thermometer, water bath, beaker, and spatula.

2.1.4. Phytochemical investigation

The phytochemistry of herbal drugs is embraced through consideration of these chemical entities that are termed as constituents. As herbal drugs contain so many chemical compounds, it is essential to single out those responsible for the therapeutic effect to be called active constituents, so the phytochemical investigation used for the attendance of different chemical constituents such as fixed oil, behenic acid, linoleic acid, alkaloids, and flavonoids was carried out.

2.2. Methods

2.2.1. Formula of herbal ointment

Table 1 Formula of herbal ointment

Sr. No.	Ingredient	Concentration	Quantity
1.	Chaulmoogra oil	10% w/w	10 gm
2.	Neem oil	8%w/w	8 gm
3.	Karanj oil	30%w/w	30 gm
4.	White soft paraffin (Base)	86%	26 gm
5.	Cetosteryl alcohol (Base)	10%	14.47 gm
6.	Liquid paraffin (Base)	10%	10.73 gm

2.2.2. Preparation of ointment

- Melt the cetosteryl alcohol, liquid paraffin and white soft petrolatum on a hot plate.
- Bring this mixture to 70°C.
- Dissolve neem oil, chaulmoogra oil, and karanj oil in water and heat the solution to 50°C.
- Add the oleaginous phase slowly to the aqueous phase, stirring constantly.
- Remove it from the heat and stir the mixture until it congeals.

2.2.3. Evaluation of ointments

Consistency

Herbal ointment or cream Rough the skin with your hands for 5 minutes, then check the smoothness and solidity.^(9,10)

Melting point

The measurement can also be made continuously with an operating process. For instance, oil refineries evaluate the freeze point of diesel fuel online, meaning that the taster is taken from the process and measured automatically. This allows for more frequent measurements as the sample does not have to be manually collected and taken to a remote laboratory (not less than 11 °C).^(2,4)

2.3. Atopic dermatitis induction

The backs of mice were shaved with an electric clipper and depilatory cream, and washed with sterile applied with 0.1 mL of 1% DNCB in vehicle or vehicle alone was attached to the lacking hair backs of the animals for 2 days on day 0 and 3. On days 7 and 10, 0.1 mL of 0.2% DNCB in the vehicle or vehicle was used to challenge for a day as a previous sensitization.

2.3.1. Experimental protocol

The following treatment was started and continued for 21 days.

2.3.2. Treatment Table

Table 2 Treatment of atopic dermatitis

Sr No.	Groups	Treatment
1	Normal control group	No treatment
2	Toxic control group	2,4-dinitrochlorobenzene(DNCB) (1%)
3	Standard group	DNCB (1%) + Tacrolimus ointment
4	Test group	DNCB(1%) + PHF

2.3.3. Skin lesion evaluation

Skin lesions were observed and evaluated as a total score of skin severity was defined as a sum of the individual scores (0, no symptoms; 1, mild; 2, moderate; 3, severe) for all of the subsequent four signs and symptoms: erythema/hemorrhage; edema; excoriation/erosion; and dryness. The total dermatitis score was defined as the individual scores indicated above (maximum score: 12).^(11,12)

2.3.4. Serum IgE levels are measured(13,14).

Blood samples were collected on the 0th and 14th days via retro-orbital puncture and sent to pathology for analysis.

2.3.5. Histopathological examination

For histopathological examinations, skin samples from the back of each mouse were taken and sent to pathology for evaluation.

2.4. Allergic contact dermatitis induction

On days 1 and 2, mice weighing 25–30 g were sensitised by applying 50 uL of a 2% w/v oxazolone in acetone solution to the lacking hair abdomen.

2.4.1. Experimental protocol

After 3 days, following treatment was started and continued for 21 days.

2.4.2. Treatment Table

Table 3 Treatment of allergic contact dermatitis

Sr. No	Groups	Treatment
1	Toxic control group	Oxazolone(2%w/v) in acetone (applied topically)
2	Standard group	Oxazolone(2%w/v) in acetone+cortisone cram
3	Test group	Oxazolone(2%w/v) in acetone+herbal ointment

2.4.3. Patch test

The skin samples were taken from the animal and sent to a healthcare lab centre for evaluation.

2.4.4. Histopathological analysis

For histopathological examinations, skin samples from the back of each mouse were taken and sent to pathology for evaluation.

3. Results and discussion

Herbal remedy, at times referred to as herbalism or botanical medicine, is the use of herbs for their therapeutic and medicinal value. An herb is a plant or plant ingredient valued for its medicinal, fragrant, or aromatic qualities. Herb plants generate and include a variety of chemical substances that operate on the body.

Many herbal drugs play a curative role in many diseases, most of them speeding up the natural healing process. A review of literature indicates that a number of plant extracts and compounds isolated from various plant sources and minerals have shown activity against the eczema. In the present investigation, we have studied anti-inflammatory activity and antibacterial activity.

3.1. Preliminary phytochemical studies

The phytochemical studies were investigated that the neem, chaulmoogra, and karanj oils show the presence of alkaloids, fixed oil, saponins, and flavonoids, etc.

3.2. Evaluation of ointment

3.2.1. Consistency

Smoothness and no solid particles.

3.2.2. Melting point

Not less than 110c.

3.3. Atopic dermatitis

3.3.1. Skin lesion evaluation test

Table 4 Effect on skin lesion after 15th days

Sr.No.	Groups	Skin lesion evaluation test
		On 15th days
1.	Normal control	00
2.	Toxic control	8.50±0.645
3.	Standard	5.00 ±0.406
4.	Test	6.75±0.250

3.3.2. Effect on IgE level serum

Table 5 Effect on IgE level serum on 0 day and on 14th days

Groups	IgE level serum (ng/ml)	
	On 0 days	On 14th days
Normal control	51.3±1.49(ng/ml)ns	51.5±1.04(ng/ml)***
Toxic control	53.3±1.49(ng/ml)ns	275±1.78(ng/ml)***
Standard	53.5±0.83(ng/ml)ns	115±2.17(ng/ml)***
Test	54.8±0.62(ng/ml)ns	184±1.49(ng/ml)***

Statistical method: One way ANOVA followed by Tukeys multiple comparison test, n=5 values are expressed as mean±S.E.M.*P<0.05

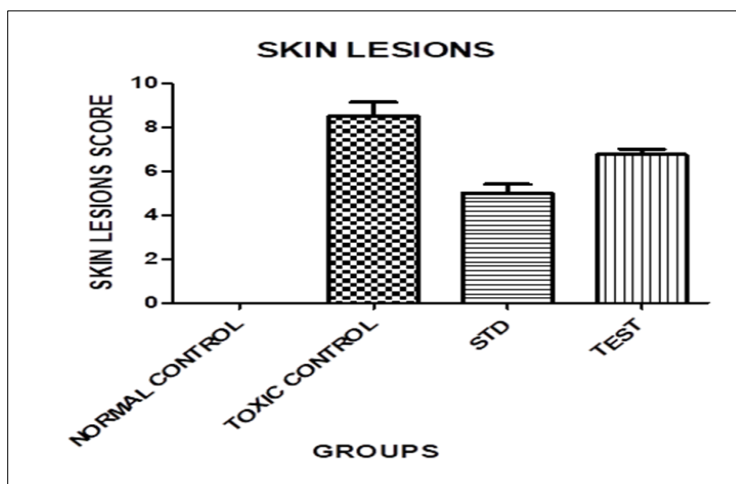


Figure 1 Effect on skin lesion after herbal ointment 14th days

The animal normal control group no treatment, toxic control group induced DNCB (1%) after 14th day was 8.5 ± 0.645 , standard group treatment tacrolimus ointment 0.03% was 5.0 ± 0.408 and test group treatment herbal ointment 6.75 ± 0.250 .

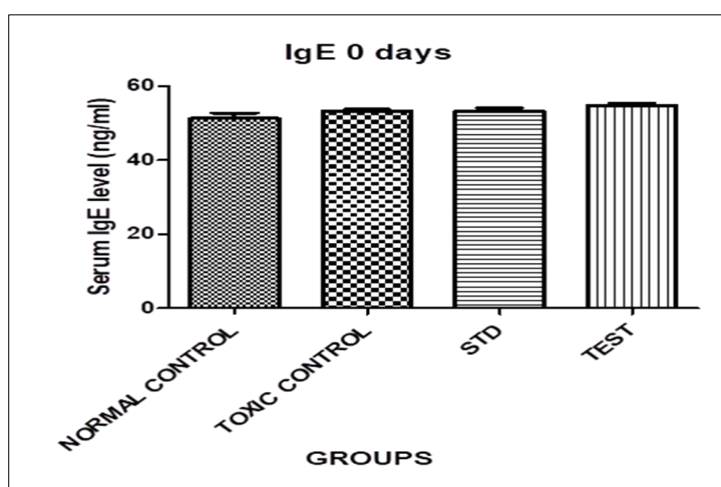


Figure 2 Effect on IgE level serum on 0 day

The animal normal control group was 51.3 ± 1.49 , toxic control group induced DNCB (1%) after 14th day was 53.3 ± 0.47 , standard group treatment tacrolimus ointment 0.03% was 53.5 ± 0.83 and test group treatment herbal ointment was 54.8 ± 0.62 .

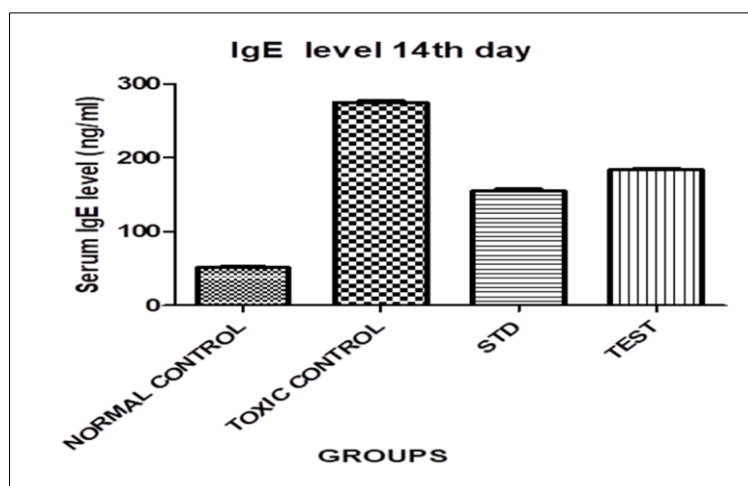


Figure 3 Effect on IgE level serum on 14th days

The animal normal control group was 51.5 ± 1.04 , toxic control group induced DNCB (1%) after 14th day was 275 ± 1.78 , standard group treatment tacrolimus ointment 0.03% was 116 ± 2.17 and test group treatment herbal ointment was 184 ± 1.49 .

3.4. Allergic Contact Dermatitis

3.4.1. Effect of patch test

Table 6 Effect on Patch Test on 20th days

Groups	Patch test reaction	Clinical criteria	Patch test result in mice
Toxic control	Extreme positive (+++)	Erythema, infiltration, possibly papules	+++
Standard	Doubtful (+/-)	Erythema, infiltration, possibly papules	+/-
Test	Weak positive (+)	Faint erythema only	+

- The animal toxic control group shows characteristics like extreme positive +++ (erythema, infiltration, possibly papules).
- The standard control group treated cortisone cream as doubtful +/- (erythema, infiltration, possibly papules).
- The test group treated herbal ointment like a weak positive + (faint erythema only)

3.4.2. Histopathological Study

Allergic contact dermatitis begins as acute spongiotic dermatitis, and it may evolve into subacute or chronic spongiotic dermatitis before resolving. Typically, the spongiosis is extensive. Spongiotic intraepidermal vesicles may form. Lymphocyte exocytosis is generally present. Papillary dermal edoema may also be present. The dermis classically displays a superficial, perivascular, lymphocytic inflammatory infiltrate with varying numbers of eosinophils. The presence of numerous eosinophils in the inflammatory infiltrate helps to differentiate allergic contact dermatitis from other types of spongiotic dermatitis.

Histopathological examination of normal control group animals showed no change in architecture and no abnormalities.

In the control group, the animals had hematoxylineosin-damaged epidermal hyperplasia and immune cell infiltration in the dermis.

However histopathological examination of animals treated with standard tolbutamide showed very much decrease in toluidine blue to count the number of mast cells.

In the test group where the experimental animals were treated with Herbal ointment structure was observed.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare that they have no conflict of interest.

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