



### *Comparative study of efficacy of aragvadha (cassia fistula linn.) Patra lepa and clonate ointment in dinitrofluorobenzene induced allergic eczema in balb/c mice*

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#### **Abstract:**

Skin is the largest organ of the integumentary system. Skin play an important immunity role in protecting the body against pathogen and excessive water loss. Under the heading of 'Kushtha' Ayurveda has described all skin diseases. In Ayurveda, there are Kushtha, Kshudra roga and Visarpa are three major groups of diseases in which skin get affected. Vicharchika is one among eleven kshudra kushtha which has a chronic course. It is considered as difficult to cure and even if is cured relapses are found to be common. Vicharchika can be simulated with the diseases Eczema/ Dermatitis. Eczema or Dermatitis is a common problem in all age group all over the world. Visha, especially toxins created in our body from unusual dietary habits are considered to be a major causative factor for Vicharchika.

Presently, the potent topical or systemic corticosteroids and anti-histaminic are the main stay in this condition however; they have serious adverse effects and have limitations for a long term therapy. Hence, there is a need for drugs having good efficacy with low toxic profile in this debilitating disorder. Aragvadha patra (Cassia fistula leaves) Lepa, is one such formulation which is used in vicharchika.

To avail a new easier formulation to mankind for vishajanya vicharchika, Aragvadha patra lepa topic has been selected for the study.

**Keywords:** Aragvadha Patra and Skin diseases, Allergic dermatitis Vicharchika, Aragvadha lepa.

#### **1. INTRODUCTION:**

Ayurveda is a holistic system of medicine. Principles of Ayurveda considers

complete body as a whole. This is different from systemic approach. Hence, every disease is described in the text according to its *nidanpanchak*.

Balanced State of *tridosha* determines health of an individual. Imbalance of *tridosha* leads to disease. In *kushtha* all the *tridoshas* are considered as *dushya*. *Charak samhita* quotes,

सर्वं त्रिदोषजं कुष्ठं दोषाणां तु बलाबलम्  
यथास्वैर्लक्षणैर्बुद्ध्वा कुष्ठानां क्रियते क्रिया॥३१॥  
दोषस्य यस्य पश्येत् कुष्ठेषु विशेषलिङ्गमद्रिक्तम्  
तस्यैव शमं कुर्यात्ततः परं चानुबन्धस्य॥३२॥

*Tridosha* are involved in pathogenesis of all types of *kushtha*. Depending on *dosha* predominant symptoms are manifested and treatment should be decided on the basis of symptoms shown in specific *kushtha*. The *dosha* presenting important and specific symptoms should be alleviated firstly followed by the treatment of associated *dosha*.<sup>[1]</sup>

*Vicharchika* is described under *kshudra kushtha* in which vitiation of all the *three dosha* takes place but predominantly *kapha dosha* is vitiated which causes *vicharchika* which is quoted as ,

कफप्राया विचर्चिका॥३०॥

In *charak samhita*. *Vagbhata* and *Sharangdhar* also quotes predominacy of *kapha* while *Acharya Sushruta* describes *vicharchika* as *pitta* predominant skin disease.<sup>[2]</sup>

- *Vagbhata* – *kandu*, *pidaka*, *shyava*, *lasikadhya*
- *Charaka* – *kandu*, *pidaka*, *shyava*, *bahusrava*

- *Sushruta- raji*, *atikandu*, *atiruja*, *ruksha*

तद्यथा-

श्वेत्यशैत्यकण्डूस्थैर्यगौरवस्नेहसुप्तिक्लेदोपदेहबन्ध  
माधुर्यचिरकारित्वानि श्लेष्मणः कर्माणि;  
तैरन्वितं श्लेष्मविकारमेवाध्यवस्येत्॥१८॥

*Kandu* is main symptom of *vicharchika* and it is due to *kapha dosha*<sup>[3]</sup>, eruptions occur due to *rakta dushti* or *pitta dushti*. *Shyavata* occurs due *vata dushti* and *pitta dushti* and *srava* and *lasika* is the features of *pitta* and *kapha*. Basically, *sapta dushyas* ie *vata*, *pitta*, *kapha*, *rakta*, *mamsa*, *tvaka* and *lasika* are involved in the manifestation of all *kushtha*.

*Aragvadha* is a plant which is known as killer of disease plant. In case of *kushtha dosh dushya sammurchhana* take place inside the body and it is represented over skin so *Ayurveda* describes two therapeutic modalities *Antahparimarjana* and *Bahirparimarjana* for management of *kushtha*. *Aragvadha* is a kind of drug which is described in the management of *kushtha* for *Antahparimarjana* and *Bahirparimarjana*. *Aragvadha* is a drug which helps to balance *tridosha* by means of its *sansran karma* also shows localized action in the *kushtha* by cleansing action.

In the 3<sup>rd</sup> chapter of *Charaka Samhita sutrasthan*, *Aragvadha* is the first word to start the chapter also, name of the chapter is given by *aragvadha* i.e. *Aragvadhiya adhyaya*. *Aragvadha* is placed at first position to highlight its importance in the management of *kushtha*. In this chapter 32 different *lepa* are described so, *Aragvadha*

for external use in the management of *kushtha* is given lot of importance.

*Aragvadha* for current research project was selected from following reference

शैरीषी त्वक् पुष्पं कापस्या राजवृक्षपत्राणि।  
पिष्टा च काकमाची चतुर्विधः कुष्ठनुल्लेपः॥  
च.चि.७/९६

It contains anthraquinone derivatives, very little tannin, sennosides A&B, rhein and its glucoside. Among which anthraquinone is researched and proven for its following medicinal Properties of Anthraquinones: [4]

1. Antiviral
2. Antifungal
3. Antibacterial
4. Laxative
5. Insecticidal
6. Antioxidant

These six properties which makes it suitable to be used in the treatment of various ailments.

For pre-clinical study of Eczema Balb/c mouse is generally used (Ref.-In vivo hair growth stimulating effect of medicinal plant extract on balb/c nude mice. Dep. of Agriculture & Biotech. South Korea 23 Jan. 2010)

According to the description in Michael Feastings list of inbred strains of mice, Balb/c mice are particularly well known for the production of monoclonal antibodies. The IgE response following topical application has been used to predict which chemicals may have the potential to cause sensitization. This mouse can be used to support research in many areas including Immunology and Inflammation research, Neurobiology research, Cancer research.(Ref. JAX-R Mi For pre-

clinical study of Eczema Balb/c mouse is generally used). Hence, efforts were made to assess efficacy of *aragvadha patra* through preclinical study.

## **2. AIM and OBJECTIVES**

### **AIM:**

To study the efficacy of *Aragvadha* (*cassia fistula linn.*) *patra lepa* compared with clonate ointment in dinitrofluorobenzene induced allergic eczema in balb/c mice.

### **OBJECTIVES:**

#### **PRIMARY OBJECTIVE:**

To evaluate the efficacy of *aragvadha* (*cassia fistula linn.*) *Patra lepa* in dinitrofluorobenzene induced allergic eczema in balb/c mice

#### **SECONDARY OBJECTIVE:**

1. To observe any adverse side effect of *aragvadha patra lepa*
2. To compare the effect of *aragvadha patra lepa* and clonate ointment.

## **3. MATERIAL AND METHODS**

### **MATERIAL:-**

- a. Contents of *Aragvadha patra lepa*
- b. Procurement of mice:-

It was from renowned laboratory

### **METHODS:-**

Authentication and standardization of drugs were done.

#### **1. METHOD OF SELECTION OF STUDY SUBJECT**

##### **A. Inclusion Criteria**

- Mice of weight: 22-25 g
- Age of mice: 6- 8 weeks
- Healthy mice
- In female non pregnant mice
- Balb/c mice
- Both male and female mice

##### **B. Exclusion criteria**

- Mice weight less than 20g and more than 25g
- Age of mice less than 6 weeks and more than 8 weeks
- Unhealthy mice
- In female pregnant mice
- Other than balb/c mice

## 2. OPERATIONAL DEFINITION

Allergic Eczema – *Vicharchika* was simulated with allergic eczema/dermatitis was considered.

## 3. APPROPRIATE METHODS OF MEASUREMENT

Criteria of assessment was according to **BUREAU OF INDIAN STANDARDS** Amendment No.3 March 2013 and **International Contact Dermatitis Research Group Scale (ICDRG SCALE)**.

1. **Itching (*Kandu*)** - Itching was observed and graded based on daily observation of the animal for duration of 2 hrs.

Sr. no.	Effect	Duration 2hrs/day	Grade
a.	No <i>kandu</i>	No itching	0
b.	Mild	2-5times	I
c.	Moderate	6-10times	II
d.	Severe	Continuous	III

## 2. Erythema (*Raga*)-

Sr. no.	Cause	Effect	Grade
a.	Normal skin colour	No raga	0

b.	Pink macules	Mild	I
c.	<i>Araktavarna</i>	Moderate	II
d.	<i>Raktavarnya</i>	Severe	III

## 3. Oedema (*Shotha*)-

Sr.no.	Oedema	Grade
1)	no oedema	0
2)	very slight oedema	1
3)	slight oedema (edges of area well defined by definite raising)	2
4)	moderate oedema (raised upto 1mm)	3
5)	severe oedema /wrinkle /scale	4

## Scaling (*Kakalodbhav*)

Sr.no	Scaling	Grade
1)	Dryness shiny	+1
2)	Fine scale	+2
3)	Moderate	+3
4)	Severe with large flakes	+4

## DATA COLLECTION TOOLS

Duration was approximately one month.

## Group of lesion in animal experiment:-

<b>GROUP 1</b>	<b>Normal control</b>	No treatment
<b>GROUP 2</b>	<b>Disease control</b>	Local application of DNFB
<b>GROUP 3</b>	<b>Standard control</b>	Local application of standard drug
<b>GROUP 4</b>	<b>Test control</b>	Local application of <i>ARAGVADHA patra lepa</i>

#### 4. METHODOLOGY:

**STUDY DESIGN:** It is a pre-clinical animal experiment.

##### Plan of Work:

##### PROCEDURE OF EXPERIMENT

1. All animals, except Normal control were sensitized and challenged with 30 µl DNFB (0.1%, v/v) in acetone: olive oil on the dorsum of each ear for three consecutive days.
2. Three days later, dorsa of mice were shaved with the help of trimmer.
3. On 6<sup>th</sup> day no sensitization was done.
4. Then on 7<sup>th</sup> and 8<sup>th</sup> day mice were challenged by painting 50 µl DNFB (0.2%, v/v) on the shaved dorsa.
5. The skin reactions were observed, during each induction of DNFB and mice were randomly divided into 4 groups as mentioned above.
6. Application from 8<sup>th</sup> day till 15<sup>th</sup> day DNFB was applied on every alternate day and *aragvadha patra lepa* was applied every day to test group and clonate ointment was applied every day to the standard group.
7. Disease control animals were sensitized and challenged with DNFB.

8. Normal control animals were not challenged with DNFB.
9. Mice were observed daily for effect for effect of *aragvadha patra lepa* and clonate ointment over DNFB induced allergic eczema.
10. Rating of skin reaction was evaluated as per the Bureau of Indian standard Amendment No. 3 March 2013, and International contact dermatitis Research Group Scale (ICDRG Scale).
11. At the end of experiment, in order to observe the overall degree of damage, mice were sacrificed and the skins of the dorsa were observed using photographs.
12. After observation of the skins of the dorsa, tissues were resected and paraffin- embedded. Sections were stained with hematoxylin and eosin (H&E) for histopathological observation.

**During Observation:** - Itching (I), Erythema (E), Oedema (O), Scaling(S) of the wound was predominantly in all mice.

#### 5. OBSERVATION AND RESULTS

##### Observation analysis and interpretation

**TABLE 01: OBSERVATIONS OF ANIMAL EXPERIMENT**

Group	Sex	Marking	Day 8				Day 11				Day 14			
			I	E	O	S	I	E	O	S	I	E	O	S
NC	M	H	-	-	-	-	-	-	-	-	-	-	-	-
	M	T	-	-	-	-	-	-	-	-	-	-	-	-
	M	W	-	-	-	-	-	-	-	-	-	-	-	-
	F	HT	-	-	-	-	-	-	-	-	-	-	-	-

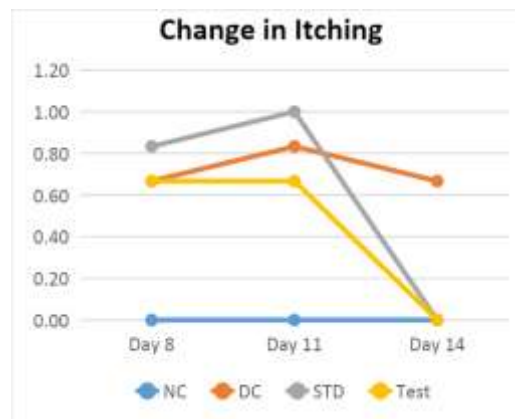
	F	FLS	-	-	-	-	-	-	-	-	-	-	-	-
	F	HLS	-	-	-	-	-	-	-	-	-	-	-	-
DC	M	RF	-	+	+	++	+++	+++	++	+++	-	+	+	++
	M	RH	+	++	-	++	-	+	-	-	-	+	+	+
	M	RLS	-	+	+	++	+	-	+	++	+	++	++	++
	F	LF	++	-	+	+	-	-	+	+	+	+	+	++
	F	LH	-	+	-	+	+	+	+	+	+	++	++	+++
	F	LLS	+	-	++	-	-	-	-	+	+	++	++	+++
STD	M	H-RF	+	+	+	++	+	-	-	+	-	+	++	+
	M	H-RH	-	++	-	+	+	+	+	+	-	-	+	+
	M	H-RLS	++	+	+	++	+	+	+	+	-	-	+	-
	F	H-LF	+	+	-	+++	+	+	++	++	-	-	+	+
	F	H-LH	-	+	-	+	+	+	+	+	-	-	-	+
	F	H-LLS	+	+	+	++	+	-	-	+	-	-	+	+
TEST	M	H-FLS	+	+	+	++	+	+	+	+	-	-	+	+
	M	H-HLS	+	+	+	++	+	+	+	++	-	-	-	+
	M	H	+	+	+	+	-	-	-	+	-	-	+	-
	F	T-RF	-	-	-	+	+	+	++	+	-	+	-	+
	F	T-RH	+	+	+	++	+	+	+	+	-	+	-	-
	F	T-RLS	-	+	-	-	-	-	-	-	-	-	-	-

**\*I: Itching; E: Erythema; O: Oedema; S: Scaling**

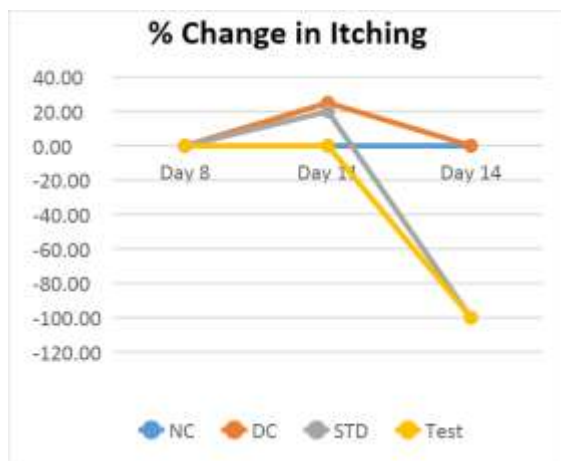
### Effect on Itching

After induction of DNFB oedema started in three groups. On 8<sup>th</sup> day gradation of itching higher in STD group. On 11<sup>th</sup> day gradation of itching is higher in STD group. On 14<sup>th</sup> day gradation of itching higher in DC group. Itching remain same at each follow up for test group on day 8 and day 11 and after that it was significantly decreases on day 14. Statistically it was found that P

value is less than 0.05. Hence, concluded that there was significant difference between 4 groups and here best group was test group in reduced itching as compare STD group.

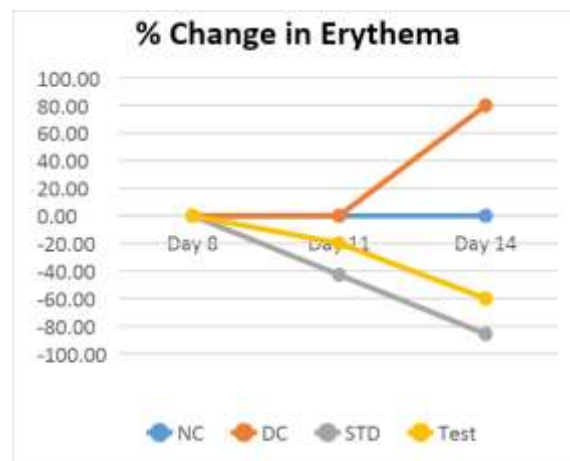
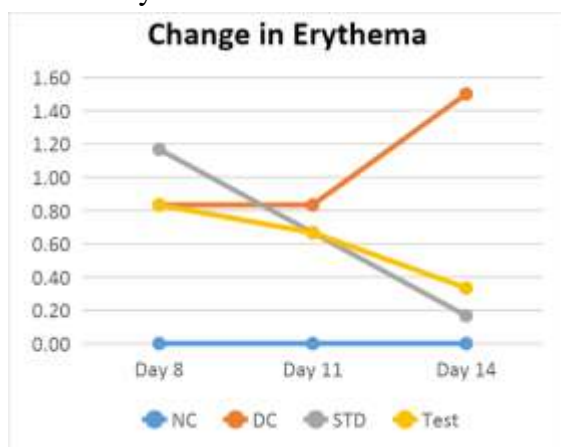






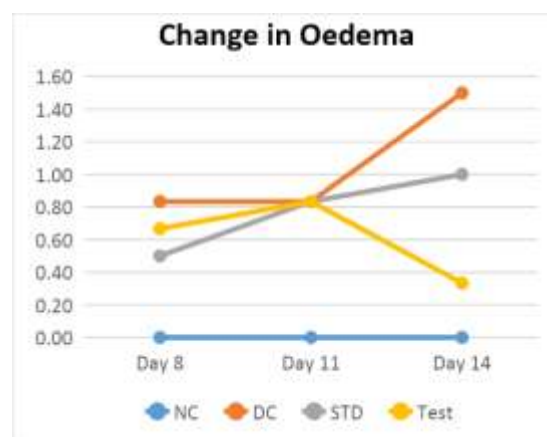
**Graph 1: Change in Itching  
Effect on Erythema**

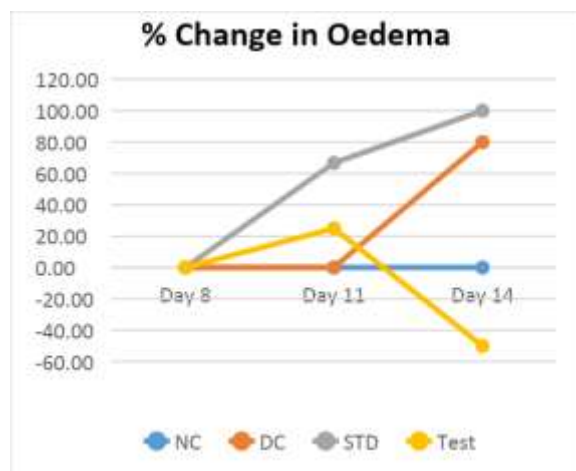
After induction of DNFB Erythema started in three groups. On 8<sup>th</sup> day gradation of erythema higher in STD group and on day 11<sup>th</sup> and day 14<sup>th</sup> it is significantly increased in DC group. And day 11<sup>th</sup> to day 14<sup>th</sup> it was significantly decreased in STD group and test group. It was significantly decrease in erythema in both STD group and test group at each follow up. Statistically it was found that P value is less than 0.05. Hence, concluded that there was significant difference between 4 groups and here best group is STD group as compared test group reduced erythema.



**Graph 2: Change in Erythema  
Effect on Oedema**

After induction of DNFB oedema started in three groups. On 8<sup>th</sup> day gradation of oedema higher in DC group. On 11<sup>th</sup> day gradation of oedema is same DC group, STD group, test group. On 14<sup>th</sup> day oedema is higher in DC group.in DC group and STD group on 14<sup>th</sup> day gradation of oedema increased and it is significantly decreasing in test group. Statistically it was found that P value is less than 0.05. Hence, concluded that there was significant difference between 4 groups and here best group was test group in reduced oedema as compare to STD group.

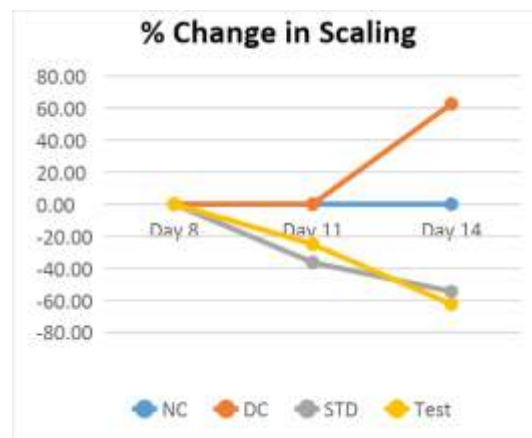
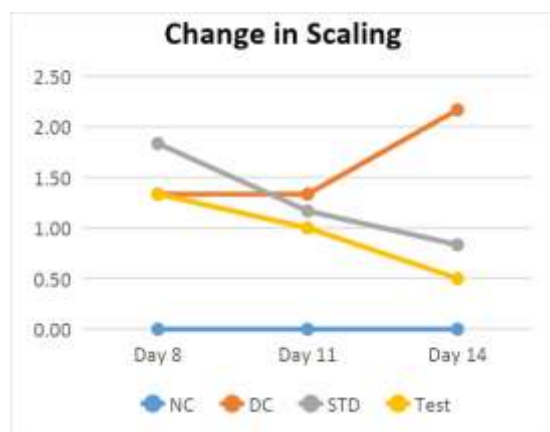




**Graph 3: Change in Oedema**

### Effect on Scaling

After induction of DNFB scaling started in three groups. On 8<sup>th</sup> day gradation of scaling higher in STD group. On 11<sup>th</sup> day gradation of scaling higher in DC group and on 14<sup>th</sup> day gradation of scaling higher in DC group. Scaling significantly decreasing at each follow up in STD and test group. Statistically it was found that P value is less than 0.05. Hence, concluded that there was significant difference between 4 groups and here reduced scaling in Test group is slightly more than STD group on 14<sup>th</sup> day.



**Graph 4: Change in Scaling**

On the basis of statistical analysis of assessment criteria and histopathology reports conclusion was drawn *Aragvadh patra lepa* is useful against DNFB induced allergic eczema in Balb/c mice.

### On observation-

After induction of eczema by DNFB on dorsum of mice. Skin lesion such as Itching, Erythema, Oedema and Scaling were observed in all three groups with varying intensity of lesion with dose of DNFB.

Healing of lesion after application of *Aragavdha Patra* and clonate ointment was observed but healing process was different in this two group.

- Different modified dosage also can be designed with *Aragvadh Patra* as its chief constituent and trials can be done on that.

## 6. DISCUSSION

In this project efforts were done to assess efficacy of traditional *lepa Kalpana* which was prepared from *Aragvadh patra*.



Overall project study was a nice experience revealed many interesting and research provoking findings. These findings were put here and discussed thoroughly.

### Conceptual study

Conceptual foundation is first step towards any scientific research. For this research project a skin disease *vicharchika* was selected and drug *Aragvadha patra* was chosen to assess its efficacy in *vicharchika*.

### Pre-clinical study

- General observations
- Experimental parameter evaluation

### Mode of Action of *Aragvadha Patra Lepa*:

- Skin is *Vata and Pitta* Sthana, in case of *Vicharchika Kapha dosha* is vitiated which vitiates these residing normal *dosha*. *Aragvadha* helps to exhales vitiated *kapha dosha*.<sup>[5]</sup>
- Aragvadha Patra Lepa* is intended for its penetration through the skin for its therapeutic action. It will help to balance *Udan Vayu*. Applied *Lepa* undergoes *Pachana* by *Bhrajak Pitta*. Thereafter, *Udan Vayu* facilitates action of *lepa* from outer surface of the skin to the *Sira*.
- Sushruta* described *Tiryakgami Sira* which are four in number and further divided into small branches, these small branches of *Sira* are beneath the skin. Essential elements from, *Avagaha*, *Abyanga*, *Parisheka Lepam* after metabolized by *Bhrajaka Pitta* and *doshaghna* action of *lepa* takes place.<sup>[6]</sup>

In *Sushrut samhita* seven layers of skin are explained where layer wise diseases of skin are explained. Considering this, *lepa* forms can be designed as per state of *dosha* and severity of disease.

### PRE-CLINICAL STUDY

#### General observation

Humans and animals are similar as they have the same organ systems performing the same task. Humans share 95% of our genes with a mouse, making them an effective model for the human body. Many previous studies on dermatitis/ eczema was done on Balb/c mice and according to the description in Michael Festing's list of inbred strains of mice, Balb/c mice are particularly well known to produce monoclonal antibodies and this mouse can be used to support research in immunology and inflammation research, Neurobiology research, Cancer research. 24 Balb/c mice all male was selected for study.

Mice were divided into four groups (Normal control, Disease control, and Standard control and Test control) with 6 mice in each group. Dinitrofluorobenzene (DNFB) selected for induction of allergic eczema in Balb/c mice which is highly toxic irritant compound.

#### It was found that-

Skin lesion such as erythema, oedema, scaling was diminished by topical application of *Aragvadha patra lepa* as well as standard drug but the diminishing pattern was different.

#### Experimental parameter evaluation

Except Normal control animals, every animal was sensitized with 30 µlDNFB

(0.1%, v/v). On day 7 of the experiment animals were challenged with DNFB. The degree of skin reactions were measured as shown in table in the form of Itching, Erythema, Oedema and Scaling.

Animals were randomly divided depending upon degree of erythema and scaling on day 8 and treatment was initiated.

It was observed that degree of erythema and scaling was high and maintained upto day 14 of experiment. Treatment with standard cream formulation and test drug decreased this degree of erythema and scaling.

On the basis of degree of erythema and scaling, it can be concluded as *Aragvadha* (*Cassia Fistula* Linn.) *Patra Lepa* is useful against dinitrofluorobenzene induced allergic eczema in Balb/C mice.

## 7. CONCLUSION

The present study entitled as “COMPARATIVE STUDY OF EFFICACY OF *ARAGVADHA* (*cassia fistula* linn.) *PATRA LEPA* AND CLONATE OINTMENT IN DINITROFLUROBENZENE INDUCED ALLERGIC ECZEMA IN BALB/C MICE.” The symptoms of *vicharchika* is similar to that of eczema, which is a common inflammatory skin condition.

- On 8<sup>th</sup> day gradation of itching was higher in STD group, erythema higher in STD group, oedema higher in DC group and scaling was higher in STD group.
- On 11<sup>th</sup> day gradation of itching was higher in STD group. Erythema is higher in DC group, oedema same in DC group, STD group and test

group. Scaling was higher in DC group.

- On 14<sup>th</sup> day gradation of itching, Erythema, oedema and scaling was higher in Disease control group.

On each follow up

- % of reduction of itching is more in Test group on day 11<sup>th</sup> as compared to STD group it is increased on day 11 and on day 14<sup>th</sup> reduction of itching is same in standard control group and test group.
- % of reduction of erythema in STD group was more as compared to Test group.
- % of reduction of oedema in Test group was more as compared to STD group.
- % of reduction of scaling in STD on 11<sup>th</sup> day was more as compared to Test group but on 14<sup>th</sup> day % reduction of scaling is slightly more in Test group as compared to STD group.

Above all changes in both STD (Clonate ointment) group and Test group (*Aragvadha patra lepa*) as compared to DC group. But, from gradation it was seen that observation of STD group in both male and female mice it was NAD to minimal (+1). Test group in male mice it was NAD and in female mice it was NAD to minimal (+1). From above observation it was concluded that probably Test group (*Aragvadha patra lepa*) was equally or more effective as compared to standard group (Clonate ointment).

On the basis of all observations and results of experimental study, the null hypothesis of the study is rejected, accepting

alternative hypothesis. Thus, it is concluded that “Aragvadha patra lepa” is effective in allergic eczema.

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