EXPERIMENTAL STUDY TO ASSESS THE EFFICACY OF AYURVEDIC HERBS OVER PARTHENIUM INDUCED IMMUNOLOGICAL ODEMA.

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ABSTRACT

Background: Ayurveda, the science of life was revealed by the seers of India, thousands of years ago, flourished as a comprehensive system of healthcare among the people. Base of this ancient system of medicine can be traced in the Vedas (Atharva Veda) dated around 1200BC. This is not only a science of life but it is the experience, observation & research of many great sages in ancient period. Hence it is an external source of knowledge having multi-dimensional textual work. Ayurveda is based on tridosha & pancha-mahabhuta siddhanta which are the base for diagnosis and treatment aspects. Like-wise tri-sutra i.e. hetu (aetiology), linga (sign & symptoms) & aushadha (treatment) were given equal importance in the clinical purview. Aims & Objectives: 1) To standardize the Parthenium plant. 2) To evaluate the efficacy of Ayurvedic herbs which is selected from
Kushtagha dashemani in parthenium induced immunological oedema. **Materials & Methods:** Review of classical literature bearing the explanations of shotha (oedema) & for experimental Source wistar strain albino rats taken from Animal house of S.D.M College of Ayurveda, Udupi. **Conclusion:** Parthenium hysterophorus is allergenic and produced CMI-mediated pedal oedema (shotha) & better activity of Kushtagha Dashemani drugs was seen in CMI.

**KEYWORDS:** Parthenium, Oedema, Ayurveda, Kushtagha Dashemani etc.

**INTRODUCTION**
In present era, more emphasis is given to healthy state of an individual as well as skin care, since it has enormous cosmetic value & performs many important physiological functions in human, but it is always exposed to many elements of environment. The outward appearance of skin is dependant not only on environment, but also on complex metabolic activities undergoing within the skin & in other parts of the body. In Ayurveda, Shotha is explained pertaining to skin in understanding the various pathologies.

Shotha is classified into two types i.e. nija (Endogenous) & aganthuja (Exogenous). Nija shotha manifested by aggravation of doshas, whereas aganthuja shotha is from external factors, which involves person coming in contact with harmful leaves, creepers etc. For example, drugs like kapikacchu (Mucuna Pruriens) are been named to exemplify this phenomenon, kapi; stands for monkey & kacchu; stands for itch.

**Aims & objectives**
1) To standardize the Parthenium plant.
2) To evaluate the effect of kushtagha activity in selected dravyas of Kushtagha dashemani in parthenium induced immunological oedema.

**Shotha (oedema):** Shotha is a disease caused due to the Derangement of Doshas, which may appear in any part of the body involving Twak and Mamsa. It is characterized by swelling, pain, Redness and raised local temperature.

‘Shotha’ is found as a main symptom in much number of ailments like Visarpa (erysipelas), Pidaka (eruptions), Arbuda (abnormal mass) etc.[1] But that which is going to spread vastly, which is nodulated, equal or unequal and particularly located dosha-samuha in the Twak (skin) and Mamsadi dhatus (tissue elements) is Shotha.
*Shotha, Shwayathu, Shopha and Utsedha were termed to be synonymous to word Shotha which means marked swelling of the skin in any place of the body.*

**Bheda (Types)**

**According to Charaka:** Even though all the three *doshas* involved in the manifestation of all the types of the *Shotha*, it is on the basis of the predominance of the respective *doshas* that *vataja, pittaja and kaphaja* varieties of disease are determined and therapies are prescribed accordingly.

All the varieties of the *Shotha* are considered to be *tridoshaja* i.e. they are caused by the vitiation of all the three *doshas* even so the causes of inflammation differs from one to another according as the particular *dosha* which is predominantly vitiated. The physician should therefore determine the line of treatment according to the predominance of one *dosha* or the other.

1) On the basis of *Dosha*
   a) *Vataja* b) *Pittaja* c) *Kaphaja*

2) On the basis of *Karana*
   a) *Nija* (*endogenous*) b) *Agantuja* (*exogenous*)[2]

3) On the basis of *Sthana* (*location*)
   a) *Ekangaja* (*confined to a part*) b) *Sarvangaja* (*all over the body*)[3]

**Purva rupa (premonitory symptoms):** Acharya Charaka explains- Feeling of increased temperature, burning sensation in eyes etc. and dilatation of the vessels of the locality.[4]

**Agantuja Shotha**

**Nidana (aetiology):** *Agantuja Shotha* is one which is caused by the external factors like contact of harmful leaves, creepers and shrubs etc.[5]

**Samanya Lakshana of Shotha (general signs):** Heaviness, instability, swelling, rise in temperature, thinning of vessels, horripilation’s & discoloration of the skin over the limbs are the general signs and symptoms of *svayathu.*[6]

**Treatment:** Acharya Charaka explains- External factors generally aggravate *vayu* along with vitiated *rakta* in turn causes localised swelling with red colour. Therapies indicated for the treatment of *visarpa* and those helpful in the alleviation of aggravated *vayu* and *rakta* should be administered and if swelling from poisonous substance then anti toxic therapies
administered. \cite{7} Therapies which will be prescribed for the *visarpa* is should be followed as that of *kushta* treatment.

Considering this, drugs from Kushtaghna dashemani were selected for the experimental study against the parthenium induced immunological oedema.

**DRUG REVIEW**

Drugs included under the *kushtaghna dashemani* are *Khadira, Abhaya, Amalaka, Haridra, Arushkara, Saptaparna, Aragvadha, Karavira, Vidanga* and *Jatipravala*. \cite{8} Out of these for the experimental study purpose only 4 drugs have been selected i.e. *Khadira* (*Acacia catechu* Willd), *Aragvadha* (*Cassia fistula Linn*), *Vidanga* (*Embelia ribes Burm. F*). & *Haridra* (*Curcuma longa Linn.*).

**Parthenium Dermatitis:** In India, Parthenium hysterophorus is the most notorious compositae weed known to produce contact hypersensitivity. This plant is variously known as congress grass, carrot weed, fever few, bastard fever few & white top.

Originally a resident of Mexico, this plant was introduced into India along with wheat shipments from USA in the 1950’s & since then has spread far & wide, covering almost the whole of India except for mountainous and desert areas.

The weed grows wildly on waste lands & along canals, railway tracks & roads. Though it grows more profusely during the rainy season, the growth is almost perennial.

The first report of contact hypersensitivity to Parthenium hysterophorous in India was recorded from Pune in 1966. Since then there have been many such reports from different parts of India.

Parthenium hysterophorous has been found to contain parthenin as well as hymenin, ambrosin & coron-opilin. The various dermatitis patterns described are airborne contact dermatitis, atopic dermatitis, photo dermatitis, seborrhoeic dermatitis and even exfoliative dermatitis. Eye lid involvement is quite common & some cases in the early phase of the disease present only with eyelid dermatitis. In the initial stage, there is worsening of lesions during the summer & monsoon with partial remission during the winters, but later on the disease persists throughout the year with bouts of exacerbations. \cite{9}
MATERIALS AND METHODS

Experimental source
- Wistar strain rats taken from the Animal House of S.D.M. Centre for Research in Ayurveda & Allied Sciences, Udupi.

Test drugs
a) Whole Parthenium plant aqueous extract.
b) Kushtaghna Dashemani drugs like Aragvadh, Khadira, Vidanga & Haridra aqueous extract.

Method of collection of data: Wistar strain albino rats of either sex were selected from Animal House of S.D.M. Centre for Research in Ayurveda & Allied Sciences, Udupi. Selected rats were randomly placed under 4 groups and in each group minimum of 6 rats were included.

Drug administration: Test drugs were administered for 7 days for Cell-mediated immunity assessment.

Inclusion Criteria
1. Animals selected are adult albino rats having weight from 140-280g.
2. Healthy Wistar strain rats of either sex.

Exclusion Criteria
1. Wistar strain rats weighing less than 140g and above 280g.
2. Pregnant and diseased rats.
3. Rats used for and under trial of other experiments.

Dose Calculation
The dose of the formulations was calculated by AOT study.

Statistical analysis: The data obtained were analyzed using one way ANOVA followed by Dunnat multiple comparison ‘t’ test for determining the level of significance of the observed effects, as post-HOC test if ‘p’ value of less than 0.05 was considered as statistically significant.
ANALYTICAL STUDY

Part A: Particulars of sample submitted
Investigation to be performed: Standardization.
Sample details: *Parthenium plant*.
Packing details: Packed in plastic packet.
Analytical Study source: S.D.M. Centre for Research in Ayurveda & Allied Sciences, Udupi.

Part B: Methodology
Loss on drying at 105°C: 10 g of sample was placed in tarred evaporating dish. It was dried at 105°C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

Total Ash: 2 g of sample was incinerated in a tarred platinum crucible at temperature not exceeding 450°C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid insoluble Ash: To the crucible containing total ash, add 25ml of dilute HCl. Collect the insoluble matter on ash less filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug.

Alcohol soluble extractive: Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value.

Water soluble extractive: Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-
weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Average value can be considered.

**HPTLC:** One gram of powdered samples were dissolved in 10 ml ethanol and kept for cold percolation for 24h and filtered. 4, 8 and 12 µl of the above samples of were applied on a pre-coated silica gel F$_{254}$ on aluminium plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethylacteate (8:1). The developed plates were visualized in UV 254, 366 nm and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254 and 366 nm. R$_f$, colour of the spots and densitometric scan were recorded.

**PART C: RESULTS**

![TLC photo documentation](image)

**Figure 1.** Showing the TLC photo documentation of Alcohol extract of Parthenium plant

<table>
<thead>
<tr>
<th>At 254nm</th>
<th>At 366nm</th>
<th>At 540nm</th>
<th>Post derivatisation</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="TLC photo documentation" /></td>
<td><img src="image" alt="TLC photo documentation" /></td>
<td><img src="image" alt="TLC photo documentation" /></td>
<td><img src="image" alt="TLC photo documentation" /></td>
</tr>
</tbody>
</table>

Track 1- Parthenium Plant– 3 µl, Track 2– Parthenium Plant – 6 µl, Track 3– Parthenium Plant – 9 µl  
Solvent system: Toluene: Ethyl Acetate (9:1).
Table 1. Showing the Physico-chemical parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results n=3 %w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss On Drying</td>
<td>5.993</td>
</tr>
<tr>
<td>Total ash</td>
<td>11.652</td>
</tr>
<tr>
<td>Acid Insoluble Ash</td>
<td>1.888</td>
</tr>
<tr>
<td>Alcohol Soluble Extractive</td>
<td>6.042</td>
</tr>
<tr>
<td>Water Soluble Extractive</td>
<td>19.909</td>
</tr>
</tbody>
</table>

Table 2: Showing the Rf values of the sample Parthenium Plant

<table>
<thead>
<tr>
<th>At 254nm</th>
<th>At 366nm</th>
<th>At 540nm</th>
<th>Post derivatisation</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>0.04(F Red)</td>
<td>0.04(L Green)</td>
<td>0.04(Violet)</td>
</tr>
<tr>
<td>-</td>
<td>0.12(F L Red)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>0.22(F L Red)</td>
<td>-</td>
<td>0.16(L Violet)</td>
</tr>
<tr>
<td>-</td>
<td>0.34(F L Red)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.40(L Green)</td>
<td>-</td>
<td>-</td>
<td>0.40(L Violet)</td>
</tr>
<tr>
<td>-</td>
<td>0.42(F Red)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>0.53(F L Red)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.58(L Green)</td>
<td>-</td>
<td>0.58(L Green)</td>
<td>0.58(L Violet)</td>
</tr>
<tr>
<td>-</td>
<td>0.60(F D Red)</td>
<td>-</td>
<td>0.60(L Violet)</td>
</tr>
<tr>
<td>0.63(Green)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>0.81(F L Red)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>0.88(F Violet)</td>
<td>-</td>
<td>0.88(L Violet)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>0.92(L Green)</td>
<td>0.92(L Violet)</td>
</tr>
<tr>
<td>-</td>
<td>0.95(F L Red)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
### Track 7, ID: *Parthenium* plant

<table>
<thead>
<tr>
<th>Peak</th>
<th>Start Position</th>
<th>Start Height</th>
<th>Max Position</th>
<th>Max Height</th>
<th>Max %</th>
<th>End Position</th>
<th>End Height</th>
<th>Area</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02 Rf</td>
<td>0.0 AU</td>
<td>0.04 Rf</td>
<td>77.7 AU</td>
<td>21.07 %</td>
<td>0.05 Rf</td>
<td>1.3 AU</td>
<td>662.4 AU</td>
<td>8.82 %</td>
</tr>
<tr>
<td>2</td>
<td>0.11 Rf</td>
<td>0.1 AU</td>
<td>0.15 Rf</td>
<td>17.3 AU</td>
<td>4.69 %</td>
<td>0.17 Rf</td>
<td>0.4 AU</td>
<td>315.9 AU</td>
<td>4.03 %</td>
</tr>
<tr>
<td>3</td>
<td>0.36 Rf</td>
<td>4.1 AU</td>
<td>0.38 Rf</td>
<td>13.2 AU</td>
<td>3.59 %</td>
<td>0.42 Rf</td>
<td>0.1 AU</td>
<td>254.9 AU</td>
<td>3.29 %</td>
</tr>
<tr>
<td>4</td>
<td>0.42 Rf</td>
<td>0.3 AU</td>
<td>0.46 Rf</td>
<td>16.9 AU</td>
<td>4.58 %</td>
<td>0.46 Rf</td>
<td>4.0 AU</td>
<td>327.0 AU</td>
<td>4.22 %</td>
</tr>
<tr>
<td>5</td>
<td>0.50 Rf</td>
<td>2.3 AU</td>
<td>0.53 Rf</td>
<td>20.7 AU</td>
<td>5.61 %</td>
<td>0.57 Rf</td>
<td>0.1 AU</td>
<td>433.4 AU</td>
<td>5.60 %</td>
</tr>
<tr>
<td>6</td>
<td>0.60 Rf</td>
<td>2.1 AU</td>
<td>0.66 Rf</td>
<td>38.5 AU</td>
<td>10.43 %</td>
<td>0.66 Rf</td>
<td>23.5 AU</td>
<td>850.3 AU</td>
<td>10.98 %</td>
</tr>
<tr>
<td>7</td>
<td>0.68 Rf</td>
<td>23.8 AU</td>
<td>0.72 Rf</td>
<td>142.0 AU</td>
<td>38.50 %</td>
<td>0.76 Rf</td>
<td>2.0 AU</td>
<td>3270.8 AU</td>
<td>42.25 %</td>
</tr>
<tr>
<td>8</td>
<td>0.89 Rf</td>
<td>0.5 AU</td>
<td>0.95 Rf</td>
<td>42.5 AU</td>
<td>11.53 %</td>
<td>0.96 Rf</td>
<td>4.5 AU</td>
<td>1606.4 AU</td>
<td>20.75 %</td>
</tr>
</tbody>
</table>

**Fig 2.a** Showing the *Parthenium* Plant raw drug at 254 nm

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**Fig 2.b** Showing the *Parthenium* Plant raw drug at 366 nm
**Figure 2.** Showing the Densitometric Scan of the sample Parthenium Plant

**Part D: Remarks:** The given sample has been standardized as per standard testing protocol. Standardization parameters, HPTLC fingerprint, densitometric scan and R<sub>f</sub> values of the sample has above.

**EXPERIMENTAL STUDY**

**PHASE I**

**AOT STUDY**

Table: 3 Showing the details of animals for AOT study

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Animal species</td>
<td>Rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Strain</td>
<td>Wistar albino</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Source</td>
<td>Animal house attached to SDM Research center, SDM Ayurveda College Udyavara</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Selection</td>
<td>A total of 8 healthy either sex of body weight 160-260g Rats were selected according to AOT software.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Acclimatization period</td>
<td>All the selected animals were kept Acclimatization for 7 days before dosing.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Numbering and identification</td>
<td>The animal was marked with saturated Picric acid solution in water for proper Identification. The marking within the cages is as follows.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table: 4 Showing the identification of animals, its desired dose (AOT), body weight & calculated dose.

<table>
<thead>
<tr>
<th>Sl. no</th>
<th>Identification of animals</th>
<th>Desired dose (according to AOT)</th>
<th>Body weight (grams)</th>
<th>Calculated dose (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Head</td>
<td>175mg/kg</td>
<td>275</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>Neck</td>
<td>550mg/kg</td>
<td>246</td>
<td>1.12</td>
</tr>
<tr>
<td>3</td>
<td>Back</td>
<td>175mg/kg</td>
<td>250</td>
<td>0.72</td>
</tr>
<tr>
<td>4</td>
<td>Base of the tail</td>
<td>550mg/kg</td>
<td>192</td>
<td>0.88</td>
</tr>
<tr>
<td>5</td>
<td>Tip of the tail</td>
<td>175mg/kg</td>
<td>240</td>
<td>0.7</td>
</tr>
<tr>
<td>6</td>
<td>Fore limb</td>
<td>550mg/kg</td>
<td>226</td>
<td>1.2</td>
</tr>
<tr>
<td>7</td>
<td>Hind limb</td>
<td>2000mg/kg</td>
<td>230</td>
<td>2.3</td>
</tr>
<tr>
<td>8</td>
<td>No mark</td>
<td>550mg/kg</td>
<td>225</td>
<td>1.03</td>
</tr>
</tbody>
</table>

Husbandry condition

1. **Housing:** Rats were housed in each cage of poly propylene with stainless steel top grill. The dry paddy husk was used as bedding material and was changed every morning.

2. **Environment:** The animals were exposed to 12 hours light and 12 hours dark cycle with the relative humidity of 50 to 70% and the ambient temperature was 22 ± 03°C degrees.

3. **Diet:** Sai Durga feed Bangalore rat pellet, was provided throughout the study period except on previous night of dosing i.e. (over-night) fasting before dosing. The drinking water was given *ad-libitum* in polypropylene bottles with stainless steel sipper tube.

Preparation of Test formulation for administration

1. **Test drug:** Parthenium whole plant aqueous extract

2. **Vehicle:** Gum acacia

3. **Dose preparation:** The test drug was made in to fine suspension in vehicle with suitable concentration. All the animals were dosed constant dose volume (1 ml/ 100g body weight) 175mg/kg, 550mg/kg, 2000mg/kg.

4. **Schedule:** Single dose per animal

   a) **Administration:** The test formulation was administered through intra-peritoneal at different dose levels to respective animal through sterile disposable syringe.

   b) **Dose fixation:** According to the AOT Software.

   c) **Route:** Intra-peritoneal

   d) **Dose:** 175mg/kg, 550mg/kg, 2000mg/kg test substance

   e) **Dose volume:** 1ml/100g animal
The LD 50 value was found to be 550mg/kg with a confidence limit of 196.4 to 884mg/kg.

PHASE II
ASSESSMENT OF CELL MEDIATED IMMUNITY
A) i) Drug Used
   a) Whole Parthenium plant aqueous extract.
   b) *Kushtaghna Dashemani* drugs like Aragvadha, Khadira, Vidanga & Haridra aqueous extract.

ii) Chemicals Used
   a) Potash Alum
   b) Normal Saline
   c) Cyclophosphamide
   d) 10% Sodium Carbonate
   e) 30% SRBC solution (Sheep blood would be collected from city slaughter house in a sterilized bottle.)

iii) Equipment’s & glass ware to be used
    Glass tubes, Glass beakers, pH Meter, Syringes etc.

Dose for Rats
1) *Parthenium whole plant aqueous extract*: From AOT study
   LD50 = 550mg/kg, then 1/5\textsuperscript{th} of LD50 is 110mg/kg & 1/10\textsuperscript{th} of LD50 is 55mg/kg.

2) *Kushtaghna Dashemani drugs aqueous extract*
   Drugs like Aragvadha, Khadira, Vidanga & Haridra made into aqueous extract form. By AOT study dose has been calculated like- as LD50 is more than 2000 mg/kg, so 1/10\textsuperscript{th} of LD50 is 200mg/kg, So 200mg/kg (TED) & 400mg/kg (TEDx2).

c) Route of Drug Administration
   The test drug Parthenium was administered according to the body weight of the animals by intra-peritoneal route & *Kushtaghna Dashemani* drugs was administered according to the body weight of the animals by oral route with the help of an oral feeding tube attached to injection syringe.
d) Animals
Wistar strain albino rats of either sex weighing between 140-280g were used for experimental study with the following conditions.

Rats were used for experimental study with the following conditions.
- The animals were obtained from animal house attached to the Pharmacology Laboratory S.D.M Centre for Research in Ayurveda and Allied Sciences. ETHICS NO: SDM-CAU/IAEC/13-14-08.
- They were exposed to natural day and night cycles with ideal laboratory condition in terms of ambient temperature, humidity.
- They were fed with pellets from “Sai Durga Feeds”, Bengaluru and water ad libitum.

STOCK SOLUTION PREPARATION

a) The aqueous extract of Parthenium should be stored in a container and kept in desiccator for future usage. Dose of Parthenium which is set by AOT study is referred as a standard marker and to assess the cell mediated and humoral immunity required dose should be taken i.e. 1/10th of LD50 dose and mixed with 10ml of distilled water.

b) Two grams of individual drug extract weighed individually and mixed well using mortar & pestle and after thorough mixing, drugs were stored in a container and kept in desiccator. Later for dosage purpose daily 200mg of Kushtaghna Dashemani drugs taken and 10ml of Distilled water with 50 mg of Gum acacia used for test 1 group & 400mg of Kushtaghna Dashemani drugs taken and 10ml of Distilled water with 50 mg of Gum acacia used for test 2 group.

e) GROUPING
ASSESSMENT OF CELL MEDIATED IMMUNITY
The rats were grouped into different groups randomly irrespective of sexes and each group comprised of six animals.

Group 1: Water control + Parthenium.
Group 2: Cyclophosphamide control
Group 3: Kushtaghna Dashemani solution (TED)
Group 4: Kushtaghna Dashemani solution (TEDx2)
The rats were sensitized subcutaneously (1ml/100g body weight) on first day & seventh day of drug administration with the following solution

i. Parthenium Solution – 1ml

ii. Normal Saline – 4ml

iii. Potash Alum-1ml

iv. pH of the above reagent (i.e. potash alum adjuvant) was maintained between 5.6-6.8 using 10% sodium carbonate.

On Seventh day initial paw volume of left hind paw were noted and 0.1 ml of (Parthenium Solution – 1ml, Normal Saline – 4ml, Potash Alum-1ml) were injected into plantar aponeurosis of left hind paw, volume of immunological edema thus produced was measured by volume displacement method. After 24 hours & 48 hours with plethysmograph. Percentage increase in paw volume, which is the induced edema formation over initial value, was calculated. The values from control group were compared with the values from the test drug administered groups to assess the cell mediated immunity response of the drug.

**OBSERVATION AND RESULTS**

Cell-mediated immunity

Table: 5. Showing the Effect of *Kushtaghna Dashemani* on cell-mediated immunity in 24 & 48 hours.

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial MEAN ± SEM</th>
<th>24 hr MEAN ± SEM</th>
<th>48 hr MEAN ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parthenium control</td>
<td>0.72 ± 0.03</td>
<td>1.10 ± 0.04**</td>
<td>1 ± 0.06**</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>1.02 ± 0.06</td>
<td>1.34 ± 0.10**</td>
<td>1.21 ± 0.07*</td>
</tr>
<tr>
<td>Test 01</td>
<td>0.77 ± 0.03</td>
<td>1.13 ± 0.08**</td>
<td>1.05 ± 0.05**</td>
</tr>
<tr>
<td>Test 02</td>
<td>0.82 ± 0.01</td>
<td>1.13 ± 0.05**</td>
<td>0.94 ± 0.02**</td>
</tr>
</tbody>
</table>

Data: MEAN ± SEM ** P<0.01

Table: 6. Showing the Effect of *Kushtaghna dashemani* in cell mediated immunity (% change in 24hrs)

<table>
<thead>
<tr>
<th>Group</th>
<th>% change in 24hrs MEAN ± SEM</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parthenium control</td>
<td>54.40 ± 4.23</td>
<td>----</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>31.94 ± 9.72</td>
<td>41.28↓</td>
</tr>
<tr>
<td>Test 01</td>
<td>54.34 ± 7.35</td>
<td>0.11↓</td>
</tr>
<tr>
<td>Test 02</td>
<td>38.02 ± 3.34</td>
<td>30.11↓</td>
</tr>
</tbody>
</table>

Data: MEAN ± SEM
The data shows there was decrease in Percentage change in 24 hours in cyclophosphamide group TED & TEDx2 groups when compared to the Parthenium control group, the observed decrease was found to be statistically non-significant.

**Table: 7. Showing the Effect of *Kushtaghna dashemani* in cell mediated immunity (% change in 48hrs)**

<table>
<thead>
<tr>
<th>Group</th>
<th>% change in 48hrs MEAN ± SEM</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parthenium control</td>
<td>38.64 ± 4.74</td>
<td>----</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>26.05 ± 13.45</td>
<td>32.58↓</td>
</tr>
<tr>
<td>Test 01</td>
<td>42.28 ± 7.57</td>
<td>9.42↑</td>
</tr>
<tr>
<td>Test 02</td>
<td>15.27 ± 2.47</td>
<td>60.48↓</td>
</tr>
<tr>
<td>Data: MEAN ± SEM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The present study had objective to detect the cell mediated immunity in parthenium induced animal model.

Immunology is one which covers the study of all aspects of immune system. It deals with the physiological functioning of the immune system in states of both healthy & diseased. Malfunctions of the immune system may produce varied immunological disorders like hypersensitivity, immune deficiency disorders etc.

**Cell-mediated immunity animal model**

It is a well-known fact that to assess effect of test drug on cell mediated immunity egg albumin paw edema in pre-sensitized animals is used as a model. In this case egg albumin or similar antigenic agent is injected with adjuvant containing potassium alum. To ascertain whether such a CMI is elicited by parthenium suspension was injected in to the plantar aponeurosis of animals pre-sensitized with parthenium-potassium alum suspension. The injection of parthenium suspension elicited significant edema indicating that it has the
potential to elicit cell mediated immunity (CMI). This was though non-significant was suppressed by cyclophosphamide. TED dose of test formulation had only a marginal effect whereas TED x 2 dose produced more than 60% inhibition of 48 hour immunological oedema. Thus the study meets both the requirements mentioned in the objective. The first one clearly shows that parthenium is allergogenic and produced CMI- mediated pedal oedema like egg albumin. This may be the reason for the strong contact dermatitis many a times associated with exposure to parthenium. Secondly kushtaghna dashameni gana drugs were moderately effective like cyclophosphamide in suppressing this CMI eliciting effect. Further refinement of the formulation may provide better effect. This clearly proves that this Kushtaghna dashemani gana has good potential in the treatment of allergic dermatitis.

Analysis of the data shows in the control group weak suppression of immunological oedema at 24th and 48th hour after injection of the paw edema eliciting agent was observed. In test drug (Kushtaghna dashemani) administered group significant decrease was observed i.e. suppression of paw oedema was observed in 24th hour and stimulation in test 1 group and suppression in test 2 group of paw oedema was observed in 48th post injection. This indicates the effect and test drug formulation (kushtaghna dashemani drugs) possess very good immunological suppression effect and slight stimulation which is of higher magnitude. The immunological oedema represents expression of cell mediated immunity hence based on the results obtained it can be inferred that Kushtaghna dashemani dravyas has cell mediated immunity suppression effect. It is pertinent to recollect here that most of the contact dermatitis type of allergy is due to CMI its suppression by the test drug group may provide evidence for their efficacy against it.

CMI is elicited by two mechanisms. The first mechanism is through activation of helper T cells. The second mechanism involved in CMI elicitation is activation and increased formation of cytotoxic –T cells.

Cell mediated immunity is the responsible for delayed type hypersensitivity and certain T cells suppress antibody production. The test sample was evaluated to assess their effect on cell mediated immunity against an experimental model, which is supposed to represent cell mediated immunity. It involved producing immunological edema. TH-1 T-lymphocyte pathway controls cell mediated immunity. The first step in the reaction is the antigen processing and presentation by macrophages and other related antigen presenting cells followed by differentiation of T-cells into different types including TH-1 type. TH-1 cells
produce IL-2, tumor necrosis factor-β (TNF-β) & γ- interferon (IFN-γ). These cytokines activate macrophages enhancing their phagocytizing capacity & stimulate another subset of T-lymphocyte known as CD8+, which mature into cytotoxic cells, which will neutralize macrophages leads to generation of large amounts of chemical mediators, reactive oxygen metabolites and neutral proteases, which are responsible for the inflammation observed during this reaction.

CONCLUSION

_Shotha_ is an independent disease caused by derangement of _doshas_, which appear in any part of the body involving the _tvak & mamsa & agantuja shotha_ said to be manifested by contact of various types of poisonous herbs.

Analysis of the results clearly indicates that parthenium extract with adjuvant elicits immunological oedema in pre-sensitized animals indicating CMI eliciting effect. This effect was moderately suppressed by both cyclophosphamide and TED x 2 dose of test formulation. It can be concluded that potent activity of parthenium as an allergenic & better activity of _Kushtaghna Dashemani_ drugs was seen in CMI.

REFERENCES